Effect of cleaner fish on sea lice in Norwegian salmon aquaculture: a national-scale data analysis

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ABSTRACT

The salmon aquaculture industry has adopted the use of invertivorous 'cleaner fishes' (CF) for biological control of sea lice infestations on farmed salmon. At present, ~50 million CF are used annually in Norway alone, with variable success in experimental and industrial contexts. We used a national-scale database of lice counts, delousing treatments and CF stocking events on Norwegian salmon farms to test for evidence of CF efficacy at 488 sites that completed a grow-out cycle within 2016-2018. Our analysis revealed that sites using more CF over the duration of a grow-out cycle did not have fewer lice on average, likely because CF use is reactive and in proportion to the scale of the lice problem. Over time within sites, we found that (i) sites using more CF early in the grow-out cycle were able to wait slightly longer (conservatively, a 5.2 week delay with 5000 CF stocked week^{-1}) before conducting the first delousing treatment, and (ii) CF stocking events were followed, on average, by a small reduction in lice population growth rates. However, both effects were small and highly variable, and lice population growth rates remained positive on average, even when large numbers of CF were used (tens of thousands per site). Moreover, effects of CF on lice density tended to be short-lived, likely reflecting mortality and escape of stocked CF. Overall, the data indicate that while some sites consistently get good results from CF, there is also widespread suboptimal use. A better understanding of factors affecting CF efficacy in commercial sea cages is required to inform legislation and drive more efficient and ethical use of CF by the salmon aquaculture industry.

1. INTRODUCTION

Ectoparasitic sea lice thrive in industrial Atlantic salmon (*Salmo salar*) aquaculture due to the high density of hosts (Jones and Beamish, 2011). Planktonic infective stages originating from farmed or wild salmonid hosts enter the sea cage environment, attach to farmed salmon, and begin to feed on the mucus, skin and blood of their hosts. Infestation pressure is ubiquitous in densely farmed regions, and severe infestations can reduce the welfare of farmed salmon (Bowers et al., 2000; Øverli et al., 2014; Stien et al., 2013). Moreover, mature lice are highly fecund, and infective stages exported from sea cages can infest wild salmonids. Infestation pressure originating from salmon farms has been implicated in population declines in wild stocks (Kristoffersen et al., 2018; Krkošek et al., 2013), and as a result, farmers in lice-prone regions are required to expend considerable resources preventing and treating lice infestations (Abolofia et al., 2017).

Effective parasite control at the scale of modern commercial salmon farms is logistically challenging, with up to 200 000 salmon held per cage and several cages per site. The largest commercial sites contain >1 million salmon with an allowable biomass >8000 t (BarentsWatch database, URL: https://www.barentswatch.no/en/fishhealth/, accessed 12 Nov 2019). Chemotherapeutants were the primary delousing strategy for decades, but are now used less—particularly in Europe—following findings of drug resistance and environmental contamination (Burridge et al., 2011; Aaen et al., 2015; Bloodworth et al., 2019; Overton et al., 2019). Mechanical and thermal delousing methods are now the most widely used in Norway and are being increasingly deployed elsewhere (e.g. Scotland, Canada, Chile), but are stressful for stock and can lead to elevated mortality rates (Overton et al., 2019). New approaches that are less stressful for salmon are needed, and biological control by invertivorous 'cleaner fish' (CF herein) has become a leading contender.

The use of CF is a rare example of biological parasite control by vertebrates, and likely the only case of a vertebrate being deployed to control parasites on another vertebrate in a commercial setting. Five main CF species are now in use at industry scale: lumpfish (*Cyclopterus lumpus*), corkwing wrasse (*Symphodus melops*), ballan wrasse (*Labrus bergylta*), goldsinny wrasse (*Ctenolabrus rupestris*), and cuckoo wrasse (*Labrus mixtus*) (Norwegian Directorate of Fisheries, 2019). None of these species are specialist cleaner fishes, unlike some tropical wrasses (Vaughan et al., 2017), but do opportunistically consume attached pre-adult and adult lice stages in tank and cage environments (Bjordal, 1991; Imsland et al., 2018; Skiftesvik et al., 2013). Industrial use of CF for biological control began in the late 1980s (Bjordal, 1991; Torrissen et al., 2013), and there was a rapid expansion coinciding with a shift away from chemical treatments after 2012. Currently ~50 million CF are stocked annually in Norway (Norwegian Directorate of Fisheries, 2019), with smaller numbers also used in other countries. Most lumpfish are now produced in hatcheries (2018: 29 million, 93 %), but wrasses used as CF are almost entirely wild-caught (Norwegian Directorate of Fisheries, 2019), with measurable impacts on wild populations (Halvorsen et al., 2017).

Cleaner fish are less stressful to salmon than other delousing methods (Groner et al., 2013; Imsland et al., 2018; Powell et al., 2018), and may also be viewed by consumers as preferable to chemotherapeutants (Treasurer, 2018). However, the use of vertebrates for biological control raises unique ethical considerations, with concerns regarding poor welfare and high mortality of CF when stocked in sea cages (Mo and Poppe, 2018; Nilsen et al., 2014; Skiftesvik et al., 2014; Treasurer and Feledi, 2014). Moreover, as salmon and CF share some pathogens, stocking wild-caught CF may increase infection risk for salmon (Murray 2016). Cost-effectiveness of CF is difficult to assess at present, because while annual expenditure on CF is known (1-3 USD per fish, total 100 million USD in 2018: Directorate of Fisheries, 2019), the evidence base for their efficacy is sparse at commercial scale (Overton et al., In Press). Farmers report variable and context-dependent efficacy (Treasurer, 2018), which may reflect sub-optimal use of CF in many cases. Lice consumption is a learned behaviour in these species (Vaughan et al., 2017), and CF must acclimate behaviourally to the cage environment before effectively cleaning salmon (Imsland et al., 2019). Even once acclimated, access to alternative food sources (e.g. biofouling, feed pellets) is likely to reduce lice consumption (Imsland et al., 2015), while unsuitable environmental conditions such as extreme temperatures or high currents can result in physiological stress, reduced feeding, and elevated mortality among CF (Hvas et al., 2018; Yuen et al., 2019). Differing habitat preferences related to temperature and current velocity may also reduce spatial overlap between salmon and CF within sea cages, and lead to fewer opportunities for cleaning interactions (Tully et al., 1996; Overton et al., In Press).

Given these concerns regarding efficacy and welfare of CF in commercial sea cages, and the impacts of the wild-catch wrasse fishery, robust evidence is required to justify and guide CF use by the industry. The current experimental evidence base relies heavily on a small number of trials, most of which were conducted in tanks or small cages that do not reflect modern commercial salmon farming conditions (Brooker et al., 2018; Powell et al., 2018; Overton et al., 2020). More evidence is needed at modern commercial scale to ground-truth findings from experimental trials.

Using a large, publicly available dataset, we tested CF efficacy across the Norwegian salmon farming industry. The Norwegian regulation on salmon lice control (Norwegian Ministry of Trade Industry and Fisheries, 2012) requires each active farm site to report key lice and lice treatment parameters weekly to the Norwegian Food Safety Authority. This includes lice numbers (divided into sessile, mobile, and adult female lice), control treatments administered (i.e. chemotherapeutant and mechanical/thermal), and CF use (species and number stocked). Using these data, we summarised CF use across aquaculture production zones in Norway (Fig. 1) and conducted two main analyses: (1) between sites, to test for differences in lice abundance according to CF use, and (2) within sites, to test whether CF stocking events result in reduced lice population growth. We expected that sites using more CF would have lower lice levels overall and would delouse less frequently.

2. METHODS

2.1. Data sourcing and cleaning

The Norwegian Food Safety Authority forwards lice and lice treatment data to a publicly available fish health monitoring site (BarentsWatch, URL: https://www.barentswatch.no/fiskehelse/). There, we accessed all records for lice levels, CF stocking events, and delousing treatments at 1178 farm sites across a period spanning Jan 1, 2016 to Dec 31, 2018). We limited the data to this period because the industry underwent several key changes in the preceding years, including an increase in the use of lumpfish as CF (Norwegian Directorate of Fisheries, 2019), and the widespread introduction of mechanical and thermal delousing from 2016-2018 (Overton et al., 2019). Together, these changes make data from pre-2016 less representative of current industry practices and conditions that may affect CF efficacy.

After omitting inactive sites (1049 remaining), we noted the start and end dates for the most recent completed grow-out cycle at each site and omitted weekly records from outside that timeframe. We also omitted whole sites that (i) did not complete a full grow-out cycle (i.e. sea transfer to harvesting) within 2016-2018 or had unclear start or end dates for grow-out cycles (548 remaining); (ii) had grow-out cycles <20 weeks long (543 remaining), (iii) operated under broodstock or research license (496 remaining); or (iv) were in production zones 1 or 13 as these zones have few sites and lice infestation pressures that differ from the rest of Norway (488 remaining). The final dataset contained 33009 lice counts and 7980 lice control reports (mechanical/thermal delousing, chemotherapeutant delivery, and cleaner fish stocking events) across the 488 sites (Fig. S1). We obtained but did not analyse data on sessile (copepodid and chalimus) lice stages as these are unlikely to be consumed by CF in sufficient quantities to drive a detectable effect (Imsland et al., 2014; Skiftesvik et al., 2013). Most grow-out cycles included in the final dataset began in 2016 or 2017 (2016: 283 sites; 2017: 200 sites; 2018: 5 sites) due to the requirement to have completed the cycle by the end of 2018. Individual records (lice counts and lice control reports) peaked in 2017 (2016: 9068 records; 2017: 22932 records; 2018: 8989 records).

2.2. Between-sites analysis

For a broad view of lice levels and CF use at Norwegian salmon farming sites, we included all records during the most recent grow-out cycle, and for each site, calculated: (i) mean abundance of mobile and adult female lice stages across the grow-out cycle; (ii) the number of weeks where the site exceeded legislated lice limits (legislated lice limits are 0.2 adult female lice between weeks 16-21, and 0.5 adult female lice outside these weeks for southern Norway, while the 0.2 limit is set between weeks 21-26 for northern Norway (Ministry of Trade, Industry and Fisheries, 2012)); (iii) the total number of CF stocked (both by species, and all species combined); and (iv) the total number of chemotherapeutant and mechanical/thermal delousing treatment reports (mechanical and thermal treatments are not differentiated in the BarentsWatch database). Some delousing records are whole-site treatments while others are partial-site treatments – these are not differentiated in the analysis.

To test whether sites that used more CF tended to have fewer lice, we fitted univariate generalized linear mixed models (GLMMs) in which the response variable (Model 1: all mobile lice fish^{-1}; Model 2: adult female lice fish^{-1}; Model 3: proportion of grow-out weeks exceeding the legislated lice limit) was regressed against the number of CF stocked over the same timeframe. CF numbers were corrected for the length of the grow-out cycle (CF week^{-1}). To account for other sources of variation in lice levels, GLMMs also included terms for the number of chemical and mechanical/thermal treatment reports per week over the grow-out cycle, the duration of the grow-out cycle (weeks; scaled and centred), the nominal grow-out capacity of the site (tonnes, scaled and centred), and the production zone (zones 2-12, specified as a random intercept factor). Models were fitted using the glmmTMB package for R (Brooks et al., 2017; R Core Team, 2018). Model families were selected after inspection of response variable distributions, with model fits then checked using diagnostic plots generated by the DHARMa package (Hartig, 2019). Full models were simplified to the most parsimonious set of model terms based on Akaike Information Criterion (AIC) scores.

2.3. Within-sites analysis

To test whether CF use correlated with reduced lice population growth over time within sites, we collated all records on lice counts, cleaner fish stocking, and delousing treatments between the start and end of the most recent grow-out cycle at each site. This allowed temporal trends in lice loads to be tracked over the course of the grow-out cycle and compared with timing of lice control reports at each site. We made two main comparisons using the within-site data:

First, we tested whether the use of CF early in the grow-out cycle was associated with a longer duration to the first delousing treatment, by (1) grouping records by site, (2) omitting all records from the first delousing treatment onwards, and (3) computing the number of weeks and CF used up until the first delousing treatment. We fitted a mixed effects regression model using glmmTMB, with ‘weeks to first delousing’ as the response variable, ‘number of CF stocked’ and ‘year’ as predictors, and production zone as a random intercept term. The response variable was log(x+1) transformed, and the predictor was scaled and centred to improve model fit (checked using DHARMa diagnostic plots).

Second, we conducted a ‘sliding window’ analysis to test whether CF deployments throughout the grow-out cycle tended to be associated with reduced lice population growth rates afterwards. For each weekly report during the grow-out cycle, we computed the mean adult female lice density during a 3-week period occurring 10-12 weeks prior (startAF), and the 3-week period immediately prior (endAF). These values defined the start and end points of the 12-week sliding window. Lice population growth during the sliding window was quantified both by the slope: *endAF-startAF/12*, and by the natural log of the response ratio: *ln(endAF/startAF)*. The response ratio variable is not compatible with zero values for startAF or endAF, so a small constant ($1e^{-6}$) was added to each before computing the response ratio, rather than omitting windows for which zeros occurred. Sliding windows containing delousing reports were omitted to avoid confounding the CF effect. In two separate models, response variables were regressed against the number of CF stocked during the first 6 weeks of the sliding window. The regression models were fitted using the glmmTMB package for R, with independent variables for the number of CF stocked (scaled and centred), the year at the time of the sliding window, and the starting adult female lice density (as population growth and the CF effect may both be influenced by starting density). To account for repeated measures within sites, we included a random intercept term for site identity. To obtain interpretable effect sizes, we also fitted a simple regression model from which the intercept and slope coefficients were used to calculate percentage reductions in lice population growth associated with a given number of CF stocked.

2.4. Latitudinal effects

There is a north-south geographic divide in CF use; wrasse and lumpfish are both used in production zones 2 to 8, while only lumpfish are used in the northernmost zones (9 to 12). We repeated the analyses above on data split into zones 2 to 8 and 9 to 12 to test whether the overall patterns hold when looking within these 2 regions with differing sea temperatures and CF species use.

3. RESULTS

3.1. CF use in Norway

Lumpfish were the most-used species across the 488 sites included in our final dataset (lumpfish = 56% of all CF used) and were predominant in every production zone except zone 5, where corkwing wrasse were preferred (Fig. 1). Lumpfish were the only CF species used in the northernmost production zones 9 to 12 (also zone 13, which was not included in our analysis). Goldsinny and corkwing wrasse were the second and third most commonly used species overall (18% and 17%, respectively), followed by ballan wrasse (5%) and 'other wrasse' (4%).

Most sites (341/488, 70%) reported stocking some CF, with a mean of 90300 CF stocked per site during the most recent grow-out cycle (range = 0-672807, SD = 110131). Grow-out cycle length (estimated based on the duration of continuous lice reporting of lice levels) varied in length from 20-128 weeks (mean = 68 weeks). Particularly short grow-out cycles probably reflect transfer of fish between farms, with the apparently short cycle having been completed at a different site. Conversely, particularly long grow-out cycles may reflect staggered start and end dates for individual cages within a site. Mean site capacity was 3443 t of salmon (range = 300-8580, SD = 1443).

3.2. Do sites that use more CF have fewer lice?

We expected that sites using larger numbers of CF would have both fewer lice and fewer weeks exceeding legislated limits over the duration of the most recent grow-out cycle. Instead, we found that CF use (number stocked per week of the grow-out cycle) was uncorrelated with lice levels, whether quantified by mobile lice, adult female lice, or the proportion of weeks exceeding the legislated limits (Models 1-3: Table 1, Fig. 2).

Lice levels varied between production zones, with the random intercept term for production zone (*ZoneN*) explaining a small but non-trivial portion of variation in lice levels for Models 1 and 2 (Table 1).

3.3. How does CF use interact with other delousing treatments?

The frequency of reported delousing by mechanical/thermal or chemotherapeutant methods was positively correlated with mean lice loads (Models 1 and 2: Table 1), consistent with lice management efforts increasing in response to high lice densities. However, the data also suggest that sites using more CF were less likely to use chemotherapeutants, and vice versa (Pearson's $r = -0.15$, $p = 0.001$; Fig. 3). This may indicate a preference for one approach or the other, and was particularly apparent at sites with high lice control effort overall (Fig. 3). This negative correlation was driven by sites in the south (zones 2 to 8) and was no longer apparent when the dataset was restricted to sites in zones 9 to 12, corresponding to latitudinal differences in the dominant lice species and disease management strategies (for example, *Caligus elongatus* is more prevalent in northern Norway and may be less resistant to chemotherapeutants than *L. salmonis*: Imsland et al., 2019). Overall, chemotherapeutant use was widespread (73% of sites), but frequency of reported use was low (mean = 3.0 treatment reports over grow-out cycle). In contrast, the frequency of reported mechanical/thermal delousing (prevalence: 77% of sites; mean = 4.4 treatment reports over grow-out cycle) was weakly positively correlated with the number of CF used over a grow-out cycle ($r = 0.25$, $p < 0.0001$; Fig. 3), indicating that sites often used both CF and mechanical/thermal delousing to control lice.

3.4. How does lice abundance vary over time?

Viewed at whole-of-industry scale, abundance of mobile lice stages increased approximately monotonically over the first 70 weeks of the grow-out cycle, and then declined between weeks 70-105 (Fig. S2). This trend contains a latitudinal component, with most sites in the south harvesting within ~80 weeks (i.e. the mean reported lice density in later weeks is primarily from northern sites). The overall trend was qualitatively similar whether the sea transfer of smolts to start a grow-out cycle took place in spring or autumn. Despite the relative predictability at whole-of-industry scale, temporal changes in lice density fluctuated rapidly when viewed at the site level, with frequent lice control interventions and re-infestations causing large week-to-week variations in lice abundance.

3.5. Does CF stocking early in the grow-out cycle delay the need for delousing?

Stocking of CF early in the grow-out cycle was associated with a small but statistically significant increase in the time to first delousing (Table 2; Fig. 4). Among sites that did not stock any CF before the first delousing, the first delousing took place 23.0 weeks into the grow-out cycle on average. Stocking 2500 CF per week increased that time to 25.6 weeks, while stocking 5000 CF per week increased it to 28.2 weeks (Fig. 4), a 5.2-week delay on average.

3.6. Does CF deployment reduce the rate of lice population growth?

The 12-week sliding window analysis revealed a small reduction in adult female lice population growth rates following CF stocking, depending on whether population growth was quantified by the log response ratio or slope of lice density between the start and end of the sliding window (Table 3). The effect was highly variable but appeared to increase with the number of CF stocked, although population growth rates remained positive on average for all but the highest CF stocking efforts (Fig. 5). Sliding windows that started with higher adult female lice densities and higher sea temperatures had larger reductions in lice population growth rates (Table 3). Extracting predictions from linear fits (y = lice population growth metric, x = number of CF used) provided an estimation of interpretable effect sizes. The log response ratio for change in adult female lice density over the 12-week window was 0.58 at sites that did not stock any CF, 0.45 (22 % lower) at sites that stocked 50 000 CF, and 0.32 (44 % lower) at sites that stocked 100 000 CF during the sliding window. Importantly, these numbers only included those CF stocked in the first 6 weeks of the 12-week sliding window. The same sites may have stocked considerable quantities of CF in previous and following weeks or months, potentially reducing the apparent effect size.

Three sites in 3 production zones (3, 6, 7) used particularly large numbers of CF to combat rising lice densities (several 6-week periods during which >200 000 CF were stocked). To illustrate the relationship between lice counts, CF deployment and delousing events, we plotted the temporal patterns for these 3 sites (Fig. 6). The sites experienced differing infestation pressures and employed differing lice control strategies. Site 1 (pink) had low lice numbers for the first ~40 weeks without any reported lice control, after which adult female lice numbers increased rapidly. This increase was countered first by a series of mechanical or thermal delousing reports and then later by very large numbers of CF. Lice numbers remained under the 0.5 adult female lice per fish limit for the remainder of the grow-out cycle, with another mechanical/thermal delousing event at week 57. Site 2 (green) also had low lice numbers for the first ~40 weeks, having stocked CF regularly throughout this time. For the latter half of the cycle, this site continued to stock small numbers of CF and conducted regular delousing treatments (3 chemotherapeutant and 8 mechanical/thermal reports) and maintained lice levels below 0.2 adult female lice per fish. Site 3 (purple) had moderate lice levels throughout the grow-out cycle, with 4 early mechanical or thermal delousing reports in weeks 26-30 limiting adult female lice densities. Large numbers of CF were stocked during weeks 43-56, which was followed by a decline in lice numbers until week ~63 and then a rapid increase that continued until the end of the grow-out cycle (with 1 more mechanical/thermal treatment).

3.7. Duration of the CF effect

Cleaner fish have the potential to provide long-term suppression of adult sea lice populations over a grow-out cycle, provided that CF are kept healthy and do not escape. However, several lines of evidence indicated that the effect of CF stocking on lice population growth has a limited duration. First, an examination of records for individual sites revealed numerous cases where large CF stocking events correlated with halted or negative lice population growth during the next ~10-20 weeks (consistent with an effect of CF), before lice numbers began to increase again (see Fig. 6 for examples). Second, almost all sites that used CF still required delousing later in the production cycle. Of the 339 sites that used >10000 CF, 317 still required at least one delousing treatment (94%), while 112 of those sites recorded 10 or more treatment reports. In some cases, subsequent delousing events may have been conducted in cages not containing CF (i.e. where sites apply different lice control strategies in different cages) and as such may not represent a failure of CF to maintain low lice levels. Nonetheless, few sites (6%) were able to avoid delousing for an entire grow-out cycle by stocking CF. Finally, repeating the sliding window analysis with a longer window duration (24 weeks) revealed that the effect observed after 12 weeks is not maintained over a longer period (Fig. S3). Specifically, the log response ratio for adult female lice abundance between the start and end of the 24 week sliding window was still significantly affected by number of CF stocked in the first 6 weeks of that window (n = 3900 windows across 294 sites, estimate = -0.08, $X^2 = 16.9$, $p < 0.0001$), but the effect size was much smaller. The estimated effect of stocking 100 000 CF at a site is a 44% slowing of adult female lice population growth over a 12-week period and a 7% slowing over a 24-week period. In isolation, none of the above lines of evidence are conclusive, but together they indicate that the CF effect probably peaks within 2-4 months of stocking.

3.8. Latitudinal effects

Splitting the dataset into zones 2 to 8 (south: mixed wrasse and lumpfish use) and zones 9 to 12 (north: lumpfish only) indicated that some findings were robust to changes in CF species and/or production zones, while others were not. Our interpretation of the between-sites analysis did not change substantively – there was still no detectable effect of cleaner fish use on lice levels averaged over the full grow-out cycle, either for sites in the south or north. However, the within-sites analyses revealed some key differences between regions. The effect of CF use on delaying the time to first delousing held when looking at the southern zones only (5.4 additional weeks by stocking 5000 CF week^{-1}; $p = 0.005$) but did not hold for the northern zones only ($p = 0.46$) – i.e. for zones 9 to 12, stocking lumpfish did not decrease the time to first delousing compared to not stocking lumpfish. Similarly, the sliding window analysis indicated that slowed lice population growth rates following CF stocking only occurred in the southern zones (log response ratio; southern zones: 6435 sliding windows at 346 sites, estimate = -0.11, $p < 0.0001$; northern zones: 2528 sliding windows at 110 sites, estimate = 0.02, $p = 0.42$) – i.e. for zones 9 to 12, stocking lumpfish did not reduce lice population growth compared to other timepoints or sites where lumpfish were not stocked. All other model terms (starting adult female lice density, sea temperature, report year) remained significant for both the northern and southern datasets ($p < 0.0001$ in each case).

4. DISCUSSION

The between-sites analysis revealed that use of cleaner fish (CF) was not correlated with lice density over the course of a grow-out cycle, nor a lower proportion of grow-out weeks exceeding legislated adult female lice levels. However, looking within sites revealed evidence that stocking CF early in the grow-out cycle had a small, but significant, effect of delaying the first delousing treatment. Similarly, CF stocking events throughout the grow-out cycle were associated with a slight reduction in mean lice population growth within the 12 weeks following. While statistically significant, both of these effects were small and highly variable, and driven by sites in zones 2 to 8 (where a mix of CF species are used), not zones 9 to 12 (where only lumpfish are used).

4.1. Between-site patterns in CF use and lice densities

The apparent lack of effect when correlating CF stocking rates and mean lice density between sites could reflect site-specific infestation pressures rather than a true absence of a CF effect. This analysis cannot tell if farmers use more CF because they have more lice, have fewer lice because they use more CF, or if lice densities are unaffected by CF stocking. We observed a similar pattern for delousing treatments that are known to be highly effective (e.g. mechanical delousing), whereby frequency of delousing was positively correlated with mean lice levels over the grow-out cycle. If CF use follows a similar pattern, the lack of a detectable CF effect at the between-sites level is not evidence of inefficacy. Instead, CF may be used in proportion to the size of the lice problem at a given site, with farmers aiming to stock as many CF as necessary to keep lice at permissible levels.

Effects of CF at the between-sites level may also be masked by other lice management strategies. Farmers typically use a suite of lice control interventions, with a typical grow-out cycle being interspersed with CF stocking events and mechanical, thermal or chemotherapeutant delousing treatments. In addition, farmers may use preventative methods that do not require reporting, such as lice barriers (Grøntvedt et al., 2018; Stien et al., 2018; Geitung et al., 2019) that may reduce infestation rates or interact with CF efficacy (Gentry, K., 2018, MSc thesis, Melbourne). Nonetheless, the absence of a clear effect at the between-site level indicates that CF are not a ‘silver bullet’ for the industry’s sea lice problems. They do not appear to be markedly better than other lice control methods for the purposes of staying under legislated lice limits. CF may be preferable to other lice removal methods in terms of salmon welfare and productivity (Overton et al., 2019), but such benefits should be weighed against welfare concerns for stocked CF (Mo and Poppe, 2018; Nilsen et al., 2014) and fishing pressure on wild wrasse populations (Halvorsen et al., 2017).

4.2. Within-site patterns in CF use and lice densities

By tracking CF use and lice density over time within sites, we detected a significant effect of CF stocking on subsequent lice population growth rates. On average, CF stocking events did not reduce lice levels at the site level but did slow the rate of population increase. Mean lice population growth may have been negative within individual cages following CF stocking events, but cage-level data are not yet publicly available. Overall, the effect of CF stocking on subsequent lice population growth was smaller and more variable than expected, both among sites (time to first delousing and sliding windows analyses) and among stocking events at the same site (sliding windows analysis). Importantly, our test of the effect of CF in delaying the first delousing was vulnerable to the same weakness as the between-sizes analysis, namely that a correlative analysis comparing patterns between sites cannot account for differing infestation pressure among sites. Sites with high infestation pressure soon after sea transfer of smolts are likely to stock higher numbers of CF early on, which could partially mask the effect of CF on time to first delousing. Our estimate of this effect is therefore likely to be conservative. The sliding windows analysis is less vulnerable to these concerns because the effect is measured over time within the same site. By including a random effect for site identity, we were also able to account for some consistent site-specific conditions underlying this effect (such as lice population growth rates in sliding windows for which CF were not stocked).

The apparent variability in the CF effect is probably driven by numerous factors relating to salmon and CF traits, as well as site-specific procedures. Many potentially influential factors were not available in the database, but we did test model terms for production zone, sea temperature, nominal biomass capacity, year, and for the sliding windows analysis only, starting adult female lice density (which was significantly associated with CF efficacy). Other factors that are likely to affect CF efficacy include the initial size and condition of CF (reviewed in Brooker et al., 2018), site exposure and temperature (Hvas et al., 2018; Yuen et al., 2019), and care and handling of CF after stocking (e.g. provision of hides, supplementary feeding, disease control) (Brooker et al., 2018; Nilsen et al., 2014; Powell et al., 2018). The effect of CF on lice population growth in this analysis was strongest when adult female lice density was higher and sea temperature was higher – these conditions coincide with a range of relevant processes. For example, lice population density is influenced by water temperature, with faster development and reproduction at higher temperatures (Samsing et al., 2016), and either the higher density of lice or the warmer water may increase feeding behaviour by CF. Warmer water, depending on latitude, may also increase encounter rates between salmon and CF – lumpfish in particular tend to prefer the surface layers regardless of temperature (L Geitung et al., unpublished data), while salmon typically swim deeper to avoid cold surface layers (Oppedal et al., 2011). In the south, lumpfish experience temperature stress and high mortality during the summer but are stocked year-round regardless (Fig. S4). Wild caught CF are also available during the warmer months, although they may not differ from reared CF in their efficacy (Skiftesvik et al., 2013).

Overall, while there are probably conditions under which CF can be reliably deployed to good effect, the amount of variation in the present analysis indicates that CF use is currently far from optimal when viewed at industry scale. If CF were routinely used in an optimal manner, we would expect to see stronger and less variable effects of CF stocking on subsequent lice growth rates – i.e. in typical circumstances, effective use of CF should never result in adult female lice populations increasing in the months following stocking of tens of thousands of CF, as occurred at numerous sites in this dataset. Another point of concern is the apparently limited duration of the CF effect; by stocking CF, farms can sometimes delay delousing but seldom avoid it. Such an effect may still be of financial benefit to farms if the extra weeks of production prior to delousing or slaughtering outweigh the cost of purchasing CF, but the most likely explanations for short-lived effects are concerning. Specifically, given the available data on mortality of CF deployed into salmon cages (Mo and Poppe, 2018; Nilsen et al., 2014), it may be that the short duration of the CF effect is a direct result of rapid declines in CF welfare and survivorship, due to factors such as handling stress, disease or seasonal changes in water temperature. Escapes are also thought to be common, and are problematic because escapees that survive (e.g. those at suitable latitudes) may compete for habitat and interbreed with local conspecifics (Faust et al., 2018). Reducing escapes and boosting the welfare and longevity of cleaner fish in cages may lengthen the duration of their effect. Anecdotally, farmers report that sites that receive CF in good condition from suppliers, handle CF appropriately and have personnel dedicated to long-term CF welfare achieve the best results.

Few studies have demonstrated high efficacy of CF in sea cages with proper controls and replication, although it can be done (e.g. large cages: Imsland et al., 2018; small cages: Skiftesvik et al., 2013). However, in general, knowledge around CF use has increased considerably in recent years through research and industry trial-and-error, and efficacy may be improving (Brooker et al., 2018; Powell et al., 2018). Moreover, some commercial sites consistently achieve above-average results with CF (e.g. see SiteN random effect; Table 3); such sites should serve as a blueprint for 'best practice' use by industry. Targeted research at these sites may help explain why promising results from experimental conditions are not yet reliably replicated in the industrial context.

4.3. Recommendations for reporting and regulation of CF use

In conducting this study, we identified several areas where additional detail in weekly reporting would facilitate more precise analysis of the cleaner fish effect. Foremost, cage-level rather than site-level reporting of lice numbers and lice control measures would improve the resolution of data on lice control interventions and outcomes. Currently, lice control may be conducted in a subset of cages at a site, while reported lice levels are the mean of all cages at the site. Given that farmers already collect cage-level data, making such data available (even with anonymisation of site identity) would enable better assessment of the efficacy of lice control strategies, and facilitate testing for interactions between lice management strategies used in combination (e.g. where preventative and post-infestation control strategies are deployed simultaneously). To address the latter scenario, we recommend that commonplace lice prevention approaches such as barrier technologies be reported alongside delousing methods. Doing so would allow for detection of interactions between lice control approaches at industry scale (e.g. are cages with lice barriers typically associated with stronger or weaker CF effects?) and facilitate improved and more detailed guidelines for optimal lice management.

5. CONCLUSIONS

Our analysis of a nation-scale database detected some effects of CF use on subsequent lice population growth rates. Stocking sufficient numbers of CF reduced lice levels at many sites but not at others. This translated to a weak and short-lived general effect averaged across the industry. Moreover, the variable effects on lice density across sites and CF stocking events, both in magnitude and direction, demonstrate that while some sites have positive results from CF use, nearly as many sites do not. Most sites still had to delouse after stocking CF, even when very large numbers (tens or hundreds of thousands) of CF were used at a site. Collectively, these results indicate widespread suboptimal use of CF. Research is needed to better understand the factors that determine efficacy of CF in sea cages and optimise their use in the industry.

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Table 1. Results of mixed effects models testing effect of cleaner fish on mean salmon lice density (mobile: Model 1; adult female: Model 2), and on the proportion of grow-out weeks in which each site exceeds the allowed number of adult female salmon lice per fish (Model 3; production zone random term was trivial and was omitted). Model terms are number of cleaner fish stocked (N CF) and frequency of chemotherapeutant (Chem) and mechanical or thermal (Mech) delousing treatments per week. Model 2 also includes model terms for duration of grow-out cycle (weeks, scaled and centered) and nominal site capacity (t, scaled and centered).

Model 1: Mobile lice

Term	**Estimate**	X^2	**Model df**	**p**
N CF per week	$7e^{-6}$	1.1	1	0.30
Chem per week	0.68	15	1	0.0001
Mech per week	1.4	79	1	<0.0001

Random effect conditional model:

Groups	**Variance**
Production zone	0.02
Residual	0.04

Model 2: Adult female lice

Term	**Estimate**	X^2	**Model df**	**p**
N CF per week	$-5e^{-7}$	0.07	1	0.79
Chem per week	0.21	20	1	<0.0001
Mech per week	0.46	119	1	<0.0001
Cycle length (sc)	-0.004	2.3	1	0.13
Site capacity (sc)	-0.004	2.0	1	0.16

Random effect conditional model:

Groups	**Variance**
Production zone	0.0004
Residual	0.003

Model 3: Proportion of weeks exceeding lice limits

Term	**Estimate**	X^2	**Model df**	**p**
N CF per week	$-9e^{-5}$	0.7	1	0.42
Chem per week	4.5	1.5	1	0.22
Mech per week	4.6	1.6	1	0.21

Table 2. Results of mixed effects model testing effect of cleaner fish on time to first delousing for salmon farm sites. N = 488 sites.

Weeks to first delousing			
Term	**Estimate**	X^2	**p**
N CF per week	0.06	13	0.0004
Start year[a]	0.05	0.2	0.93

Random effect conditional model:	
Groups	**Variance**
Production zone (intercept term)	0.01
Residuals	0.11

[a]Year specified as factor; estimate is 2018 compared to 2016

Table 3. Results of mixed effects models testing effect of cleaner fish stocking on subsequent adult female lice population growth ('sliding windows' analysis). The number of CF stocked in the first 6 weeks of a given 12-week period is regressed against the rate of lice population growth from the first 3 to last 3 weeks of the 12-week sliding window (n = 8963 sliding windows across 456 sites).

Response 1: lnRR for adult female lice density			
Term	**Estimate**	**X^2**	**p**
N CF per week	-0.12	43	<0.0001
Starting lice density	-6.42	2264	<0.0001
Temperature	0.004	1.0	0.32
Year[a]	0.49	89	<0.0001

Random effect conditional model:

Groups	**Variance**
Site (intercept term)	0.39
Residuals	1.06

Response 2: Slope of change in adult female lice density			
Term	**Estimate**	**X^2**	**p**
N CF per week	-0.0002	0.74	0.39
Starting lice density	-0.06	2002	<0.0001
Temperature	0.0003	61	<0.0001
Year*	0.02	320	<0.0001

Random effect conditional model:

Groups	**Variance**
Site (intercept term)	0.00007
Residuals	0.0001

[a]Year is specified as a factor; estimate is 2018 compared to 2016

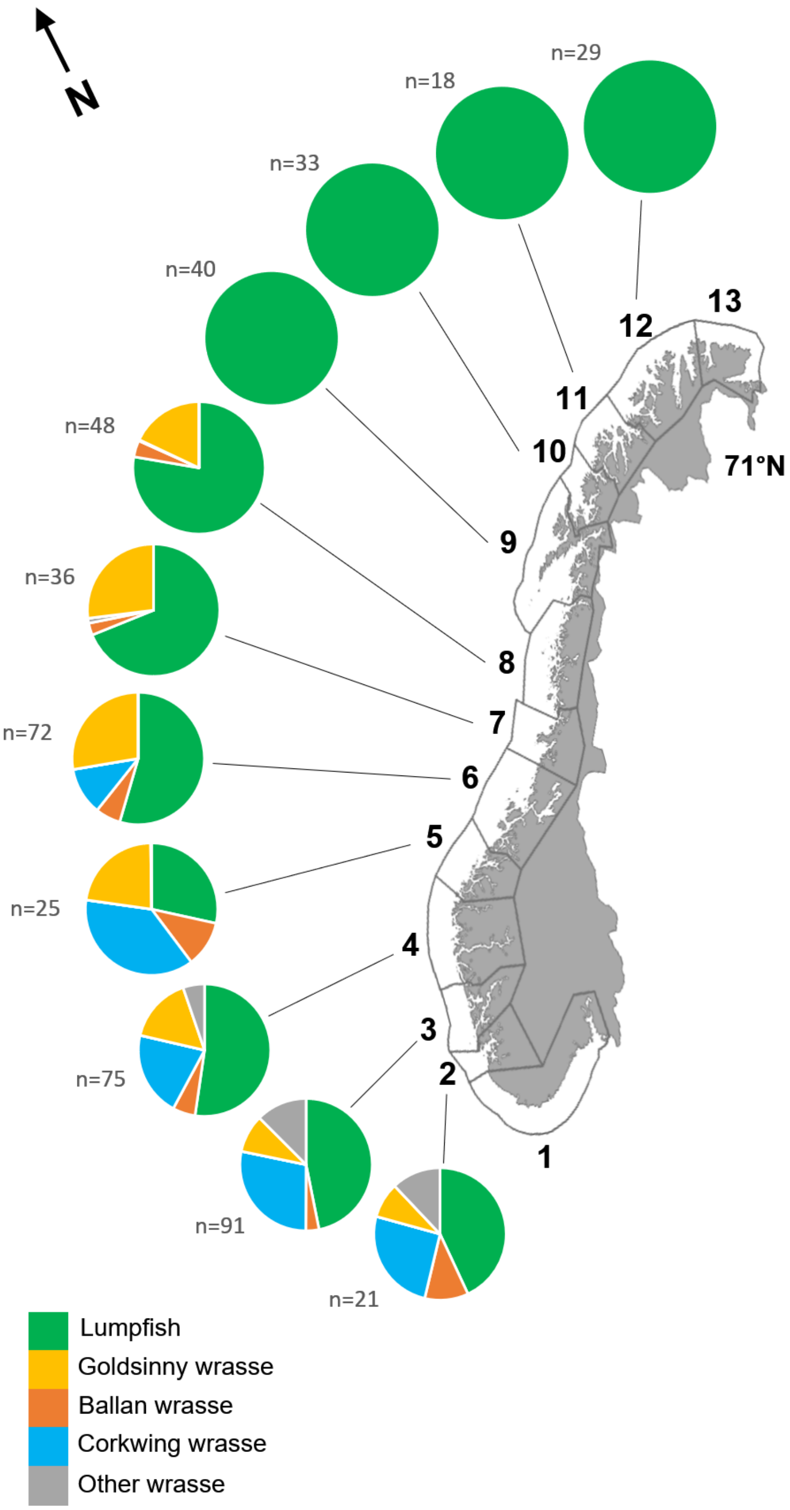

Figure 1. Use of cleaner fish taxa across Norwegian salmon aquaculture production zones 2-12 for the sites that completed a grow-out cycle within 2016-2018 (n = 488). Production zones are defined by government legislation (Norwegian Ministry of Trade Industry and Fisheries 2016). 'n' indicates the number of sites assessed in each zone. Cuckoo wrasse are recorded but not shown (<1% of cleaner fish used).

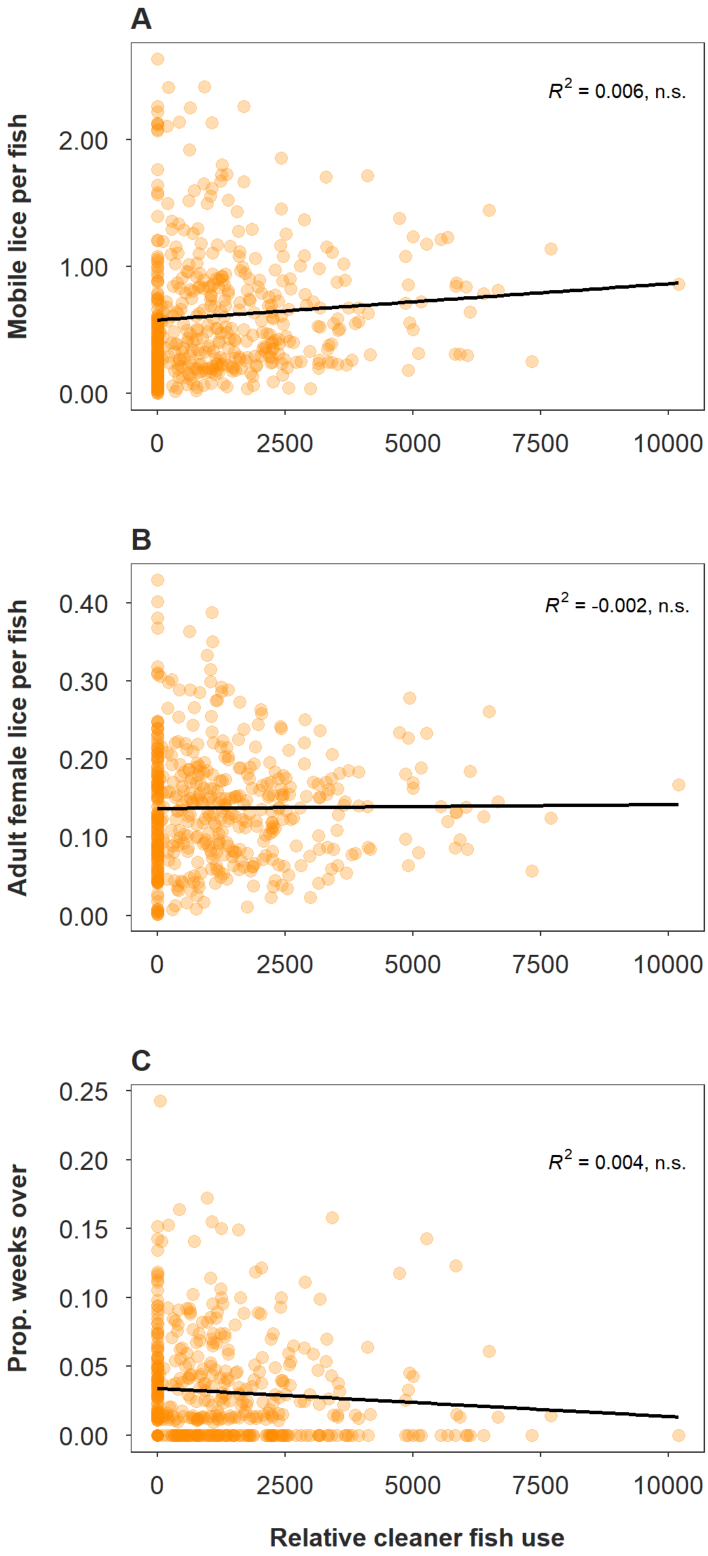

Figure 2. No correlation between the number of cleaner fish stocked per week and site-level (n = 493) lice abundance. Data are aggregated over the full duration of the most recent grow-out cycle. A: mobile lice per fish; B: adult female lice per fish; C: proportion of grow-out weeks exceeding legislated maximum permissible adult female lice density.

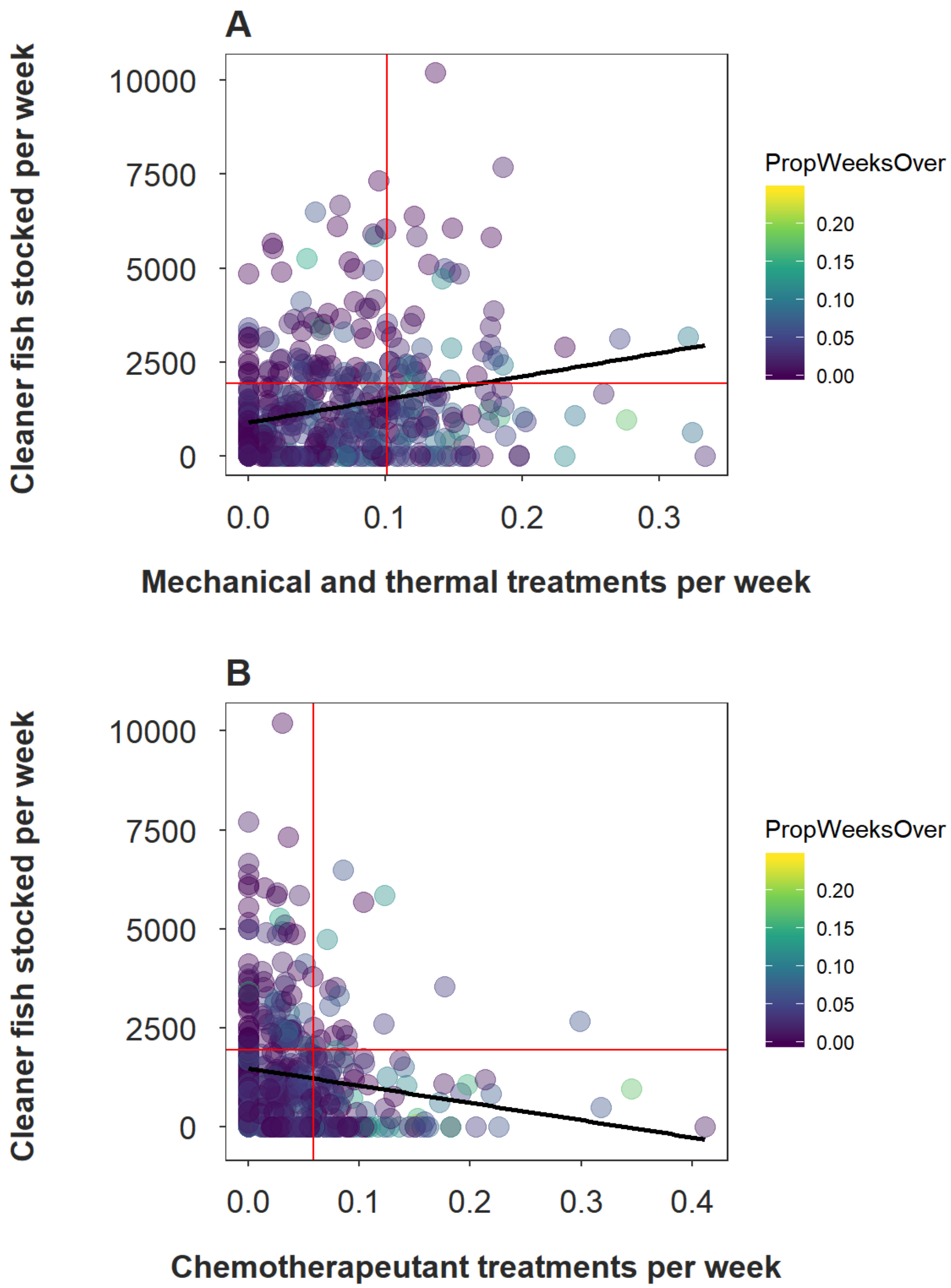

Figure 3. A: no correlation between number of CF stocked per week and the frequency of mechanical or thermal delousing treatments for a given site. B: weak negative correlation between number of CF stocked per week and the frequency of chemotherapeutant use reports for a given site. Red lines delineate the upper quartiles for CF use (horizontal) and delousing frequency (vertical). Few sites were in the upper quartile for both CF use and frequency of chemotherapeutant use.

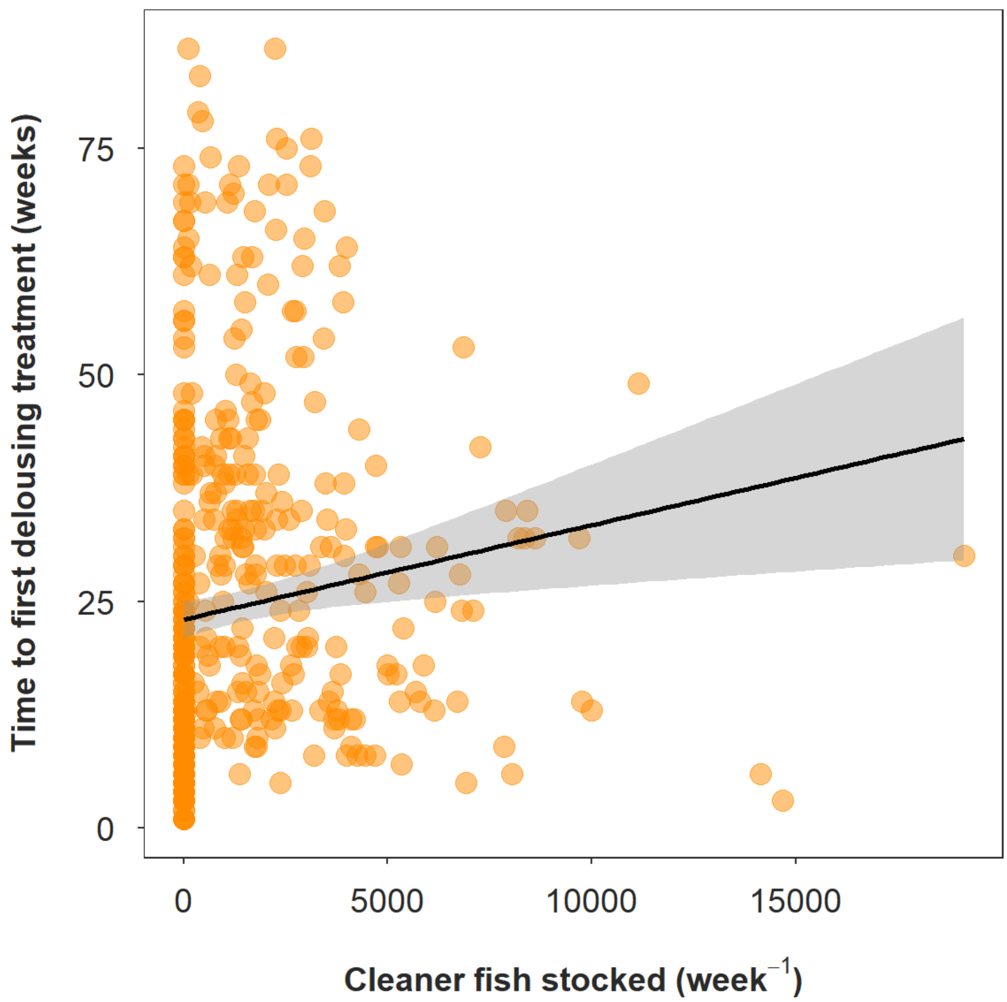

Figure 4. Weak positive correlation between the number of CF stocked per week (up to the first delousing treatment) and the duration between the start of the grow-out cycle and the first delousing treatment (n = 488 sites).

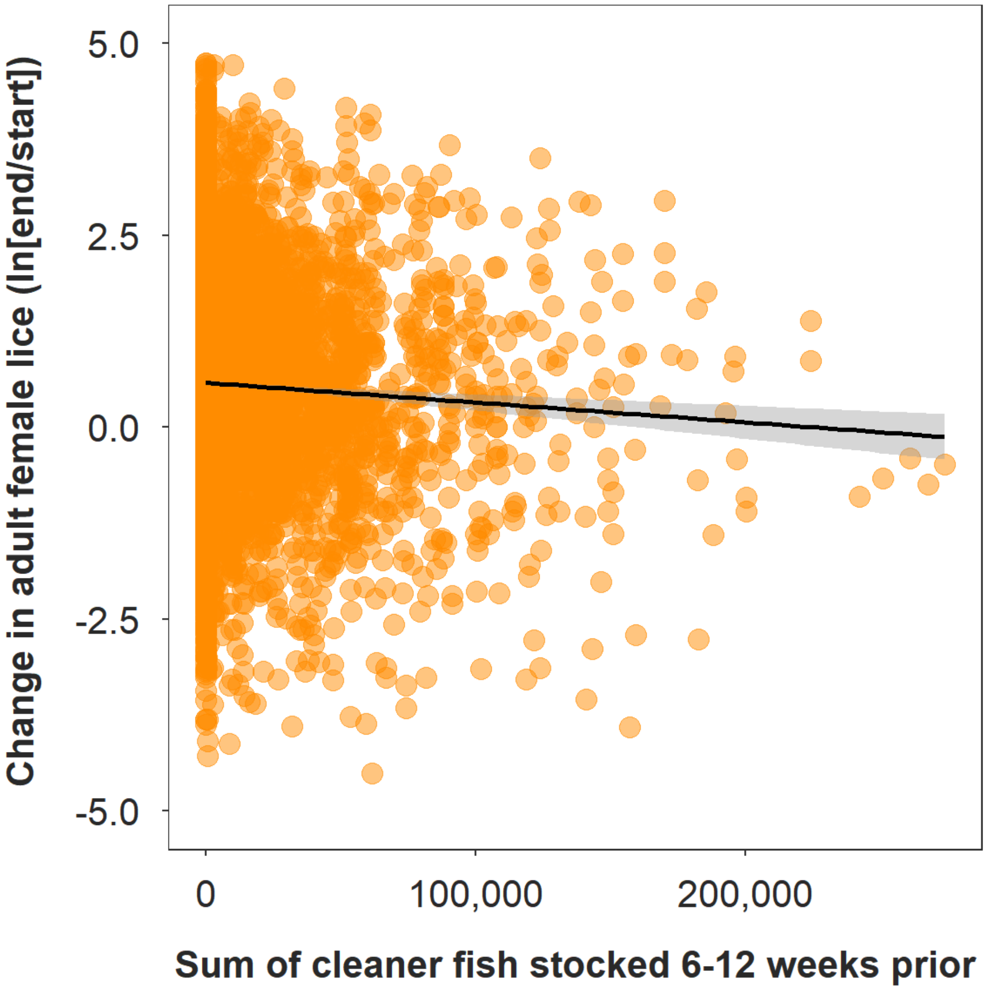

Figure 5. Weak negative correlation of cleaner fish stocking events on lice population growth during subsequent weeks. Adult female lice densities increase more slowly over a 12-week moving window when more cleaner fish are stocked in weeks 1-6 of that window (n = 8963 sliding windows across 456 sites).

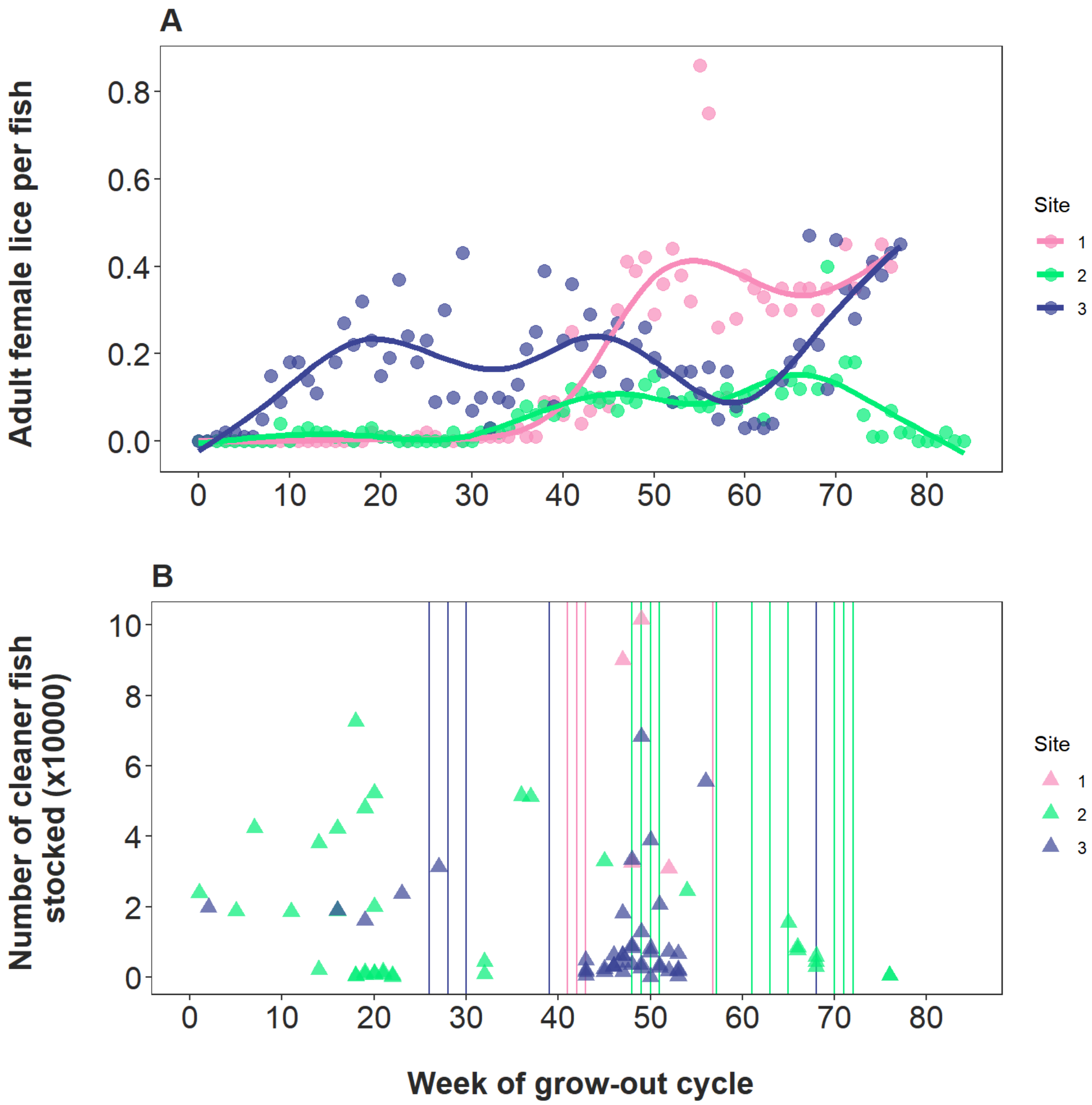

Figure 6. Temporal patterns in lice infestation and control over a grow-out cycle at 3 illustrative sites (anonymised as Sites 1, 2 and 3). These sites were selected due to the use of large numbers of cleaner fish. Panel A shows adult female lice density over time, fitted with the generalised additive model function: *lice density ~ s(time)*. Panel B shows cleaner fish stocking events (triangles) over the same time period. Vertical lines indicate timing of delousing treatments (mechanical, thermal and chemotherapeutant treatments are not differentiated). Sites 1 and 3 started their grow-out cycles in autumn 2016; Site 2 in spring 2017.