REVIEWS IN Aquaculture

Reviews in Aquaculture, 1–20 doi: 10.1111/raq.12299

Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review

Kathy Overton[1,2], Tim Dempster[2], Frode Oppedal[1], Tore S. Kristiansen[1], Kristine Gismervik[3] and Lars H. Stien[1]

1 Animal Welfare Research Group, Institute of Marine Research, Bergen, Norway
2 Sustainable Aquaculture Laboratory – Temperate and Tropical (SALTT), School of BioSciences, University of Melbourne, Parkville, Australia
3 Animal Welfare Research Group and Fish Health Group, Norwegian Veterinary Institute, Oslo, Norway

Correspondence
Lars H. Stien, Animal Welfare Research Group, Institute of Marine Research, P.O. Box 1870, NO-5817 Bergen, Norway. Email: lars.helge.stien@hi.no

Received 6 August 2018; accepted 19 September 2018.

Abstract

With the expansion of Atlantic salmon aquaculture, the economic and ecological impacts of salmon lice (*Lepeophtheirus salmonis*) has increased. Norway battles this problematic parasite with various control and preventative methods within farms. We analysed two national-level databases to examine the number of operations reported each year from 2012 to 2017 and salmon mortality rates attributable to each operation type. From 2012 to 2017, 1.4 times more operations were registered, despite only limited increases in biomass produced across this period. We detected a rapid and recent paradigm shift in the industry's approach to lice control from chemotherapeutant to non-medicinal operations. Chemotherapeutants (azamethiphos, cypermethrin, deltamethrin and hydrogen peroxide) dominated operations from 2012 to 2015 (>81%), while mechanical and thermal treatments dominated in 2016 and 2017 (>40% and >74%, respectively). Thermal operations caused greatest mortality increases (elevated mortality for 31% of treatments), followed by mechanical (25%), hydrogen peroxide (21%), and azamethiphos, cypermethrin and deltamethrin (<14%). Temperature, fish size and pre-existing mortality rates all influenced post-treatment mortality outcomes. For chemotherapeutants, mortality increased as sea temperature increased. For mechanical and thermal treatments, mortalities increased at low (4–7°C) and high (13–16°C) temperatures. Fish with high pre-existing mortality (0.25–1.0% mortality the month before treatment) experienced increased mortality after treatment, and large fish (≥2 kg) were more susceptible to increased mortality than small (<2 kg). Generally, thermal, mechanical and hydrogen peroxide operations performed better in 2017 compared to 2015 and 2016, as the percentage of mortality observations were lower. With mechanical and thermal treatments now predominant, future research and industry development should prioritise reducing mortality and improving post-treatment outcomes.

Key words: Atlantic salmon, *Lepeophtheirus salmonis*, *Salmo salar*, sea lice, treatment.

Introduction

Since its conception in mid-Norway in the late 1960s, the Atlantic salmon aquaculture industry has grappled with the pathogenic marine parasite: the salmon louse *Lepeophtheirus salmonis* (Costello 2006; Torrissen *et al.* 2013). Salmon lice are caligid copepods that attach to the skin of salmon, feeding on mucus, blood and skin (Mordue & Birkett 2009). Moderate to high infestations can lead to physical damage, skin erosion, osmoregulatory failure, secondary infections, immunosuppression and chronic stress (Bowers *et al.* 2000; Grave *et al.* 2004; Hamre *et al.* 2009). Salmon lice not only have the greatest economic impact of all parasites affecting aquaculture, but infestations in farms also negatively affect wild stocks via spillback effects (Torrissen *et al.* 2013; Vollset *et al.* 2017). Controlling this parasite is troublesome, expensive and important, not only to minimise production losses and to improve the welfare of farmed fish, but also to protect wild salmon populations.

Norway produces the most salmon worldwide, and the number of farmed salmon greatly exceeds the number of wild salmon (~728 farmed harvested salmon per wild salmon in 2015, based on the number of fish held in farms and the number of salmon estimated to return to Norwegian rivers each year: Thorstad & Forseth 2016; Norwegian Directorate of Fisheries 2018). Salmon farms are often located along natural wild salmon migration routes, which can place additional infestation pressures on out-migrating smolts (Krkošek *et al.* 2013). The main source of lice infestations originates from farms, where regular delousing operations constantly place selection pressure on resistance development (Torrissen *et al.* 2013). While the development of new treatment methods can help to reduce lice loads after being introduced, due to rapid resistance development, they are often only valuable and efficacious for limited time periods (Aaen *et al.* 2015). The principle of rotating treatments is therefore essential to follow, to try and maintain treatment efficacies for as long as possible. The development of coordinated production zones (Fig. 1), synchronized fallowing and synchronized treatments throughout Norway are other control measures (Norwegian Ministry of Trade, Industry and Fisheries 2012). The development of in-cage technologies that prevent infestations through environmental manipulation has also become increasingly popular, especially skirts around the cages (Grøntvedt *et al.* 2018; Stien *et al.* 2018), snorkel cages (Oppedal *et al.* 2017; Wright *et al.* 2017) and deep-water feeding (Frenzl *et al.* 2014).

The Norwegian lice surveillance programme requires each farm to develop a general plan for prevention and treatment of salmon lice (Torrissen *et al.* 2013). Generally, plans should include regular lice counting within the farm, methods and routines for delousing operations, routines for evaluation of treatment efficacy and routines for fallowing. All farms are required to annually re-evaluate and update their lice management plans, and also provide details to the Norwegian Food Safety Authority (Torrissen *et al.* 2013).

Permissible lice levels above which farms are required by law to initiate measures to reduce lice (Norwegian Ministry of Trade, Industry and Fisheries 2018) vary in space and time. As of March 2017, lice levels cannot exceed 0.2 mature females per salmon for 6 weeks across spring during the period when wild salmon smolts out-migrate, with the timing of the spring period different south and north of North Trøndelag (64°N, 12°E). For the rest of the year across Norway, an average of up to 0.5 mature females per salmon is permissible. Five main chemotherapeutants and three main types of non-medicinal principles are used for delousing operations by the industry.

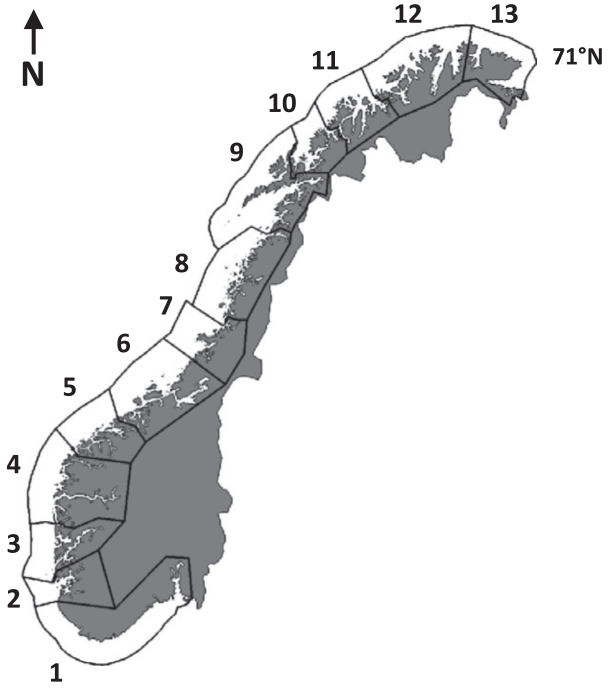


Figure 1 The 13 Atlantic salmon aquaculture production zones in Norway implemented by the Norwegian Ministry of Trade, Industry and Fisheries in 2017

Chemotherapeutants for salmon louse control

Anti-parasitic chemotherapeutants are used to treat lice infestations in most countries where salmon aquaculture is practiced (Burridge *et al.* 2010). As salmon lice reproduce throughout the year, the aim of successful control was to prevent internal infestation cycles from being established as well as preventing the presence of gravid females (Burridge *et al.* 2010).

Chemotherapeutants are used in two main ways: bath treatments and in-feed additives (Burridge *et al.* 2010). Organophosphates, pyrethroids and hydrogen peroxide are administered through bath treatments, whereas avermectins (emamectin benzoate and diflubenzuron) are administered as additives in medicated feeds (Burridge *et al.* 2010). Bath treatments are performed either by lining a sea-cage with a tarpaulin and reducing the volume of water within the cage, which normally also increases fish density (Volent *et al.* 2017), or by crowding and pumping fish into a well-boat. The recommended treatment concentration for the chemotherapeutant is added, and salmon are held in the bath for the recommended treatment time. After treatment, the tarpaulin is removed (in-cage treatment) or the fish are pumped out (well-boat treatment) and the chemotherapeutant is released into the surrounding water.

Organophosphates: azamethiphos

Organophosphates were the first chemotherapeutant introduced for salmon delousing treatments as they are water soluble (Torrissen *et al.* 2013). Their lice removal mechanism works by acting as an inhibitor of acetylcholinesterase, which causes overstimulation of the muscular and nervous systems, leading to paralysis (Walsh *et al.* 2007). Until 1995, more than 80% of all delousing operations administered in Norway were performed using organophosphates (Fallang *et al.* 2004). Dichlorvos was the main chemotherapeutant used in all salmon farming countries until the early 1990s, after which widespread resistance appeared (Torrissen *et al.* 2013). In 1994, azamethiphos was introduced, which brought an additional benefit of being 10× more effective than dichlorvos, but also safer for mammals and therefore had higher safety margins for handling (Roth *et al.* 1993; Burka *et al.* 1997; Aaen *et al.* 2015). Azamethiphos was used in Norwegian salmon aquaculture for lice control from 1994 to 1999, and from 2008 onwards (Aaen *et al.* 2015).

Azamethiphos has a rapid effect that can be observed within a few hours (Torrissen *et al.* 2013). It is effective in removing pre-adult and adult salmon lice, but not the sessile larval stages (Roth *et al.* 1993; Whyte *et al.* 2016). Treatment is administered as in-cage bathing using a tarpaulin, and baths are performed at 0.1 ppm for 30–60 minutes (Roth *et al.* 1996; Burka *et al.* 1997). Increased surface activity for salmon can occur after treatment, even at recommended therapeutic treatment concentrations (Burka *et al.* 1997). Toxicity of azamethiphos for lice and salmon is thought to increase with treatment temperature (Roth *et al.* 1996).

Pyrethroids: cypermethrin and deltamethrin

Pyrethroids are synthetic analogues of natural pyrethrins, which are the active ingredient from the flower heads of *Chrysanthemum cinerariaefolium* (Burridge *et al.* 2010). They are extremely toxic to crustaceans but are also highly degradable and non-toxic to mammals. Deltamethrin and two types of cypermethrin have been used for delousing in Norway since 1994 (Burridge *et al.* 2010; Aaen *et al.* 2015). Both deltamethrin and cypermethrin interfere with louse nerve membrane function, causing paralysis and death (Miller & Adams 1982; Burridge *et al.* 2010; Torrissen *et al.* 2013). Cypermethrin and deltamethrin are effective against all attached stages of lice, although this depends on temperature (Burridge *et al.* 2010; Torrissen *et al.* 2013).

The recommended usage for cypermethrin is brand dependent, requiring different concentrations for baths between 30 minutes to 1 hour (Burridge *et al.* 2010). For deltamethrin, bathing is recommended at 2–3 μg/L for 40 minutes. Low temperatures are reported to not have toxic effects to salmon. However, Olsvik *et al.* (2014) recommended that exposure times should not be exceeded when treating with deltamethrin below 5°C. Farmers have also applied deltamethrin and azamethiphos combined in high-concentration and short-duration as bath treatments to remove lice (Olsvik *et al.* 2014).

Hydrogen peroxide

Hydrogen peroxide has been used in aquaculture for decades, including against fungal infections in hatcheries (Burridge *et al.* 2010). It was first introduced as a delousing agent in Norwegian salmon aquaculture in 1993 and was used occasionally until 1997, before chemicals such as organophosphates and pyrethroids became the treatments of choice (Aaen *et al.* 2015). However, due to the development of resistance towards these chemicals in some Norwegian production zones, hydrogen peroxide was reintroduced for delousing in 2009 (Grave *et al.* 2004; Aaen *et al.* 2015; Helgesen *et al.* 2015). Treatment is administered either in-cage or by using a well-boat.

Hydrogen peroxide is thought to cause mechanical paralysis in lice through gas bubbles forming inside the haemolymph, causing lice to fall off the salmon (Johnson *et al.* 1993; Burridge *et al.* 2010; Aaen *et al.* 2014). It is, however, increasingly toxic to salmon with treatment time, concentration and temperature (Thomassen 1993). The Norwegian Medicines Control Authority guideline recommends treatment with a dose of 1.7 g/L for 20 minutes at temperatures below 8°C, and a dose of 1.3–1.5 g/L for temperatures between 8 and 13°C (The Norwegian Medicines Control Authority 2000). Importantly, hydrogen peroxide must not be used at temperatures above 13°C, as the safety margin becomes too narrow (The Norwegian Medicines Control Authority 2000). Unlike other chemotherapeutants, hydrogen peroxide disassociates into water and oxygen, does not bioaccumulate in the environment and is therefore considered environmentally friendly (Kiemer & Black 1997). Resistance towards hydrogen peroxide has already been observed in some areas of Norway (Helgesen *et al.* 2015).

Non-medicinal delousing operations for salmon louse control

With widespread resistance towards all available chemotherapeutants spreading throughout Norway (Aaen *et al.* 2015), the industry has developed non-medicinal alternatives to control salmon lice. Five main species of cleaner fish (wrasse: *Labrus bergylta, Ctenolabrus rupestris, Centrolabrus exoletus, Symphodus melops*; lumpsuckers:

Cyclopterus lumpus) have been used as an alternative delousing method, with more than 50 million cleaner fish used in 2017 across two thirds of all farms (Norwegian Directorate of Fisheries 2018). However, the sustainability of their use and their welfare has been of concern (Skiftesvik *et al.* 2013; Olsen 2017). Underwater lasers that shoot lice off the fish (Optical Delousing™, Stingray Marine Solutions AS, Norway) have been introduced as an alternative to cleaner fish, but so far documentation of delousing efficiency in commercial farming is anecdotal (Holan *et al.* 2017).

Freshwater treatment in well-boats has shown promising results as an alternative bath treatment, and has become utilised as a delousing treatment by the industry (Powell *et al.* 2015; Hjeltnes *et al.* 2018). However, lice could potentially develop resistance towards freshwater (Ljungfeldt *et al.* 2017).

Mechanical and thermal delousing systems are recently developed technologies used as alternatives to chemotherapeutants. While they have been used by the Norwegian industry since 2015, little published data is available about the extent of their use and their broad scale effects on post-treatment outcomes for salmon. The few reports available were written during the developmental phase prior to their widespread use by the industry (Grøntvedt *et al.* 2015; Roth 2016; Gismervik *et al.* 2017).

Mechanical treatments

Three types of mechanical delousing technologies developed by three separate companies exist: Flatsetsund (FLS) Engineering AS, SkaMik AS and the Hydrolicer®. All three technologies require the fish to be crowded and then pumped up into a treatment system where the lice are mechanically removed from fish.

Gismervik *et al.* (2017) examined fish welfare and delousing efficiency for the FLS system. Fish are first pumped into the system via a funnel and passed through two low pressure washers (0.2–0.8 bar). The spray nozzles then 'flush/spray' the lice off the fish as it passes through the pipe. The water is then filtered after treatment to prevent lice from being released into surrounding waters. Cameras within the pipes monitor fish to control spray nozzle pressure and speed during the treatment. The recommended fish size for FLS system treatment is up to 4 kg, and Gismervik *et al.* (2017) found the system removed 81–100% of mobile lice and 76–91% of adult female lice. Effects on attached lice stages are uncertain (Gismervik *et al.* 2017), but FLS claim their system removes 50–70% (Flatsetsund Enginnering AS 2018).

Less is known about the SkaMik and Hydrolicer® systems. To our knowledge, no independent reports or publications are available examining welfare and mortality from these treatment methods. However, a recent survey of farmers found that scale loss was very common, salmon mortality was common and gill bleedings and wounds were observed at least during developmental phase testing for mechanical treatments (Hjeltnes *et al.* 2018). SkaMik is similar to the FLS system but includes a brush system for removing lice. However, after developments to the system in 2017, SkaMik stated that the brushes are mainly used to steer salmon through the system rather than brush off the lice (Hjeltnes *et al.* 2018). The Hydrolicer® pumps fish into a closed pipe filled with water, where inverse water turbulence 'vacuums' the lice off the fish. At a conference in Trondheim February 2017, the producers of the SkaMik-system reported that the system removed 85–95% of the lice. At the same conference, a representative from Hydrolicer® reported 82–100% removal efficiency on mobile lice, and 70–85% on adult female lice. Effects on attached lice stages are uncertain. According to the Hydrolicer® representative, post-treatment mortality was below 0.4%.

Thermal treatments

Thermal delousing is based on inactivation of lice, as they detach from fish after short exposures to warm water (Brunsvik 1997; Grøntvedt *et al.* 2015). Salmonids can survive in water temperatures of 20–34°C for short periods of time (Elliot & Elliot 1995), and while the upper thermal limit for lice is similar, due to the size difference between the host and parasite, lice have a shorter survival time (Grøntvedt *et al.* 2015; Roth 2016). There are two competing systems for thermal treatments: the Thermolicer® and the Optilicer®. Both systems have had independent research organisations investigate their effects on fish welfare and delousing efficiency (Grøntvedt *et al.* 2015; Roth 2016), but only in the developmental phase of the technologies.

In both systems, treatment begins when fish are crowded in the sea-cage and pumped past a de-watering strainer before they enter a treatment chamber filled with warm seawater at temperatures up to 34°C. The major difference between the two systems is that while the fish are pumped through the treatment chamber in the Thermolicer®, the Optilicer® has paddle wheels that push the fish at a pre-set speed through a tank with warm water. Time in the treatment chamber is usually set to 20–30 seconds in both systems (Holan *et al.* 2017). After treatment, the water around the fish is removed so that detached lice are filtered out. The fish are finally flushed through pipes back to the same or a neighbouring sea-cage.

Grøntvedt *et al.* (2015) reported that Thermolicer® treatment at 34°C removes 75–100% of mobile lice. Attached lice were counted before, immediately after, and 1, 2 and 3 weeks after treatment, revealing that treatment was ineffective in removing attached lice. Roth (2016) reported a clear relationship between seawater temperature and treatment temperature; 99% of mobile lice were removed already at 28°C in early spring, while 33°C was needed to achieve similar effects in late summer.

Delousing operation use and effects on salmon mortality in Norway

While all these delousing operations are currently used by the Norwegian salmon aquaculture industry, there has been no comprehensive analysis of treatment use and mortality risks across Norway. The Norwegian Regulation on the operation of aquaculture production sites (Norwegian Ministry of Trade, Industry and Fisheries 2008) requires all fish farmers to report local cage identification (ID), year class, number of fish, losses due to mortality, feed use, number of fish harvested and mortality data each month to the Norwegian Directorate of Fisheries. In addition, the Regulation on the prevention of salmon lice in aquaculture (Norwegian Ministry of Trade, Industry and Fisheries 2008) requires farmers to report sea temperature, number of lice (sessile, mobile and adult females), whether they have treated, treatment method and treatment substance each week to the Norwegian Food Authorities. From 2012 to 2017, there were on average 807 (range: 788 to 828) sites in operation in the sea reporting to this database (Norwegian Directorate of Fisheries 2018).

Combining these two data sets supplied by the Norwegian Directorate of Fisheries and the Norwegian Food Authorities provided data on treatment method and timing, mortality in the month before treatment, mortality in the month that treatment occurred, average fish weight and seawater temperature. First, we documented the distribution of treatment methods used in Norway to assess how treatment use changed through time. Second, we examined if mortality associated with each treatment method was temperature dependent. Third, to determine if mortality increases associated with each treatment method varied with fish health/condition, we compared salmon mortality rates post-treatment for cages that experienced low (uncompromised) and high (compromised) mortality the month before treatment at different temperatures. Fourth, to determine if salmon size influenced salmon mortality and treatment risks at different temperatures, we compared post-treatment mortality rates for small and large fish for all delousing operations. Finally, we looked at how salmon mortality observations changed across the 2012–2017 period for each delousing operation. As we had access to databases which contained all delousing registrations and all reported salmon mortalities throughout the entire period, we therefore had data on the entire population of delousing operations in Norway from 2012 to 2017. Therefore, we have not performed any statistical analysis, as statistical inference is inapplicable to complete population studies, as sampling errors disappear altogether, and *P*-values tend to zero (Alexander 2015). This means that increases and decreases observed in the data are real and do not need to be defended with statistical inference.

Analysis of delousing operation use from 2012 to 2017

We analysed the 10,130 registrations of delousing operations in Norwegian salmon aquaculture from 2012 to 2017 provided by the Norwegian Food Authorities. The number of registrations in the database represented the number of observations for each operation. We formed four broad treatment categories consisting of: (1) chemotherapeutant bathing (azamethiphos, cypermethrin, deltamethrin); (2) hydrogen peroxide; (3) thermal (Thermolicer®, Optilicer®); and (4) mechanical (Hydrolicer®, FLS Avluser, Ska-Mik Avluser). Hydrogen peroxide was separated from bathing with other chemotherapeutants to explore the Norwegian salmon aquaculture industry's use of the chemical following its reintroduction in 2009.

Total number of registered delousing operations from 2012 to 2017

We determined the number of operations registered each year from 2012 to 2017 for each of the five delousing treatment categories both at national scale and within each of the 13 production zones in Norway. We also included an additional 'Bath other' category to include all bath treatments registered with the substance as 'other', 'freshwater' or combinations of chemotherapeutics.

Post-treatment salmon mortality by treatment method

By combining the Norwegian Food Authorities and the Norwegian Directorate of Fisheries databases, we created a new data set where registered delousing operations were paired with monthly mortality data for the sea-cages at their respective sites. Each observation included percentage mortality the month before treatment, percentage mortality the month of treatment, average fish weight, seawater temperature and treatment method used. We then applied the following filters to standardise the data set: (1) only observations where the number of fish within a cage was >50,000 were used; (2) observations with extreme (>1%) salmon mortality the month before treatment were excluded; (3) instances of combined use of azamethiphos and deltamethrin were removed to ensure that observations used were only for one chemotherapeutant; (4) treatments reported as 'other' were removed; and (5) bath treatments registered with freshwater were too few and were therefore also removed. This left a total of 41,051 valid observations for further analysis.

First, we calculated an index for increase in mortality for each observation as the percentage point (pp) difference between the month with treatment and the month before. For example, if 0.4% salmon mortality was observed in a cage the month before treatment and 1.2% mortality in the month of treatment, then the mortality increase was 0.8 pp.

For each treatment category (azamethiphos, cypermethrin, deltamethrin, hydrogen peroxide, thermal and mechanical) we then calculated the percentage of observations within five mortality categories: 1–2, 2–5, 5–10, 10–25 and 25–100 pp increase in monthly mortality. Minor pp increases in mortality (i.e. <1 pp) were excluded, so that differences between mortalities observed within temperature categories and between treatments could be easily compared.

Effect of temperature on post-treatment mortality

To determine salmon mortality observed at different temperatures, we created five broad temperature categories of 0–4, 4–7, 7–10, 10–13 and 13–16°C, which span seawater temperatures observed around Norway for all seasons. The 0–4°C temperature category was not included for thermal and mechanical treatments, as no observations were made within this range. Data were grouped in these categories so that there would be reasonable numbers of observations per category. Less than 2% of all registered delousing operations occurred at temperatures >16°C and were therefore not included. To assess possible spatial autocorrelation related to temperature in the data set, we investigated the distribution of temperatures within each of the 13 production zones (Fig. S2a).

Effect of welfare status and temperature on post-treatment mortality

During our initial analysis of pp change in salmon mortality after treatment, we assumed that percentage mortality the month before treatment would not influence the pp change in mortality the month of treatment. We tested this assumption for all chemotherapeutants and thermal and mechanical delousing across the five temperature categories by assessing if high mortality the month before treatment resulted in a higher mortality in the month of treatment. For this analysis, the database was separated into two groups: 'uncompromised' fish with low mortality the month before treatment (<0.25%), and 'compromised' fish, due to the presence of disease or other stressors, with high mortality the month before treatment (0.25–1%). Data were grouped into these two categories as 0.25% mortality the month before treatment was just below the median and therefore allowed for reasonable numbers of observations per group. To assess possible spatial autocorrelation related to welfare status in the data set, we investigated the distribution of compromised and uncompromised fish within each of the 13 production zones (Fig. S2b,c).

Effect of salmon weight, welfare status and temperature on post-treatment mortality

As salmon mortality the month before treatment affected pp change in mortality after treatment, we included this in all further analysis. To determine if pp change in mortality for each treatment method varied with fish weight, the database was further separated into two groups: small (<2 kg) and large (≥2 kg). The data were split into these two categories as the median in all production zones was approximately 2 kg (Fig. S2d). We then determined the percentage of observations for small or large, compromised or uncompromised fish within each of the given mortality categories. To assess possible spatial autocorrelation related to salmon weight in the data set, we investigated the distribution of fish weights within each of the 13 production zones (Fig. S2d).

Mortality outcomes per delousing operation from 2012 to 2017

We investigated if treatment outcomes for each delousing operation improved through time. As salmon weight influenced salmon mortality observations for both uncompromised and compromised fish, we included this in our analysis. We conducted two analyses which separated compromised and uncompromised fish. We determined the percentage of observations with salmon mortality change ≥1 pp for each treatment each year, for both small and large fish. To ensure sufficient data for interpreting patterns, only categories where more than 20 observations were made at each temperature for each year were included.

Results

Total number of registered delousing operations from 2012 to 2017

Spatial autocorrelation related to temperature in the data set appeared limited, with temperature distribution broadly similar across the 13 production zones (Fig. S2a), with the exception that warmer temperatures above 10°C were infrequent in the most northern production zones (11–13).

The overall number of registered delousing operations reported in the Norwegian Food Authorities database

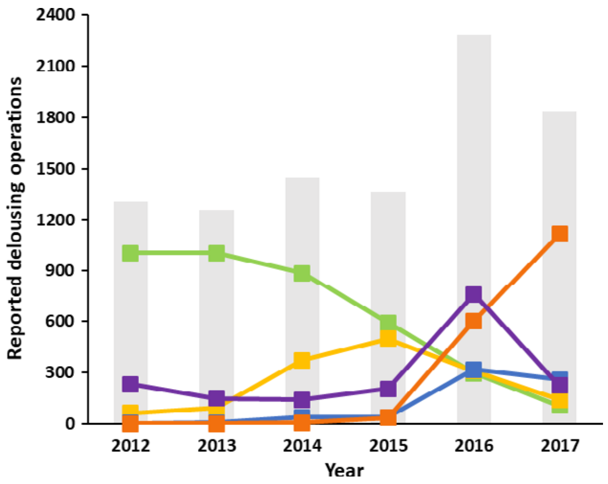


Figure 2 Number of reported delousing operations undertaken by chemotherapeutant bathing (azamethiphos, cypermethrin, deltamethrin), hydrogen peroxide bathing, mechanical treatment, thermal treatment, and 'bath other' treatments (unspecified combinations of chemotherapeutics, freshwater)(lines), and total number of reported delousing operations (grey bars) from 2012 to 2017 in all production zones in Norwegian Atlantic salmon aquaculture. ▬ Total; -■- Chemotherapeutant; -■- Hydrogen peroxide; -■- Mechanical; -■- Thermal; -■- Bath other.

increased 1.4 times from 2012 to 2017 (Fig. 2), while the tons of salmon produced was approximately the same; 1,232,000 tons in 2012 vs. 1,234,000 tons in 2016 (Norwegian Directorate of Fisheries 2018). Adjusting for tons of salmon produced, this represents a real increase in treatment registration frequency as biomass produced by the industry was relatively stable across 2012–2016. This equates to 1 reported treatment every 1150 tons of salmon produced in 2012, increasing to 1 reported treatment for every 763 tons of salmon produced in 2016.

Bathing with chemotherapeutants dominated delousing operations in 2012 and 2013 (Fig. 2). From 2014 onwards, their usage rapidly declined from constituting more than 80% of all reported delousing operations in 2013 to only 6% in 2017 (Fig. 2). Hydrogen peroxide use increased from 2012 to a peak in 2015, where 36% of all delousing operations were reported as hydrogen peroxide, but has since decreased to only 8% of reported delousing operations in 2017 (Fig. 2).

Mechanical and thermal delousing operations are relatively new, and although there were some registered treatments prior to 2016, 2016 was the first year they came into general use. In 2017, they became the dominant delousing operations used; 14% of all registered treatments in 2017 were mechanical, and 61% were thermal (Fig. 2). 'Bath other' reached a peak in 2016, making up 33% of all delousing operations (Fig. 2). However, bathing with these unspecified substances and freshwater decreased in 2017 to 12% of all reported delousing operations (Fig. 2).

Changes in treatment methods used within each production zone in Norway are evident (Fig. 3). From 2012 to 2015, bathing with chemotherapeutants dominated production zones 9 to 13, with more than 80% of all treatments registered as chemotherapeutants (Fig. 3a). Chemotherapeutant use was also high in production zones 2 to 8, although to a lesser extent at 40–72% of all registered treatments (Fig. 3a). Hydrogen peroxide was the second most used delousing operation, comprising between 17% and 33% of all observations in production zones 3 to 8 (Fig. 3a). There were only a few registrations of thermal treatments in production zones 2 (7% of all treatments), 3 (2% of all treatments) and 5 (0.3% of all treatments) (Fig. 3a). Similarly, reported mechanical delousing treatments were also low in zones 6 (2% of all treatments), 7 (11% of all treatments) and 8 (3% of all treatments) (Fig. 3a).

In contrast, bathing with chemotherapeutants in 2016 to 2017 accounted for less than 20% of treatments in production zones 2 to 8 (Fig. 3b). Thermal treatments dominated in zones 2 to 6 (>53% of all treatments). Mechanical treatment use also increased, particularly in zones 6 (33% of all treatments), 7 (48% of all treatments) and 8 (28% of all treatments).

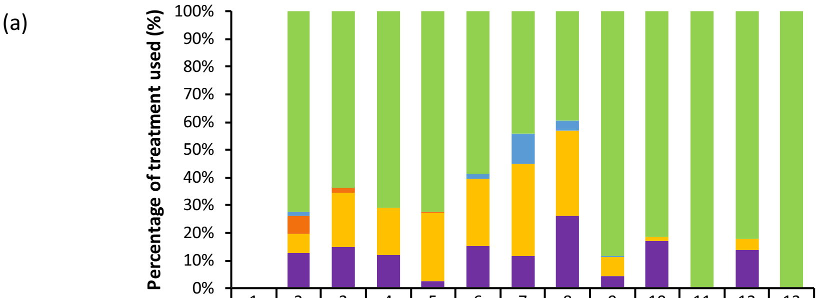
Effect of temperature on post-treatment mortality

Spatial autocorrelation related to welfare status in the data set appeared limited, with distributions of the mortality pp increases broadly similar for the compromised and uncompromised fish across the production zones (Fig. S2b,c).

Overall, thermal treatments had the greatest level of increased monthly mortality compared to the month before treatment, with 31% of treatments increasing registered mortality rates. Mechanical (25%), hydrogen peroxide (21%), azamethiphos (13%), deltamethrin (13%) and cypermethrin (12%) also increased registered mortality rates, although to a lesser extent than thermal.

Mortality rates from bathing with azamethiphos, cypermethrin, deltamethrin or hydrogen peroxide increased with temperature, as did the percentage of observations with increased mortality generally (Fig. 4). At the highest temperature category (13–16°C), the percentage of observations with increased mortality (≥1 pp) was >35% for hydrogen peroxide, 27% for azamethiphos and <20% for cypermethrin and deltamethrin (Fig. 4d). Hydrogen peroxide and azamethiphos also had relatively high numbers of observations with very high mortality increases (≥10 pp) (Fig. 4a,d). Although the percentage of observations with increased mortality generally increased with temperature, for deltamethrin there was an increase for the lowest temperature category (0–4°C) (Fig. 4c), and for hydrogen peroxide the lowest

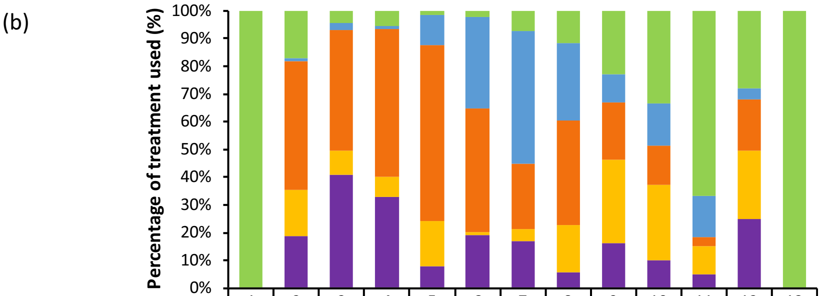
(a)



	1	2	3	4	5	6	7	8	9	10	11	12	13
🟩 Chemotherapeutant bathing	0	233	769	631	254	423	210	202	369	200	39	154	3
🟦 Mechanical	0	4	2	0	0	11	51	17	2	0	0	0	0
🟧 Thermal	0	21	19	0	1	0		0	0	0	0	0	0
🟨 Hydrogen peroxide	0	22	235	152	86	176	158	157	28	4	0	7	0
🟪 Bath other	0	41	182	105	9	110	55	134	19	42	0	26	0

Production zone

(b)



	1	2	3	4	5	6	7	8	9	10	11	12	13
🟩 Chemotherapeutant bathing	5	55	42	41	4	15	18	39	45	66	40	27	1
🟦 Mechanical	0	3	23	7	29	236	121	94	20	30	9	4	
🟧 Thermal	0	149	404	397	172	317	59	127	41	28	2	18	0
🟨 Hydrogen peroxide	0	53	81	54	44	7	11	57	59	53	6	24	0
🟪 Bath other		60	378	243	21	137	43	19	32	20	3	24	0

Production zone

Figure 3 (a) Percentage of delousing operation registrations and total number of reported delousing events used in each production zone for Norwegian salmon aquaculture from 2012 to 2015. (b) Percentage of delousing operations and total number of reported delousing events used in each production zone for Norwegian salmon aquaculture from 2016 to 2017

percentage of observations with increased mortality was at an intermediate temperature category (7–10°C; Fig. 4d).

Similarly, mortalities were also highest at low (4–7°C) and high (13–16°C) temperatures for mechanical and thermal treatments, but lower at intermediate temperatures (7–13°C; Fig. 4e,f). Both mechanical and thermal treatments were associated with higher levels of salmon mortality across all temperature categories relative to chemotherapeutant treatments, excluding hydrogen peroxide (Fig. 4).

Effect of welfare status and temperature on post-treatment mortality

Spatial autocorrelation in the data set related to fish weight appeared limited, with distributions similar across production zones, aside from production zone 13, where relatively little farming occurred and few observations were recorded ($n = 4$) (Fig. S2d).

For all delousing operations other than thermal, compromised salmon (defined as salmon in sea-cages with 0.25–1% mortality in the month before treatment) had higher increases in post-treatment mortality than uncompromised salmon (defined as salmon with <0.25% mortality in the month before treatment) (Fig. 5). For azamethiphos, cypermethrin and deltamethrin, the positive relationship between increasing temperature and increasing percentage of observations with high mortality (Fig. 4a,b,c) held for both compromised and uncompromised salmon (Fig. 5a,b,c). Hydrogen peroxide had marked increases in mortality for compromised fish across all temperatures (Fig. 5d). Further, compromised fish treated at 10–13°C

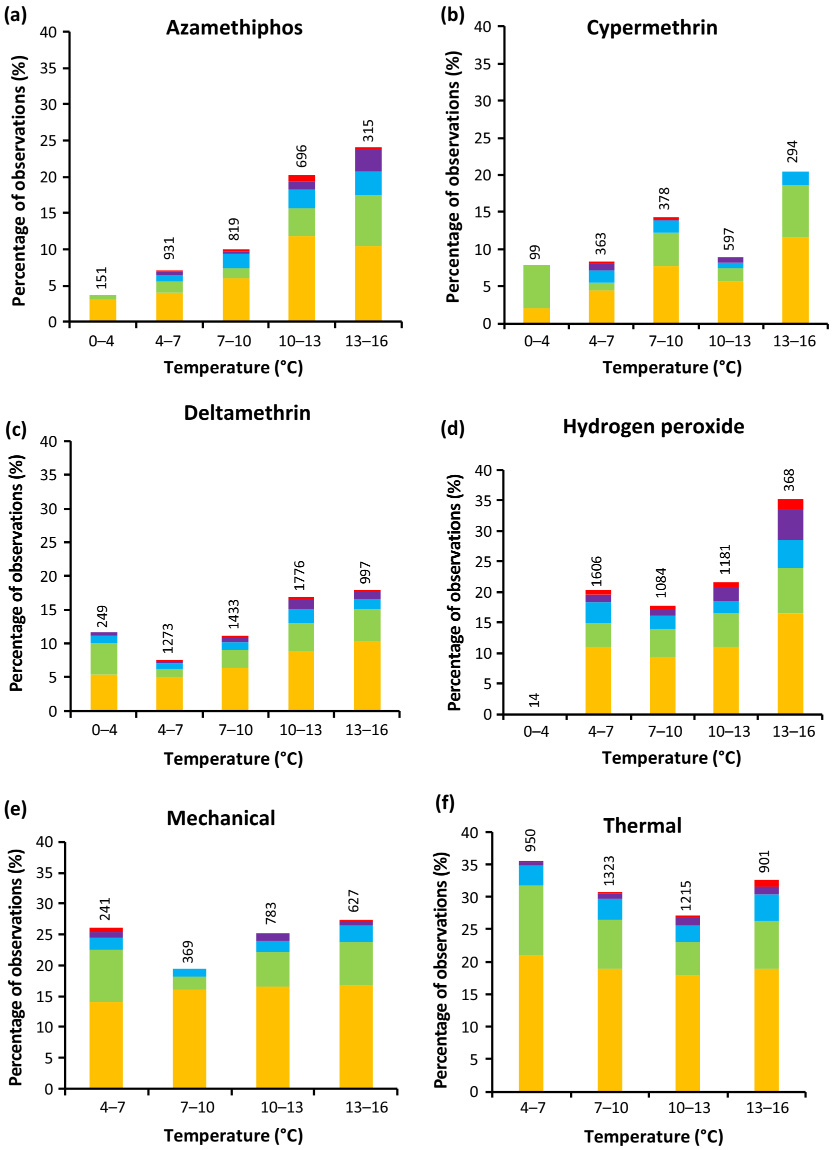


Figure 4 Percentage frequencies of salmon mortalities observed for the percentage point (pp) changes in the mortality categories 1–2.5, 2.5–5, 5–10, 10–25 and 25–100 pp at 4–7, 7–10, 10–13 and 13–16°C for: (a) Azamethiphos (b) Cypermethrin (c) Deltamethrin (d) Hydrogen peroxide (e) Mechanical (f) Thermal. The number of observations (including 0–1 pp) for each temperature category is listed above the bar. 🟥25–100; 🟪10–25; 🟦5–10; 🟩2.5–5; 🟨1–2.5.

and 13–16°C had 6% and 10% of observations with high increases in mortality (≥10 pp), respectively. In contrast, uncompromised fish treated with hydrogen peroxide within the same temperature categories had <2% of observations ≥10 pp (Fig. 5d). Mechanical treatment had similar mortality levels as hydrogen peroxide. Mortalities were higher at 4–7°C and 10–13°C compared to the other two temperature categories for uncompromised fish, but increased as temperature increased for compromised fish (Fig. 5e). For thermal treatment, both compromised and uncompromised fish had similar mortalities at 4–7°C. Uncompromised fish had lower mortalities from 7 to 16°C compared to compromised fish, and increased mortalities were observed at low (4–7°C) and high (13–16°C) temperatures for compromised fish, with mortalities lowest in the 10–13°C temperature category.

Effect of salmon weight, welfare status and temperature on post-treatment mortality

Dividing the mortality data further into small (<2 kg) and large (≥2 kg) fish revealed an even more

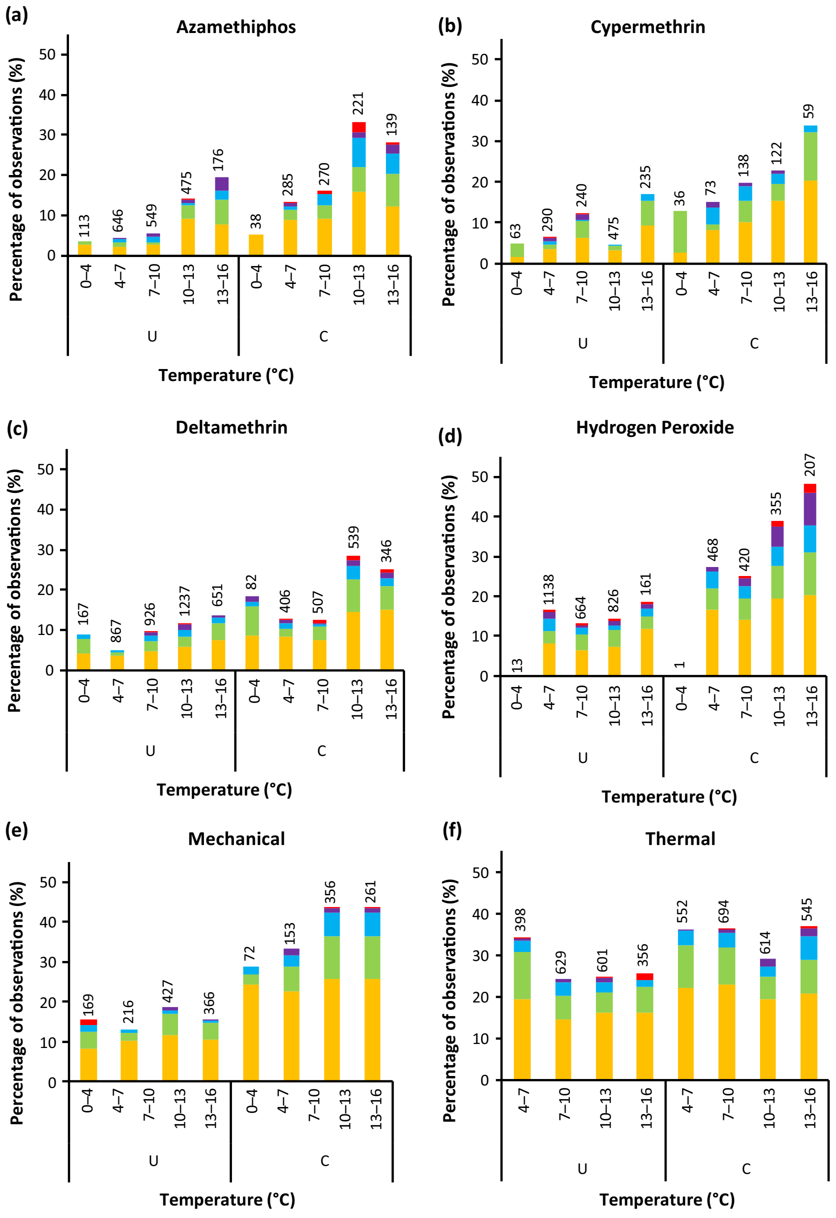


Figure 5 Percentage frequencies of salmon mortalities observed for the percentage point (pp) changes in the mortality categories 1–2.5, 2.5–5, 5–10, 10–25 and 25–100 pp at 4–7, 7–10, 10–13 and 13–16°C, where U = less than 0.25% salmon mortality observed the month before treatment (uncompromised) and C = 0.25–1% salmon mortality observed the month before treatment (compromised), for: (a) Azamethiphos (b) Cypermethrin (c) Deltamethrin (d) Hydrogen peroxide (e) Mechanical (f) Thermal. The number of observations (including 0–1 pp) for each temperature category is listed above the bar. 🟥25–100; 🟪10–25; 🟦5–10; 🟩2.5–5; 🟨1–2.5.

complicated picture (Fig. 6). For azamethiphos, large fish had a marked increase in post-treatment mortality at high temperatures compared to small fish (Fig. 6(i and ii)a). For cypermethrin, salmon mortalities for uncompromised fish were unsystematic across temperatures and between sizes, although 1–2.5 and 2.5–5 pp mortality observations were particularly high for small fish at 13–16°C (Fig. 6(i)b). However, for compromised fish, the percentage of observations with increased mortality increased with temperatures for both small and large fish (Fig. 6(ii)b). Overall, both small and large compromised fish experienced higher mortalities compared to uncompromised fish.

Mortality from deltamethrin in both uncompromised small and large salmon exhibited high mortalities at 0–4°C but decreased at 4–7°C (Fig. 6(i)c). Mortality then

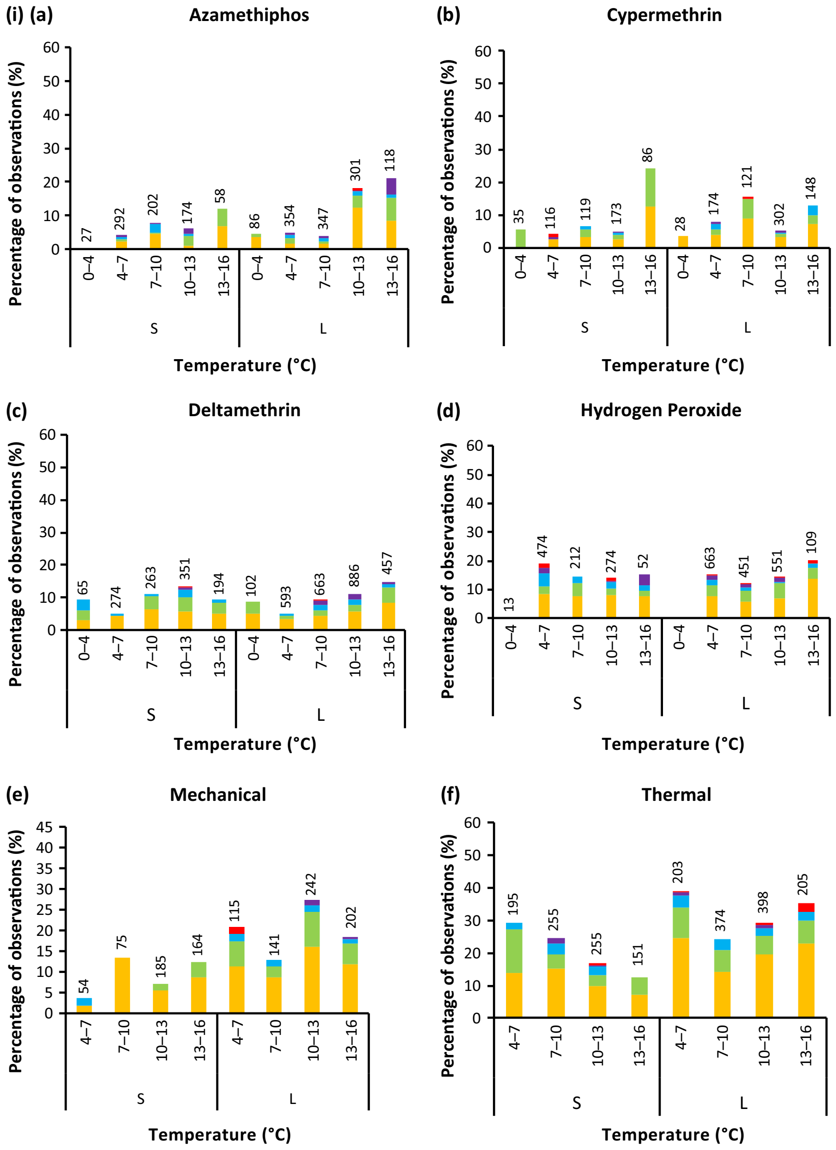


Figure 6 Percentage frequencies of salmon mortalities observed for the percentage point (pp) changes in the mortality categories 1–2.5, 2.5–5, 5–10, 10–25 and 25–100 pp, at 4–7, 7–10, 10–13 and 13–16°C. S = fish weighed <2 kg during treatment (small) and L = fish weighed ≥2 kg during treatment (large) when: (i) salmon mortality the month before treatment was between 0% and 0.25% and (ii) salmon mortality the month before treatment was between 0.25% and 1%, for: (a) Azamethiphos (b) Cypermethrin (c) Deltamethrin (d) Hydrogen peroxide (e) Mechanical (f) Thermal. The number of observations (including 0–1 pp) for each temperature category is listed above the bar.🟥25–100; 🟪10–25; 🟦5–10; 🟩2.5–5; 🟨1–2.5.

increased from 7 to 10°C upwards. A similar distribution was also observed for small and large compromised salmon (Fig. 6(ii)c), with 0–4°C and 10–13°C having the highest percentage of observations with mortality increases after treatment.

Salmon mortality was similar at all sea temperatures for hydrogen peroxide for uncompromised small and large salmon (Fig. 6(i)d). Mortality was highest at 13–16°C for compromised small and large fish (Fig. 6(ii)d), with 14% and 6% of all observations within this temperature category having 10–25 pp increase in salmon mortality respectively. Again, mortality increased as temperature increased for both uncompromised and compromised fish.

Among mechanical treatments, there was no systematic effect of temperature on salmon mortality for uncompromised fish (Fig. 6(i)e). However, mortality was

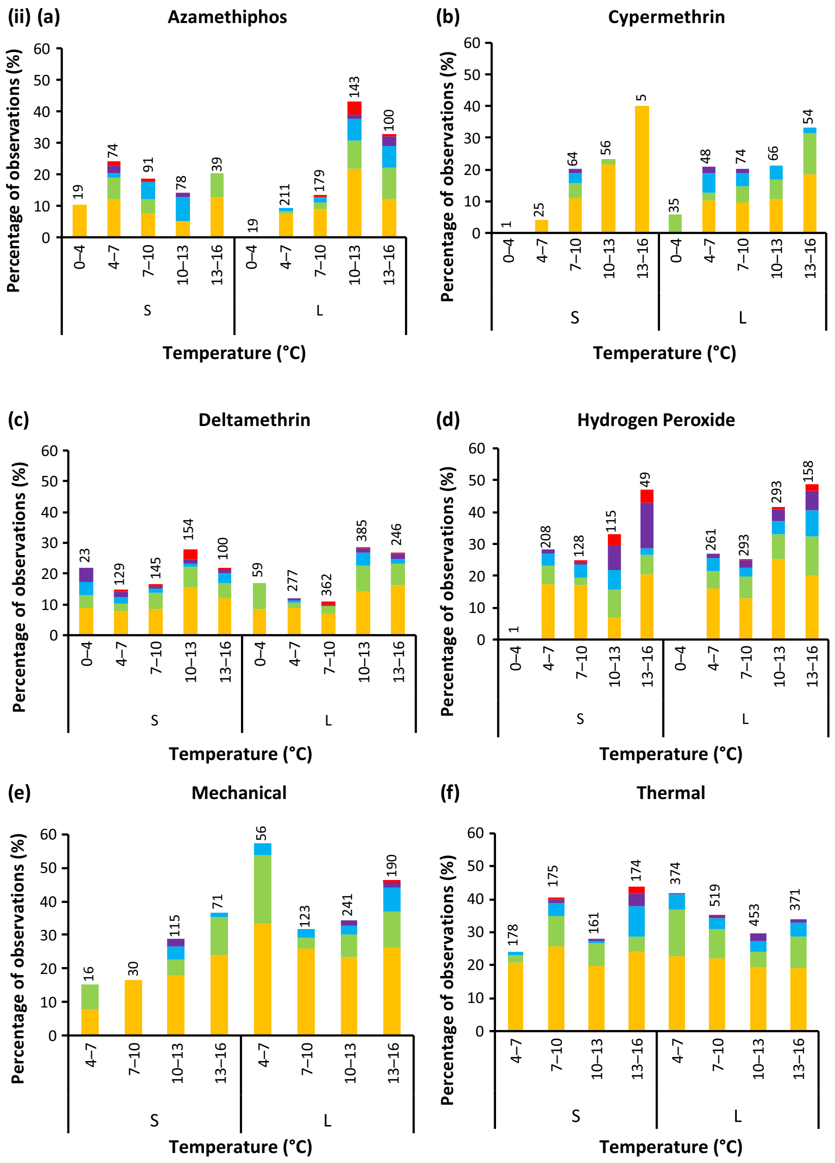


Figure 6 Continued

higher at all temperature categories for uncompromised large fish compared to uncompromised small fish (Fig. 6(i)e). For compromised fish, large salmon exhibited highest mortality at 4–7°C and 13–16°C, and for small compromised fish, mortality increased as temperature increased (Fig. 6(ii)e).

As temperature increased, thermal treatment resulted in decreased mortality for uncompromised small fish (Fig. 6(i)f). However, for large fish, higher mortality observations at 4–7°C and 13–16°C were detected. No clear effect of temperature on mortality in small fish post-treatment was evident for compromised fish (Fig. 6(ii)e), although mortality was generally high, particularly at 13–16°C. For compromised large fish, pp increase in mortality post-treatment was generally also high for all temperatures (Fig. 6(ii)e).

Mortality outcomes per delousing operation from 2012 to 2017

The percentage of mortality observations ≥1 pp for uncompromised and compromised fish across the different temperature categories varied between years, with a few clear trends (Fig. 7; Fig. S1). For the main treatments used in 2017 (hydrogen peroxide, mechanical and thermal), the percentage of mortality observations ≥1 pp was

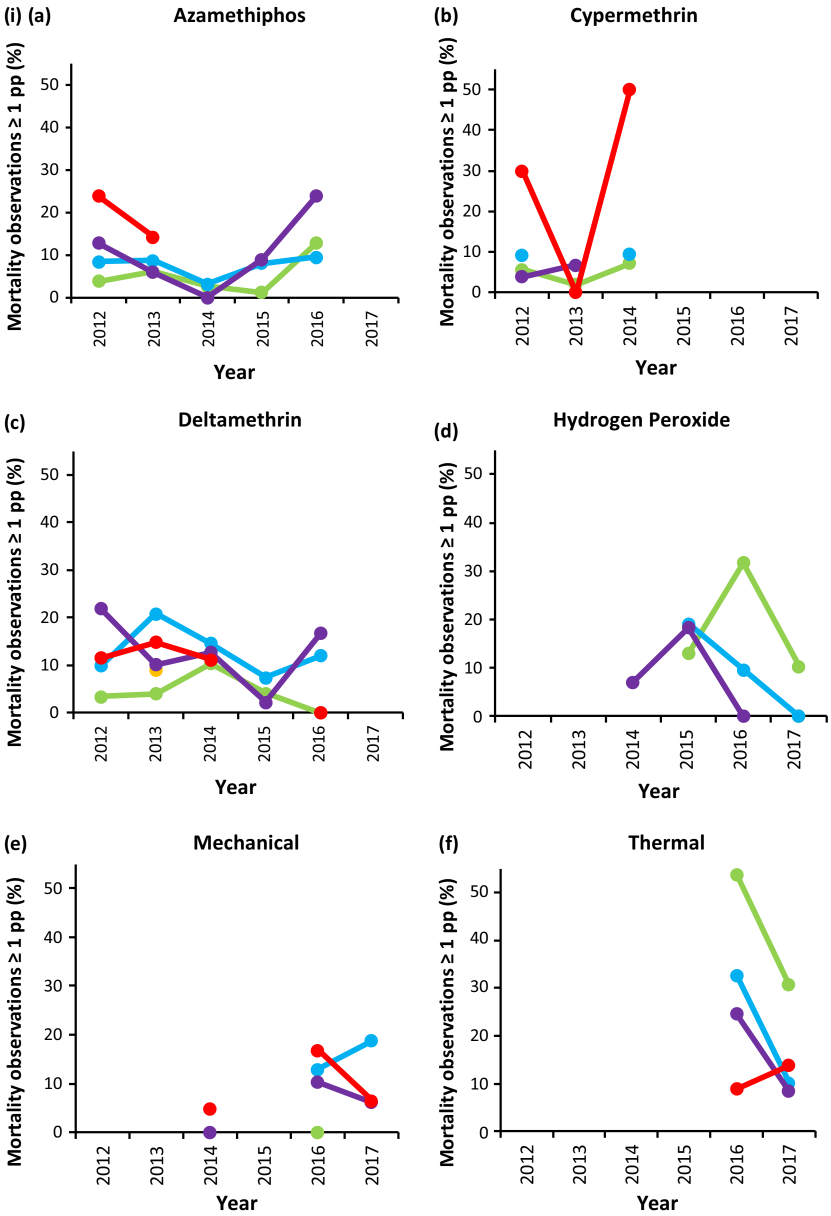


Figure 7 Percentage of mortality observations ≥1 percentage point (pp) for uncompromised (0–0.25% mortality the month before treatment) at 4–7, 7–10, 10–13 and 13–16°C from 2012 to 2017, for: (i) small (<2 kg) fish and (ii) large (≥2 kg) fish, for: (a) Azamethiphos (b) Cypermethrin (c) Deltamethrin (d) Hydrogen peroxide (e) Mechanical (f) Thermal. —●—0–4; —●—4–7; —●—7–10; —●—10–13; —●—13–16.

generally lower than in previous years (Fig. 7; Fig. S1). The decrease was especially clear for hydrogen peroxide, where mortality more than halved from 2016 to 2017 for both small and large fish below 10°C (Fig. 7(i)d,(ii)d). Outcomes of mechanical treatments improved for small and large fish at temperatures between 10°C and 16°C but were worse at 7–10°C (Fig. 7(i)e,(ii)e). Similarly, thermal treatment outcomes improved for both small and large fish for all temperature categories from 2016 to 2017, except for 13–16°C, which had increased mortality observations (Fig. 7(i)f,(ii)f).

Discussion

Rapid and recent paradigm shift in anti-salmon lice operations from 2012 to 2017

The data demonstrates an abrupt and dramatic shift in salmon lice treatment strategy by the Norwegian salmon

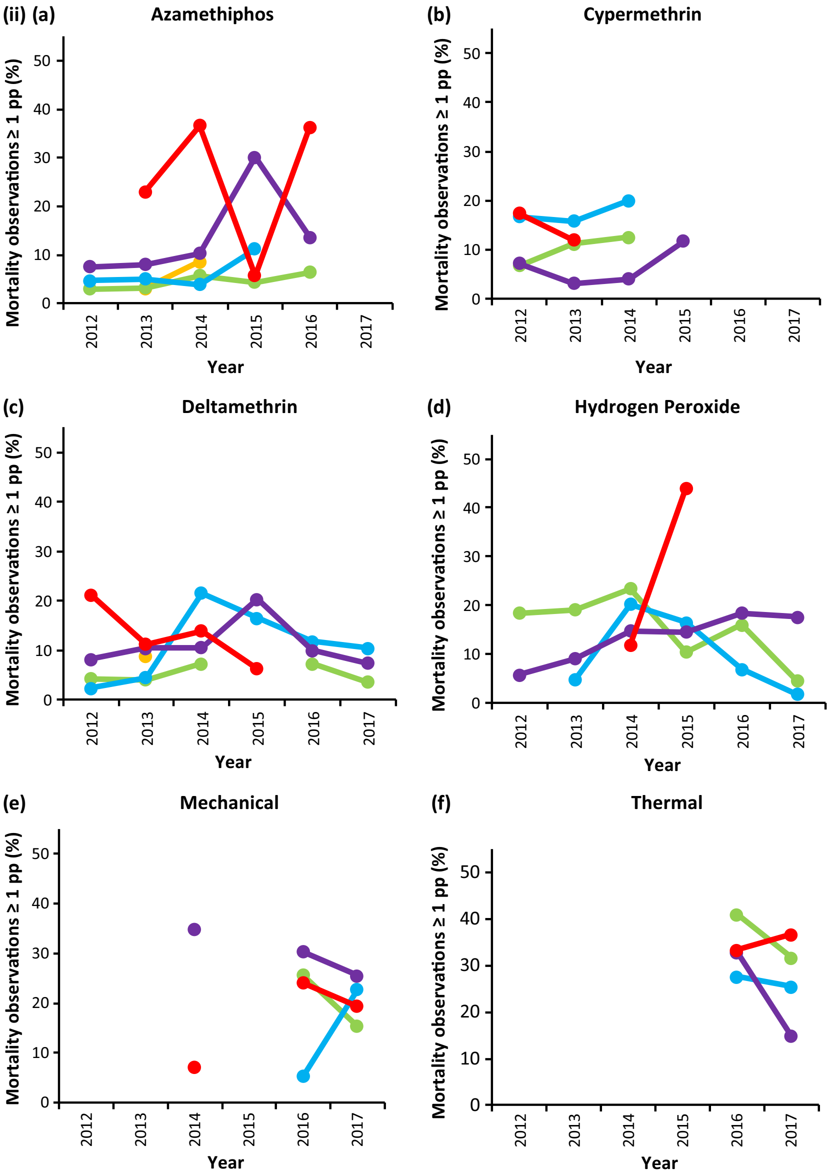


Figure 7 Continued

aquaculture industry. Bathing with chemotherapeutants (including hydrogen peroxide) diminished from comprising 79% of treatments in 2015 to 13% in 2017, with newly introduced thermal and mechanical treatments filling the void. Multiple factors likely contributed to this shift in treatment strategy, including the widespread development of resistance to chemotherapeutants (Aaen *et al.* 2015; Helgesen *et al.* 2017) rendering them less effective, and the increased availability and capacity of mechanical and thermal delousing systems.

The data also revealed that treatment frequency increased 1.4 times in real terms from 2012 to 2017. Furthermore, use of medicated feeds against salmon lice also increased by 2.7 and 1.4 times from 2012 to 2016 and 2012 to 2017, respectively (Hjeltnes *et al.* 2018). Across this period, the industry simultaneously implemented new cage-based preventative technologies (e.g. skirts, snorkels and deep lights and feeding; Frenzl *et al.* 2014; Stien *et al.* 2016; Oppedal *et al.* 2017; Wright *et al.* 2017; Stien *et al.* 2018; Grøntvedt *et al.* 2018) and functional feeds (Jensen *et al.* 2015) to prevent lice from infecting fish. Further, control methods that continuously reduce lice within cages have also increased. Anti-lice lasers are now in use at several locations (Kyst.no 2018), and nearly four times more cleaner fish were used in 2017 (50 million) than in 2012 (13 million; Norwegian Directorate of Fisheries 2018). Set against the backdrop of measures to explicitly reduce cage-based salmon lice loads, the increased treatment frequency could reflect an industry dealing with an escalating problem. However, thermal and mechanical treatments are reportedly most effective against mobile and adult lice stages, and less so against attached stages. In contrast, cypermethrin and deltamethrin, which were the most used chemotherapeutant treatments in 2012–2014, are effective across all life history stages. Reduced efficacy of treatments against the early attached stages means that female lice levels return more rapidly to pre-existing levels, which in turn means the need to re-treat fish occurs more rapidly. It is also possible that lice that have detached during crowding or are not caught by lice filters in thermal or mechanical systems could re-infect fish in neighbouring cages or farms (Gismervik *et al.* 2017). Farmers may also have changed decision points on when to treat cages i.e. before they reach regulated levels, and implemented delousing on a cage-by-cage basis rather than whole-farm basis. These three processes have the potential to increase treatment registrations, without necessarily reflecting an escalating salmon lice problem. Further, in farms with amoebic gill disease (AGD) and high lice levels, farmers have treated with hydrogen peroxide or freshwater to tackle both parasites at the same time. The peak observed in 2016 for 'Bath other' might therefore be a result of increased AGD treatments that year (Fig. 2). The 'Bath other' peak may also reflect a combination of chemotherapeutants used in an attempt to increase efficacy. This practice is now greatly reduced after an information campaign by the Norwegian Food Safety Authorities addressing 'off-label use' of chemotherapeutants (Mattilsynet 2018).

Our analysis also revealed that treatment strategy depended strongly upon production zones throughout Norway (Fig. 3). Chemotherapeutants were only frequently used in the most southern and northern production zones where there are relatively few treatments registered by only a small number of farms. The rise of mechanical and thermal delousing and rapid shift in treatment use within most production zones could be explained by multiple possibilities, including:

1. The biology of the system within each zone. Production zones 3 and 4 lie in the south, and hence factors such as higher seawater temperatures in the summer and autumn can impact chemotherapeutant use, with higher mortalities at higher temperatures (e.g. azamethiphos: Roth *et al.* 1996; hydrogen peroxide: Thomassen 1993). While chemotherapeutant bathing dominated from 2012 to 2015, hydrogen peroxide use was also high in zones 3, 4, 5, 6, 7 and 8 (19, 17, 25, 24, 33, 31%, respectively) compared to other zones. This correlated to reduced treatment efficacies in these production zones for azamethiphos, cypermethrin and deltamethrin (Helgesen *et al.* 2017). However, with widespread resistance to hydrogen peroxide across Norway (Helgesen *et al.* 2015, 2017), increases in mechanical and thermal treatment use can also be attributed to lack of effective treatments available.
2. The physical location of the headquarters of the producers of thermal and mechanical delousers. Thermolicer® and Optilicer® are produced in production zones 3 and 5 respectively. Hydrolicer®, FLS and SkaMik® headquarters are located in production zones 6, 6 and 7 respectively. This corresponds directly to the dominance of thermal treatment in zones 3 to 5, and high use of mechanical treatment in zones 6 and 7.
3. A company with many farmers or a group of farmers within a production zone could together purchase a thermal or mechanical treatment machine, sharing this technology within the group. Other farmers in the area would see this group using the new technology, and also begin to use it due to the farming cultural norms of the area.
4. High cost of initial capital investment in mechanical and thermal delousers. If a company or group of farms have invested in expensive equipment, they will likely attempt to maximise its use. Because of this, high use of a certain type of delousing technology within production zones with sufficient farming to warrant investment in these delousers could arise. The opposite is also true in that these technologies may be too costly for production zones (e.g. 1 and 9) with limited production.

Salmon mortality risks associated with salmon weight, welfare status and temperature

Sea temperature, fish size and pre-existing mortality rates prior to treatment all exhibited distinct patterns with treatment, and complex interactions among these three factors appeared for most treatment types. Because the data set only enables correlative analyses, we cannot partition how much mortality is due to each of these factors.

Increasing sea temperature correlated with increasing mortality after treatment across most delousing methods. All delousing operations crowd fish before treatment, which introduces stress and risk of hypoxic conditions (Oppedal *et al.* 2011; Skjervold *et al.* 2001). Salmon have decreased stress tolerance at high water temperatures due to the combined effect of both higher oxygen demand (Remen *et al.* 2013; Hvas *et al.* 2017a) and lower oxygen solubility in warmer water (Jonsson & Jonsson 2009). Further, in summer and autumn gill health problems (e.g. infectious gill diseases, damages from algae or cnidaria; Hjeltnes *et al.* 2018) can arise, which can reduce the metabolic capacity of salmon (Hvas *et al.* 2017b). It is also possible that other diseases (e.g. pancreas disease) can play a role in mortality at high seawater temperatures.

Mortality the month before delousing emerged as a major predictor of the risk associated with delousing.

Comparing Fig. 6(i) and (ii) one can broadly say that the risk more than doubled across temperatures and fish sizes for all treatments. Mortality the month before treatment is a critical parameter that should be considered before delousing, as it reflects the health status of the fish group. Strategies beyond choice of method could include expediting planned delousing if mortality is low, and postponing if mortality is high, while still maintaining lice levels below legal limits. If postponing the lice treatment is not possible, slaughtering out or euthanizing fish can be a more welfare friendly strategy, although economic motivations are often prioritised over fish welfare needs (Hjeltnes *et al.* 2018).

Chemotherapeutants

For all chemotherapeutants, mortality increased as sea temperature increased. For azamethiphos and hydrogen peroxide, toxicity increases with temperature (i.e. azamethiphos: Roth *et al.* 1996; hydrogen peroxide: Thomassen 1993). While pyrethroids are not believed to be toxic to fish (Burridge *et al.* 2010), salmon exposed to pyrethroids increased oxygen consumption by 50% compared to unexposed fish and 40% more oxygen compared to salmon treated with azamethiphos (F Oppedal, unpublished data). The combined handling stress, increased oxygen consumption and increased toxic effect may explain why compromised fish exhibited a higher increase in mortality after chemotherapeutant bathing than uncompromised fish. In addition to a general lesser resilience, some of the compromised fish may also have been suffering from poor gill health, making it difficult for them to extract sufficient oxygen from the water during handling, as seen in heavily AGD scored fish (Hvas *et al.* 2017b). For the uncompromised fish, the effect from increasing temperature when treated with cypermethrin, deltamethrin or hydrogen peroxide was much less pronounced; there was also no clear difference between large and small fish, indicating that healthy fish are not severely affected by these treatments. Treating large fish led to substantially higher mortality at temperatures above 10°C. This may have been due to large fish at higher temperatures needing more oxygen, and the combination of increased ventilation rate and larger gill surface area could cause an increased toxic effect. Large fish are also often stocked at higher biomasses, which could in turn also impact mortality.

Thermal and mechanical treatments

While case study-based reports on thermal and mechanical treatments have documented the post-treatment outcomes for salmon welfare (i.e. Grøntvedt *et al.* 2015; Gismervik *et al.* 2017; Poppe *et al.* 2018), there has been no comprehensive assessment on how sea temperature, fish size and welfare status influence salmon mortality. For thermal treatments, we detected a clear pattern of diminishing mortality with increasing sea temperature for small uncompromised fish. This may be due to reduced temperature shock; even though farmers may adjust treatment temperatures downwards at low sea temperatures as recommended by Roth (2016), in general the difference between ambient seawater temperature and treatment temperature decreased with increasing ambient temperature.

The decline in mortality with decreasing temperature difference was, however, neither not present for large uncompromised fish, nor for the compromised large and small fish. The temperature difference may be less important than risks to large fish from traumatic injury during crowding and when being transported through the system, and/or at higher risk of hypoxia. Therefore, this could explain why mortality rates for both large uncompromised and compromised fish treated with thermal treatment experienced increased mortality. Increased mortalities at low and high temperatures was less clear for small compromised fish, although this could have been due to the smaller number of observations compared to other thermal observation groups.

While mechanical delousing has emerged as the second most important method to thermal, the number of observations from which to draw patterns is relatively low. In general, for uncompromised small and large fish, mortalities were low with no distinct interaction with temperature. For compromised fish, a clear effect of temperature emerged for small fish, with mortalities increasing with temperature. For large compromised fish, increased mortalities at low and high temperatures emerged, likely for the same reasons as thermal delousing. A complicating factor that makes general analysis of the effects of mechanical delousing difficult is that the three different systems in use likely dominate in different production zones, where the ambient seawater temperatures will also differ.

Mortality outcomes per treatment from 2012 to 2017

Overall, treatment outcomes for all treatment methods improved from 2015 to 2017, particularly so for the three most used delousing operations in 2016–2017: thermal, mechanical and hydrogen peroxide. Numerous possibilities could explain these improved outcomes, including a range of advances in technology and improvements in their use through time as operators become more experienced with their deployment. Further, the introduction and widespread use of thermal and mechanical treatments in 2016 and 2017 may have reduced the need to use hydrogen peroxide treatments in high-risk conditions. Prior to the introduction of thermal and mechanical treatments in 2015, hydrogen peroxide may have been the only option at times when other chemotherapeutants were unsuitable, regardless of risks to fish welfare. For example, hydrogen peroxide may be used regardless of risky conditions where lice are resistant to other chemotherapeutants, or when delousing is required shortly before harvesting (food safety regulations mandate minimum times between chemical delousing and harvest).

Uncertainties in the database

The database used relies on reported delousing events to the Norwegian Food Authorities and the difference in monthly mortality reported to the Norwegian Directorate of Fisheries the month before and the month of delousing. Each data point has inherent uncertainties, including: (1) number of fish in a cage is estimated by the farmer; (2) actual cause of fish mortality after treatment; (3) date of delousing relative to reporting dates at the end of each month; (4) crowding time during delousing (not reported); and (5) variation in delousing protocols and reporting accuracy among companies. However, we have no reason to believe that these uncertainties differentially affected the patterns identified for sea temperature, fish size or pre-existing mortality. Further, the size of the data set, which is based on thousands of observations across 6 years, reduces the relative influence of any anomalous events on overall trends.

Nevertheless, interpretation of certain aspects of the dataset should be cautious, especially for groups where the number of observations is relatively low. There may also be differences in when mortality occurred after the different delousing operations (i.e. acute or delayed). Ideally, targeted, controlled experiments should explicitly test for relationships between mortality outcome, treatment method, temperature and fish size. Moreover, these analyses should include sub-lethal effects on fish welfare.

Conclusion

Broadly, our results illustrate that the salmon farming industry in Norway has largely retreated from chemotherapeutant use in favour of thermal and mechanical delousing treatments. With such a dramatic shift in delousing strategy, the outcomes of mechanical and thermal delousing treatments, in terms of both short and long-term impacts on salmon welfare and mortality combined with their lice removal efficacy, must be a clear focus for future research and development. Improved knowledge is required to decrease the risks associated with these treatments, as our findings demonstrate that high mortality rates can occur in specific circumstances.

Further, our findings illustrate the importance of national databases of this type, which allow in-depth analysis of industry-scale processes. Previous database analyses have been used to analyse lice populations (Revie *et al.* 2003), and also identify factors associated with delousing operations and changes in delousing operation use (Lees *et al.* 2008; Murray & Hall 2014; Murray 2016). Recently, salmon mortality databases in Scotland have been made public for full transparency. When national-level databases of cage-based mortality rates become publicly accessible, it will enable a greater level of analysis to understand underlying mechanisms.

Our analyses of factors that correspond with increased mortalities are limited to those currently reported and may not tell the full story. To improve databases for more sophisticated analyses likely to yield greater benefit to the industry, we recommend that policymakers consider additional reporting requirements, including: (1) underlying disease status prior to treatment; (2) consistent reporting of chemotherapeutant dosages; (3) consistent reporting of mechanical, thermal and freshwater method use, including treatment temperature and water chemistry; (4) crowding time and duration during treatments; (5) cage-by-cage rather than whole farm reporting; (6) detailed scoring of the health and welfare status of the fish (e.g. SWIM, Stien *et al.* 2013; Folkedal *et al.* 2016) prior to and after treatment to enable assessment of the sub-lethal effects of treatment on fish welfare; (7) Daily mortality rates instead of monthly, which will allow better identification of mortality caused by delousing treatments and other operations on the farm. Daily mortality rates are already registered by companies and could be easily integrated from their farm management software.

Acknowledgements

We would like to thank the Norwegian Marine Data Centre, particularly Arnfinn Morvik, for providing access to the Norwegian Food Authorities and Norwegian Directorate of Fisheries databases. This research was funded by the Research Council of Norway Project 267664 REGFISH-WELH.

References

Aaen SM, Aunsmo A, Horsberg TE (2014) Impact of hydrogen peroxide on hatching ability of egg strings from salmon lice (*Lepeophtheirus salmonis*) in a field treatment and in a laboratory study with ascending concentrations. *Aquaculture* **422–423**: 167–171.

Aaen SM, Helgesen KO, Bakke MJ, Kaur K, Horsberg TE (2015) Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends in Parasitology* **31**: 72–81.

Alexander N (2015) What's more general than a whole population? *Emerging Themes in Epidemiology* **12**: [Cited 25 Aug 2018]. Available from https://doi.org/10.1186/s12982-015-0029-4.

Bowers JM, Mustafa A, Speare DJ, Conboy GA, Brimacombe M, Sims DE *et al.* (2000) The physiological response of Atlantic salmon, *Salmo salar* L., to a single experimental challenge with sea lice, *Lepeophtheirus salmonis*. *Journal of Fish Diseases* **23**: 165–172.

Brunsvik PS (1997) Miljømessig avlusing av laks. Småtrykk 261, Gildeskål Forsøksstasjon AS, 30 p (in Norwegian)

Burka JF, Hammell KL, Horsberg TE, Johnson GR, Rainnie DJ, Speare DJ (1997) Drugs in salmonid aquaculture – A review. *Journal of Veterinary Pharmacology and Therapeutics* **20**: 333–349.

Burridge L, Weis JS, Cabello F, Pizarro J, Bostick K (2010) Chemical use in salmon aquaculture: a review of current practices and possible environmental effects. *Aquaculture* **306**: 7–23.

Costello MJ (2006) Ecology of sea lice parasitic on farmed and wild fish. *Trends in Parasitology* **22**: 475–483.

Elliot JM, Elliot JA (1995) The effect of the rate of the temperature increase on the critical thermal maximum for parr of Atlantic salmon and brown trout. *Journal of Fish Biology* **47**: 917–919.

Fallang A, Ramsay JM, Sevatdal S, Burka JF, Jewess P, Hammell KL *et al.* (2004) Evidence for occurrence of an organophosphate-resistant type of acetylcholinesterase in strains of sea lice (Lepeophtheirus salmonis Krøyer). *Pest Management Science* **60**: 1163–1170.

Flatsetsund Enginnering AS (2018). FLS Delousing System [Brochure]. Frei, Norway: Flatsetsund Engineering AS. [Cited Apr 29 2018] Available from https://www.fls.no/wp-content/uploads/2018/01/Trykk-FLS_trifoldENG.pdf

Folkedal O, Petersen JM, Bracke MBM, Stien LH, Nilson J, Martins C *et al.* (2016) On-farm evaluation of the Salmon Welfare Index Model (SWIM 1.0): theoretical and practical considerations. *Animal Welfare* **25**: 135–149.

Frenzl B, Stien LH, Cockerill D, Oppedal F, Richards R, Shinn A *et al.* (2014) Manipulation of farmed Atlantic salmon swimming behaviour through the adjustment of lighting and feeding regimes as a tool for salmon lice control. *Aquaculture* **424–42**: 183–188.

Gismervik K, Nielsen KV, Lind MB, Vijugrein H(2017) Norwegian Veterinary Institute's Repoert Series: 6-2017. Mekanisk avulsing med FLS-avlusersystem = dokumentasjon av fiskevelferd og effekt mot lus. Norwegian Veterinary Institute (in Norwegian).

Grave K, Horsberg TE, Lunestad BT, Litleskare I (2004) Consumption of drugs for sea lice infestations in Norwegian fish farms: methods for assessment of treatment patterns and treatment rate. *Diseases of Aquatic Organisms* **60**: 123–131.

Grøntvedt RN, Nerbøvik IKG, Viljugrein H, Lillehaug A, Nilsen H, Gjevre AG (2015) Norwegian Veterinary Institute's Report Series: 13. Thermal de-licing of salmonid fish – documentation of fish welfare and effect. Norwegian Veterinary Institute.

Grøntvedt RN, Kristoffersen AB, Jansen PA (2018) Reduced exposure of farmed salmon to salmon louse (*Lepeophtheirus salmonis* L.) infestation by use of plankton nets: estimating the shielding effect. *Aquaculture* **495**: 865–872.

Hamre LA, Glover KA, Nilsen F (2009) Establishment and characterization of salmon louse (*Lepeophtheirus salmonis* (Krøyer 1837)) laboratory strains. *Parasitology International* **58**: 451–460.

Helgesen KO, Romstad H, Aaen SM, Horsberg TE (2015) First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse *Lepeophtheirus salmonis* in Norway. *Aquaculture Reports* **1**: 37–42.

Helgesen KO, Jansen PA, Horsberg TE, Tarpai A (2017) The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*) in Norway 2016. Norwegian Veterinary Institute.

Hjeltnes B, Bang-Jensen B, Bornø G, Haukaas A, Walde CS (Ed.) (2018) The Health Situation in Norwegian Aquaculture 2017. Norwegian Veterinary Institute 108 p.

Holan AB, Roth B, Breiland MSW, Jelena K, Hansen ØJ, Iversen A *et al.* (2017) Beste praksis for medikamentfrie metoder for lakseluskontroll (MEDFRI). Rapport 10/2017, Nofima, 116 p (in Norwegian).

Hvas M, Folkedal O, Imsland A, Oppedal F (2017a) The effect of thermal acclimation on aerobic scope and critical swimming speed in Atlantic salmon *Salmo salar*. *Journal of Experimental Biology* **220**: 2757–2764.

Hvas M, Karlsbakk E, Mæhle S, Wright D, Oppedal F (2017b) The gill parasite *Paramoeba perurans* compromises aerobic scope, swimming capacity and ion balance in Atlantic salmon. *Conservation Physiology* **5**: [Cited 1 Feb 2018]. Available from https://doi.org/10.1093/conphys/cox066.

Jensen LB, Proyan F, Larssen E, Bron JE, Obach A (2015) Reducing sea lice (*Lepeophtheirus salmonis*) infestation of farmed Atlantic salmon (*Salmo salar* L.) through functional feeds. *Aquaculture Nutrition* **21**: 983–993.

Johnson SC, Constible JM, Richard J (1993) Laboratory investigations on the efficacy of hydrogen peroxide against the salmon louse *Lepeophtheirus salmonis* and its toxicological and histopathological effects on Atlantic salmon *Salmo salar* and Chinook salmon *Oncorhynchus tshawytscha*. *Diseases of Aquatic Organisms* **17**: 197–204.

Jonsson B, Jonsson N (2009) A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. *Journal of Fish Biology* **75**: 2381–2447.

Kiemer MCB, Black KD (1997) The effects of hydrogen peroxide on the gill tissues of Atlantic salmon, *Salmo salar* L. *Aquaculture* **153**: 181–189.

Krkošek M, Revie CW, Gargan PG, Skilbrei OT, Finstad B, Todd CD (2013) Impact of parasites on salmon recruitment in the Northeast Atlantic Ocean. *Proceedings of the Royal Society of London B: Biological Sciences* **280**:20122359.

Kyst.no (2018) Solgte over 50 luselasere første kvartal - Nå får vi belønning for utholdenheten. Kyst.no. (in Norwegian) [Cited 27/04/2018] Available from https://www.kyst.no/article/solgte-over-50-luselasere-foerste-kvartal-naa-faar-vi-beloenning-for-utholdenheten/

Lees F, Baillie M, Gettinby G, Revie CW (2008) The efficacy of emamectin benzoate against infestations of *Lepeophtheirus salmonis* on farmed Atlantic salmon (*Salmo salar* L) in Scotland, 2002-2006. *PLoS ONE* **3**: e1549. https://doi.org/10.1371/journal.pone.0001549.

Ljungfeldt LER, Quintela M, Besnier F, Glover KA (2017) A pedigree-based experiment revleas variation in salinity and thermal tolerance in the salmon louse, *Lepeophtheirus salmonis*. *Evolutionary Applications* **10**: 1007–1019.

Mattilsynet (2018) Legemiddelkampanjen: Sluttrapport etter Mattilsynets tilsynskampanje på legemiddelbruk i oppdrettsnæringen. Mattilsynet.no (in Norwegian).

Miller TA, Adams ME (1982) Mode of action of pyrethroids. In: Coats JR (ed) *Insecticide Mode of Action*, pp. 3–27. Academic Press, New York.

Mordue AJ, Birkett MA (2009) A review of host finding behavior in the parasitic sea louse, *Lepeophtheirus salmonis* (Caligidae: Copepoda). *Journal of Fish Diseases* **32**: 3–13.

Murray AG (2016) Increased frequency and changed methods in the treatment of sea lice (*Lepeophtheirus salmonis*) in Scottish salmon farms 2005-2011. *Pest Management Science* **72**: 322–326.

Murray AG, Hall M (2014) Treatment rates for sea lice of Scottish inshore marine salmon farms depends on local (sea loch) farmed salmon biomass and oceanography. *Aquaculture Environment Interactions* **5**: 117–125.

Norwegian Directorate of Fisheries (2018) Laks, regnbueørret og ørret. [Cited 02 Jul 2018] Available from http://www.fiskeridir.no/Akvakultur/Statistikk-akvakultur/Akvakulturstatistikk-tidsserier/Laks-regnbueoerret-og-oerret (in Norwegian).

Norwegian Ministry of Trade, Industry and Fisheries (2008) Forskrift om drift av akvakulturanlegg (akvakulturdriftsforskriften), FOR-2008-06-17-822. Norwegian Ministry of Trade and Fisheries. (in Norwegian). [Cited Mar 23 2018] Available from https://lovdata.no/dokument/SF/forskrift/2008-06-17-822?q=FOR-2008-06-17-822

Norwegian Ministry of Trade, Industry and Fisheries (2012) Forskrift om bekjempelse av lakselus i akvakulturanlegg. Norwegian Ministry of Trade and Fisheries. (in Norwegian). [Cited Aug 24 2018] Available from https://lovdata.no/dokument/SF/forskrift/2012-12-05-1140

Fisheries (2017) Forskrift om produksjonsområder for akvakultur av matfisk i sjø av laks, ørret og regnbueørret (produksjonsområdeforskriften). Norwegian Ministry of Trade, Industry and Fisheries. (in Norwegian) [Cited 5 Mar 2018] Available from https://lovdata.no/dokument/SF/forskrift/2017-01-16-61.

Norwegian Ministry of Trade, Industry and Fisheries (2018) Forskrift om bekjempelse av lakselus i akvakulturanlegg. Norwegian Ministry of Trade, Industry and Fisheries. (in Norwegian) [Cited Mar 5 2018] Available from https://lovdata.no/dokument/SF/forskrift/2012-12-05-1140

Olsen S (2017) Næringen trenger ikke enda flere å spise wienerbrød med, de trenger rett og slett også litt tyggemotstand. iLaks. (in Norwegian). [Cited Aug 24 2018] Available from https://ilaks.no/naeringen-trenger-ikke-enda-flere-a-spise-wienerbrod-med-de-trenger-rett-og-slett-ogsa-litt-tyggemotstand/

Olsvik PA, Ørnsrud R, Lunestad BT, Steine N, Fredriksen BN (2014) Transcriptional responses in Atlantic Salmon (*Salmo salar*) exposed to deltamethrin, alone or in combination with azamethiphos. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* **162**: 23–33.

Oppedal F, Dempster T, Stien LH (2011) Environmental drivers of Atlantic salmon behavior in sea-cages: a review. *Aquaculture* **311**: 1–18.

Oppedal F, Samsing F, Dempster T, Wright DW, Bui S, Stien LH (2017) Sea lice infestation levels decrease with deeper 'snorkel' barriers in Atlantic salmon sea-cages. *Pest Management Science* **73**(9): 1935–1943.

Poppe TT, Dalum AS, Nord ERÅ, Nordgreen J, Helgesen KO (2018) Termisk behandling av laks. Norsk veterinærtidsskrift nr.3. pp. 148-156 (in Norwegian).

Powell MD, Reynolds P, Kristensen T (2015) Freshwater treatment of amoebic gill disease and sea-lice in seawater salmon production: considerations of water chemistry and fish welfare in Norway. *Aquaculture* **448**: 18–28.

Remen M, Oppedal F, Imsland AK, Olsen RE, Torgersen T (2013) Hypoxia tolerance thresholds for post-smolt Atlantic salmon: dependency of temperature and hypoxia acclimation. *Aquaculture* **416–417**: 41–47.

Revie CW, Gettinby G, Treasurer JW, Wallace C (2003) Identifying epidemiological factors affecting sea lice *Lepeophtheirus salmonis* abundance on Scottish salmon farms using general linear models. *Diseases of Aquatic Organisms* **57**: 85–95.

Roth B (2016) Avlusing av laksefisk med Optilicer: Effekt på avlusing og fiskevelford. Rapport 59/2016. Nofima, Norway, 41 p (in Norwegian).

Roth M, Richards RH, Sommerville C (1993) Current practices in the chemotherapeutic control of sea lice infestations in aquaculture: a review. *Journal of Fish Diseases* **16**: 1–26.

Roth M, Richards RH, Dobson DP, Rae GH (1996) Field trials on the efficacy of organophosphorus compound azamethiphos for the control of sea lice (Copepoda: Caligidae) infestations of farmed Atlantic salmon (*Salmo salar*). *Aquaculture* **140**: 217–239.

Skiftesvik AB, Blom G, Agnalt A-L, Durif CMF, Browman HI, Bjelland RM *et al.* (2013) Wrasse (Labridae) as cleaner fish in salmonid aquaculture – The Hardangerfjord as a case study. *Marine Biology Research* **10**: 289–300.

Skjervold PO, Fjæra SO, Østby PB, Einen O (2001) Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture* **192**: 265–280.

Stien LH, Bracke M, Folkedal O, Nilsson J, Oppedal F, Torgersen T *et al.* (2013) Salmon Welfare Infex Model (SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. *Reviews in Aquaculture* **5**: 33–57.

Stien LH, Dempster T, Bui S, Glaropoulos A, Fosseidengen JE, Wright DW *et al.* (2016) 'Snorkel' sea lice barrier technology reduces sea lice loads on harvest-sized Atlantic salmon with minimal welfare impacts. *Aquaculture* **458**: 29–37.

Stien LH, Lind MB, Oppedal F, Wright DW, Seternes T (2018) Skirts on salmon production cages reduced salmon lice infestations without affecting fish welfare. *Aquaculture* **490**: 281–287.

The Norwegian Medicines Control Authority (2000) Behandling mot lakselus i oppdrettsanglegg. The Norwegian Medicines Control Authority, 43 p (in Norwegian).

Thomassen JM (1993) Hydrogen peroxide as a delousing agent for Atlantic salmon. In: Boxshall GA, Defaye D (eds) *Pathogens of Wild and Farmed Fish: Sea Lice*, pp. 85–91. Ellis Horwood, Chichester.

Thorstad EB, Forseth T (2016) Status of Norwegian salmon stocks in 2016. The Norwegian Institute for Nature Research (NINA). [Cited Mar 19 2018] Available from https://brage.bibsys.no/xmlui/handle/11250/2394052

Torrissen O, Jones S, Asche F, Guttormsen A, Skillbrei OT, Nilsen F *et al.* (2013) Salmon lice – impact on wild salmonids and salmon aquaculture. *Journal of Fish Diseases* **36**:171–194.

Volent Z, Birkevold J, Stahl A, Lien A, Sunde LM, Lader P (2017) Experimental study of installation procedure and volume estimation of tarpaulin for chemical treatment of fish in floating cages. *Aquacultural Engineering* **78**: 105–113.

Vollset KW, Dohoo I, Karlsen Ø, Halttunen E, Kvamme BO, Finstad B *et al.* (2017) Disentangling the role of sea lice on the marine survival of Atlantic salmon. *ICES Journal of Marine Science* **75**: 50–60.

Walsh TK, Lyndon AR, Jamieson DJ (2007) Identification of cDNAs induced by the organophosphate trichlorphon in the parasitic copepod *Lepeophtheirus salmonis* Copepoda; Caligidae. *Pesticide Biochemistry and Physiology* **88**: 26–30.

Whyte SK, Westcott JD, Revie CW, Hammell KL (2016) Sensitivity of salmon lice (*Lepeophtheirus salmonis*) in New Brunswick, Canada, to the organophosphate Salmosan® (w/w 50% azamethiphos) using bioassays. *Aquaculture* **464**: 593–600.

Wright DW, Stien LH, Dempster T, Vågseth T, Nola V, Fosseidengen J-E *et al.* (2017) 'Snorkel' lice barrier technology reduced two co-occurring parasites, the salmon louse (*Lepeophtheirus salmonis*) and the amoebic gill diseases causing agent (*Neoparamoeba perurans*), in commercial salmon sea-cages. *Preventative Veterinary Medicine* **140**: 97–105.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Percentage of mortality observations ≥1 percentage point (pp) for compromised (0.25–1% mortality the month before treatment) at 4–7, 7–10, 10–13 and 13–16°C from 2012 to 2017, for: (i) small (<2 kg) fish and (ii) large (≥2 kg) fish, for: (a) Azamethiphos (b) Cypermethrin (c) Deltamethrin (d) Hydrogen peroxide (e) Mechanical (f) Thermal.

Figure S2. Boxplots of the distribution of (a) temperature; (b) uncompromised fish mortality pp; (c) compromised fish mortality pp; (d) fish weight in all 13 Norwegian Atlantic salmon production zones.