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Sea lice prevention strategies affect cleaner fish delousing efficacy in commercial Atlantic salmon sea cages

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ABSTRACT: Over the last 2 decades, cleaner fishes have been employed to remove external sea lice parasites from Atlantic salmon *Salmo salar* in sea cages. Norway, Scotland, Ireland, and the Faroe Islands combined now use ~60 million cleaner fish per year. While small-scale experiments demonstrate the efficacy of cleaner fishes, industrial-scale sea cages have multiple structures and conditions that create different environments, which may impact cleaner fish efficacy and welfare. Here, in commercial sea cages, we investigated if 4 different anti-lice strategies impacted the delousing efficacy, physical condition, and behaviour of cleaner fish (corkwing wrasse *Symphodus melops*). The strategies tested were: (1) cleaner fishes only; (2) cleaner fishes and functional feed; (3) cleaner fishes, functional feed, and deep lights and feeding; and (4) cleaner fishes, functional feed, deep lights and feeding, and lice skirts. Corkwing wrasse were sampled from 3 cage-level replicates of each anti-lice strategy 3 times over 2 mo. Lice levels on salmon were recorded every 3 to 4 wk. Only 11% of corkwing wrasse had salmon lice in their gut, with individual wrasse having up to 72 lice in their stomach. Wrasse in cages encircled by lice skirts consumed one-ninth as many lice as those in other anti-lice treatments and had less overall impact on the number of lice per salmon. Fin, skin, mouth and eye condition, K factor, and observed cleaning behaviours of corkwing wrasse were similar across all anti-lice strategies. Our results demonstrate that different in-cage anti-lice strategies altered the magnitude of lice consumption in corkwing wrasse at this site and for this production period. Moreover, while a small proportion of corkwing wrasse appear to target lice as prey, most individual corkwing wrasse were ineffective biological control agents in a full-scale farm setting.

KEY WORDS: Biological control · Fish welfare · *Lepeophtheirus salmonis* · Lice skirts · *Salmo salar* · *Symphodus melops*

1. INTRODUCTION

The hunt is on for ethical, effective, and cost-efficient solutions that will protect the world's most farmed marine fish, Atlantic salmon *Salmo salar*, from salmon louse *Lepeophtheirus salmonis* infestations. Salmon lice are ectoparasitic copepods that feed on salmonid tissues, causing lesions which can lead to immunosuppression, osmoregulatory failure, and even death (Costello 2006, Fast 2014). They proliferate in the dense host populations generated by aquaculture and may spillback to wild fishes with population-level consequences (Krkošek et al. 2013). Treatment with chemotherapeutants dominated salmon lice control for decades until the resistance to most chemotherapeutants emerged (Aaen et al. 2015), and public concern regarding the effects of chemotherapeutants on non-target organisms led to

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political reform favouring 'green' salmon production (Hersoug 2015). At present, treatments that physically remove lice by heat shock or mechanical abrasion are most common but can be detrimental to salmon welfare (Overton et al. 2019).

Biological control via the use of cleaner fish is an alternative, widely used method for lice control in sea cages (Treasurer 2018). Cleaner fishes (many species of wrasse, e.g. *Centrolabrus exoletus*, *Ctenolabrus rupestris, Labrus bergylta, Symphodus melops*, and *Tautogolabrus adspersus*, and lumpsuckers *Cyclopterus lumpus*) eat salmon lice directly off the skin of salmon. Wrasses and lumpsuckers were discovered as biological control agents in the 1980s (Bjordal 1988), and in tank trials, wrasse can maintain lice numbers at <0.5 lice per salmon when stocked at 5–10% of salmon numbers (Leclercq et al. 2014). Compared to chemical treatments, cleaner fish are regarded by some as an economically and ecologically sound solution to the salmon lice problem (Liu & Bjelland 2014). Over 54 million cleaner fishes were stocked in 65% of Norway's salmon farms in 2017, a 30-fold increase since 2008 (Norwegian Directorate of Fisheries 2018), while ~6.5 million are stocked each year in Scotland and Ireland (Munro & Wallace 2017, 2018, Bolton-Warberg 2018).

Compared to tank trials, the evidence basis for the delousing effect of cleaner fishes in commercial-scale sea cages is limited (Overton et al. 2020), with few full-scale studies conducted with suitable replication and a non-confounded experimental design (Table 1). One replicated study found lumpsuckers successfully controlled lice levels on a salmon farm from late autumn to early spring (Imsland et al. 2018), but research over the high lice incidence summer months and across cleaner fish species is lacking. Gut analyses reveal that 14–36% of lumpsuckers and 16% of goldsinny wrasse *Ctenolabrus rupestris* eat lice in sea cages (Deady et al. 1995, Imsland et al. 2014, Eliasen et al. 2018). Variation in delousing efficacy in sea cages may be due to genetics (Imsland et al. 2016), learning and experience (Imsland et al. 2015), or environmental factors such as temperature (Sayer & Reader 1996) and light (Loew et al. 2016). Furthermore, the additional complexities and large scale of commercial salmon farms may contribute to deteriorating condition (Skiftesvik et al. 2013), low winter survival (Darwall et al. 1992, Sayer & Reader 1996), increased disease risk (Gulla & Bornø 2018), and exceedingly high (48–100%) mortality of cleaner fishes held in commercial net pens (Nilsen et al. 2014, Olsen 2017). At present, it is unclear how an array of relatively new sea lice prevention strategies, which further alter commercial sea cage environments, interact with cleaner fish condition and delousing efficacy (Overton et al. 2020).

Prevention technologies reduce opportunities for louse attachment to salmon. The infectious copepodid stage of salmon lice is positively phototactic and most abundant in the upper surface layer, so a number of prevention strategies encourage deep swimming in salmon (Oppedal et al. 2017). Submerged lights and submerged feeding can draw salmon deeper at night, but not consistently during sunlight hours (Frenzl et al. 2014). Additionally, salmon need to surface to fill their swim bladder (Dempster et al. 2009, Korsøen et al. 2009), so they are still susceptible to lice in surface waters. Alternatively, lice skirts — fine mesh nets that encircle sea cages — act as a barrier to salmon lice. Lice skirts are widely used, with >900 lice skirts sold by a single company in 2017, with 2 to 3 skirts used depending upon cage size (Nodland 2017). With good conditions, they can reduce lice loads by 30% (with a 5 m skirt; Grøntvedt et al. 2018) to 80% (10 m skirt; Stien et al. 2018). However, lice skirts can reduce dissolved oxygen content in cages (Stien et al. 2012, 2018), which can lead to poor welfare, reduced growth, and altered behaviour of salmon (Oppedal et al. 2011, Oldham et al. 2017, Solstorm et al. 2018). The effects of lice skirts on cleaner fish is unknown. Functional feed is a passive method for prevention of infestation that can lower lice loads on salmon by 20% compared to standard diets (Jensen et al. 2015). Functional feeds may work by including ingredients that thicken the mucosal cell layer of salmon epidermis, promote healing of damaged tissue, and repel copepods. It is considered a positive potential solution that promotes welfare and incurs little effort by the farmer. Other lice-prevention strategies include snorkels, which limit salmon surface access within a narrow tarpaulin tube (Stien et al. 2016), situating new farms in waters with low connectivity (Samsing et al. 2017), fallowing (Werkman et al. 2011), and floating closed containment cages (Nilsen et al. 2017).

Cage-based prevention strategies cause considerable changes to the sea cage environment, to which both salmon and cleaner fishes will respond. For instance, the use of submerged lights and feeding zones can attract salmon, leading to deeper swimming (Frenzl et al. 2014). Similarly, light is likely an important environmental gradient determining cleaner fish distribution, as they use vision to forage and generally occupy shallow habitats (wild corkwing wrasse *Symphodus melops*, <5 m; Quignard & Pras 1986). In sea cages, submerged lights may draw

Table 1. Design and results of past commercial-scale studies investigating cleaner fish delousing efficacy. CF: cleaner fishes; goldsinny *Ctenolabrus rupestris*; corkwing *Symphodus melops*; rock cook *Centrolabrus exoletus*; ballan *Labrus bergylta*; lumpsuckers *Cyclopterus lumpus*

Experimental design	Number and species of cleaner fish	Number of salmon	Period of trial	Results	Source
Summary of 13 farm trials. Trials had various designs, the highest level of replication was 13 cages without CF, 2 cages with goldsinny, and 1 cage with corkwing	Various wrasses	Various, up to 25 000 per cage	1988–1991	- 6/13 trials interrupted by disease, escapees, or mortality of CF - Number of chemical treatments required could be more, less, or the same with CF	Darwall et al. (1991)
1 cage with CF. 26 without CF but with unidentified chemical treatments which confounds comparison with CF cages	150 wild goldsinny	15 000 smolts in treatment cage, up to 47 000 1 yr olds in control cages	May–Oct 1991	- One sample date had lower lice numbers in CF cages. The same lice levels as cages without CF for rest of experiment - High mortality of CF	Deady et al. (1995)
2 'groups' of N = 8 cages. 4 cages with goldsinny wrasse. 1 cage with corkwing wrasse. 2 cages with mix of goldsinny and corkwing wrasses. 8 cages without CF but with unidentified chemical treatments which confounds comparison with CF cages	Total 871 goldsinny, 343 corkwing (1 CF:100 salmon)	5000 to 8000 smolts per cage	May–Sep 1992	- CF and non-CF cages experienced similar lice numbers (<5 mobile stages per salmon), except for 1 non-CF cage - High losses (either from escape or mortality) of CF	Deady et al. (1995)
4 cages with CF (then 6, after 1 mo); 1 (then 3, after 4 mo) without CF	Goldsinny and rock cook (1 CF:90 salmon)	13 000 to 24 000 smolts in treatment cage, 20 000 in control cages	Jun 1992–Jul 1993	- Chemical treatments still required, CF did not reduce lice loads	Tully et al. (1996)
Unknown number of cages. Compared a farm with CF to a farm without CF, 5 km distant	Wild-caught mix, mostly ballan wrasse 1 CF: 25 salmon	25 000 per cage	Sep 2010 to May 2011	- Fewer treatments at site with CF - Lice numbers at site with CF lower in September/October, no statistical analyses - Lice number analogous between sites from November	Treasurer (2013)
N = 3 cages 1 with CF 2 without CF	650 farmed ballan wrasse, 300 wild-caught mix	54 000 per cage	10 wk, 2012	- One sample taken after 10 wk. No lice on salmon in cage with CF, 0.23–0.3 on salmon in cages without CF	Treasurer (2013)
N = 8 cages 6 cages stocked at 4, 6, and 8% density of CF 2 cages without CF	Lumpsuckers	193 304 per cage	Oct 2015 to May 2016	- Pre-adult lice were more common on salmon in cages without CF for from February to March ($p < 0.05$) - 6 and 8% densities of lumpfish kept adult female lice levels lower than control from January to April ($p < 0.05$)	Imsland et al. (2018)

cleaner fishes deeper and extend lice-hunting hours. However, substantial improvements to delousing may be limited by lack of shelter, given that hides are often not placed near lights and do not extend the full depth of the sea cage. Lice skirts can reduce surface dissolved oxygen saturation (Stien et al. 2012), and salmon can swim deep to avoid hypoxic water (Oldham et al. 2017). Cleaner fishes may respond similarly, but lice skirts also reduce surface flow (Frank et al. 2015), and cleaner fish may prefer shallow, low-flow water that mimics their wild habitats (Skiftesvik et al. 2015, Yuen et al. 2019), leading to salmon swimming at depth and cleaner fishes near the surface, thus reducing encounter rates. Furthermore, if functional feed successfully lowers the lice load on salmon, cleaner fishes will have fewer opportunities to consume lice. Each prevention strategy could reduce the cleaner–host encounter rate and limit opportunities to interact with and delouse salmon.

Prevention strategies may also impact cleaner fish welfare. In standard sea cages, cleaner fish condition worsens with time (Skiftesvik et al. 2013). With lice skirts, this may be exacerbated if low oxygen levels decrease fish metabolism and impair foraging behaviour. To counter poor cleaner fish welfare, the industry has assembled best practice procedures (FHF 2016). The procedures stipulate that farmers must feed cleaner fishes regularly, a practice now widely implemented (e.g. all Mowi farms; Henrik Trengereid pers. comm). If anti-lice strategies are effective, and cleaner fishes rely on lice as the principal component of their diet, then condition may decrease. Moreover, current best practice procedures do not account for the impact of new sea cage technologies.

Here, in a replicated commercial-scale trial, we compared the delousing efficacy, physical condition, and behaviour of corkwing wrasse *S. melops*, a widely used wild-caught wrasse in Norway, under different lice prevention strategies. This is important because innovative prevention technologies change the sea cage environment, and farmers must demonstrate that cleaner fishes remain effective in removing lice and that their welfare is secured in new cage arrangements. By understanding how cleaner fishes deal with the complexities of a full-size salmon farm, the industry can improve cleaner fish survival and welfare and lower lice infestation rates. Achieving this will subsequently reduce the economic, welfare, and environmental costs of alternative sea lice control measures.

2. MATERIALS AND METHODS

2.1. Location, experimental design, and environmental conditions

The experiment was conducted at the Centre for Aquaculture Competence's full-scale research and development facility at Vindsvik, in Jøsenfjorden, western Norway (~59° N) from 25 August (Day 1) to 11 October (Day 49) 2017. A specific animal ethics approval was not required under Norwegian law, as normal production procedures were followed. As cleaner fish are part of standard production (used in 65% of cages in Norway; Norwegian Directorate of Fisheries 2018) and were present in all cages at this site, their use was not regarded as an experimental treatment. Sampling of fish with acceptable humane endpoints to determine their welfare status or sample organs is within animal welfare regulations as this forms part of normal farming operations. The site was operational since September 2016, with all anti-lice strategies (see below) in place by January 2017, with continuous monitoring of the salmon for a 13 mo period (Bui et al. 2020). This full-scale farm has 12 circular sea cages that are 120 m in circumference and 35 m deep, arranged in 2 rows parallel to the coastline (Fig. 1a,b). Each sea cage contained ~65 000

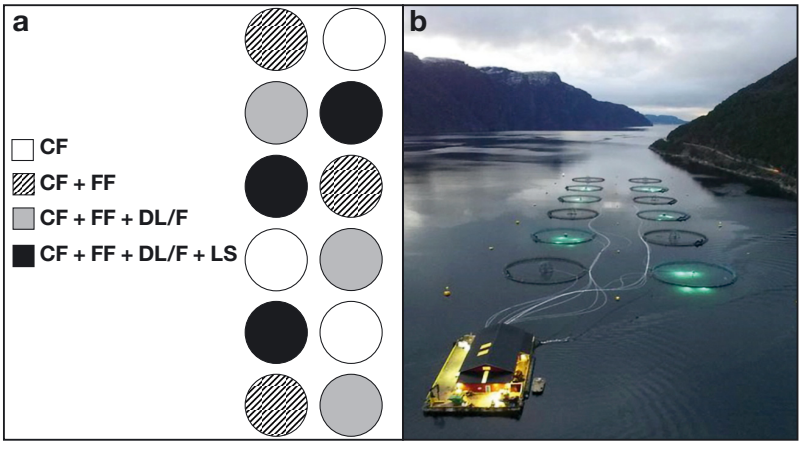

Fig. 1. (a) Diagrammatic representation of the Vindsvik study site and experimental design. Each circle represents a 120 m circumference sea cage, with shade representing anti-lice strategy. Strategies were allocated to sea cages in a randomised block design. CF: cleaner fish, FF: functional feed, DL/F: deep light and deep feeding, LS: lice skirts. (b) Aerial view of the study site at dusk with underwater light treatments clearly visible in cages

Atlantic salmon (mean ± SE, 1.9 ± 0.08 kg) and ~5000 cleaner fishes. New wild-caught cleaner fishes were added to cages when mortality estimates were high, as determined by the site manager (see Table S1 in the Supplement at www.int-res.com/articles/suppl/q012p067_supp.pdf). At the time of this study, corkwing wrasse (~84% of all cleaner fishes caught in pots) were the most abundant species, but lumpsuckers *Cyclopterus lumpus* (<1%), rock cook *Centrolabrus exoletus* (<1%), goldsinny (~4%), and both wild and farmed ballan wrasse *Labrus bergylta* (~12%) were also present. Three hides for cleaner fish shelter (~1 m diameter) made of black plastic strips and extending from the surface to 6 m depth hung in each cage.

One of 4 anti-lice strategies was applied to each of 3 replicate sea cages (N = 12 sea cages): (1) cages containing cleaner fishes and salmon that were fed a standard salmon feed (Skretting Optiline, 9 mm), henceforth referred to as cleaner fish only; (2) cages containing cleaner fishes and salmon that were fed a functional feed (Skretting's Shield, 9 mm); (3) cages containing cleaner fishes and salmon that were fed functional feed, plus deep lights (Aurora SubLED Combi, 120 W UV LED light, violet colour, at 5-7 m depth; AKVA Group) and deep feeding (AKVA SubFeeder; AKVA Group); and (4) cages containing cleaner fishes and salmon that were fed functional feed, plus deep lights/feeding, plus a skirt to prevent sea lice (mesh size 350 µm, 6 m deep, Norwegian Weather Protection Aquaculture). Treatments were allocated to sea cages using a randomised block design (Fig. 1a,b).

Cleaner fishes and salmon in all cages were fed using standard husbandry procedures during the experiment, including supplementary feed for cleaner fish. Environmental profiles of the water column were collected from a reference point on the farm using a conductivity, temperature and depth (CTD) sensor (Model SD204, SAIV AS, Norway). The CTD probe was set to measure every minute, moving between 0 and 40 m in depth.

2.2. Swimming depths of Atlantic salmon and cleaner fish

The swimming depth distributions of salmon in each cage were continuously recorded using a PC-based echo integration system (CageEye). The transducers were positioned below the centre of each cage at 35 m depth, facing upwards with a 15° acoustic beam. Full details of the system are given by Bjordal et al. (1993). Echo intensity, which is directly proportional to fish density, was recorded at 0.5 m depth intervals from 0 to 35 m and converted to relative echo intensity in each interval. A mean value of the echo observations per minute (60 pings min^{-1}) was recorded and used to calculate a relative density on a scale from 0 to 1. All data were condensed to cage averages per 1 m depth interval, to create a mean salmon school depth, prior to analysis.

As the echosounder system did not provide data on wrasse swimming depths, we directly observed wrasse with an ROV prior to the first sampling period during daytime to set the depth for wrasse pots.

2.3. Delousing efficacy of cleaner fish

Delousing efficacy was measured in 2 ways: the number of lice per corkwing wrasse gut, and a calculated 'cleaner fish effect' (CFE). To determine the number of lice consumed by wrasse, 8 to 10 corkwing wrasse from each sea cage were caught using prawn-baited wrasse pots deployed from 09:00 to 12:30 h at 7 m depth, then euthanised with an overdose of tricaine methanesulfonate (Finquel®, Scan Aqua AS; 5 g 10 l^{-1}). Each wrasse was dissected and their gut contents visually inspected. The number of salmon lice were counted, scales were recorded as present or absent, and the other content was identified as either crustaceans, feed, algae, completely digested/unidentifiable, or empty.

Cleaner wrasse primarily consume the mobile stages of lice (preadult and adult) as they are large enough to detect (Skiftesvik et al. 2013). Thus, to measure CFE, we compared the expected mean number of mobile lice $salmon^{-1}$ (µ(expected mobile)) to the actual mean number of mobile lice $salmon^{-1}$ (µ(actual mobile)). Assuming no chemical delousing and equal attrition across cages, the µ(expected mobile) for sample *t* should equal the µ(actual mobile) at sample time *t*, unless cleaner fishes are removing mobile lice. Thus, a high CFE occurs when cleaner fishes consume many lice, whereby there is a low µ(actual mobile) compared to high µ(expected mobile).

To calculate $\mu(\text{expected mobile})_t$, 20 salmon were collected from every cage using a 3 m ring net pulled up from ~5 m near the centre of the cage 3 wk before the experiment (2 August) and close to Sample 1 and Sample 2 (28 August and 13 September). Salmon were not sampled at Sample 3 (6 October) due to lice treatments. Each sample spanned over 3 consecutive days (see Table S1). Salmon were transferred using a dip net to a bath containing an overdose of anaesthesia. After euthanasia, individuals were assessed

for their infestation status whereby lice were counted and staged as chalimus I, chalimus II, preadult I, preadult male, preadult female, adult male, adult female, adult female with eggstrings, or *Caligus elongatus*. Then, the mean number of lice per salmon across replicate cages was determined for each lice management strategy. At 12.5°C (the average temperature during the experimental period), it takes 3 wk for attached lice (i.e. copepodids, chalimus 1, and chalimus 2) to become mobile lice (i.e. pre-adult 1 stage and later) (Samsing et al. 2016; Hamre et al. 2019), when they can be consumed by cleaner fishes (Brooker et al. 2018). Thus, to calculate $\mu(\text{expected mobile})_t$, we used the following formula:

$$\mu(\text{expected mobile})_t = \mu(\text{attached})_{t-3\text{weeks}} + \mu(\text{actual mobile})_{t-3\text{weeks}} \quad (1)$$

where $\mu(\text{expected mobile})_t$ is the expected mean number of mobile lice per salmon for that lice management strategy at sample time *t*, assuming no mortality or emigration; $\mu(\text{attached})_{t-3\text{weeks}}$ is the mean number of attached lice salmon^{-1} for that management strategy, 3 wk prior to sample time *t*; and $\mu(\text{actual mobile})_{t-3\text{weeks}}$ is the mean number of mobile lice per salmon for that management strategy, 3 wk prior to sample time *t*.

To calculate the CFE for each lice management strategy and sample point, we used the following formula:

$$\text{CFE} = \mu(\text{expected mobile})_t - \mu(\text{actual mobile})_t \quad (2)$$

where $\mu(\text{expected mobile})_t$ is the expected mean number of lice per salmon for that lice management strategy at sample time *t*, assuming no mortality or emigration; $\mu(\text{actual mobile})_t$ is the actual mean number of mobile lice per salmon for that management strategy at sample time *t*, which was recorded from the physical inspection of experimental fish.

2.4. Physical condition of cleaner fish

Length (*L*, ±0.05 cm) from snout to caudal fin and weight (*W*, ±0.05 g) were measured, from which Fulton's condition factor ($K = W / [L]^3 \times 100$) was calculated to estimate corkwing wrasse condition. Eye, fin, skin, snout, opercula and gill damage were recorded as present or absent, as were deformities.

2.5. Behaviour of cleaner fish

In each sea cage at all 3 sampling times, behaviour was filmed for 15 min on 2 underwater cameras (GoPro Hero 5) at 7 m depth. A remotely operated underwater vehicle (Deep Trekker DTG2) checked the cameras were not tangled and that one was facing a hide. Video recordings were analysed afterwards to determine the prevalence of the following behaviours: swimming with salmon, inspecting salmon, feeding on lice, alternative feeding, salmon avoidance, inter/intra-specific aggression (see Table S2). Behaviours were counted as present or absent in each 15 min film replicate.

2.6. Statistical analyses

To determine if corkwing wrasse lice consumption differed between prevention strategies, a zero-inflated generalised linear mixed model (function 'glmmTMB' in package 'glmmTMB', RStudio v. 3.4.2; R Core Team 2018) analysed lice count per corkwing gut data, where group was fixed and cage and sample time were random effects. As there were more fish with several or no lice in their guts, and only a few with 10s of lice gut^{-1}, a negative binomial family was specified.

CFE fitted a normal distribution so was analysed using a standard linear model (function 'lm'), with both group and sample as fixed effects. Sample was treated as a fixed effect because there were not enough levels to treat it as a random effect.

To test if corkwing wrasse condition differed between different anti-lice strategies, *K*-scores fitted a normal distribution and therefore were analysed using a linear mixed-effects model estimated by maximum likelihood, with group as a fixed effect and cage and sample time as random effects (function 'lmer' in 'lme4').

To test if physical damage differed between prevention strategies, fin, skin, eye, snout, gill, and opercula damage were fitted to a generalised linear mixed model with a binomial distribution, where group was a fixed effect and sample time and cage were random effects (function 'glmmTMB' in package 'glmmTMB', R). Stomach contents and behaviour data were analysed using the same model and distribution.

Mean school depth was calculated for each replicate cage from 30 August to 23 September. A 1-way ANOVA (function aov, R) was used to test if salmon schooled at different depths when subjected to different lice-prevention strategies. Post-hoc analysis was conducted using Tukey's test (function TukeyHSD, R) where $p < 0.05$ was considered statistically significant.

Homogeneity of variance and normality of errors were confirmed with visual inspection of residual plots for both *K*-score and CFE (function 'plot', R). All models were compared to corresponding null models, excluding group as a factor in likelihood ratio tests (function 'anova', R) to attain p-values. Where $p < 0.05$, Tukey's test (function 'lsmeans' in package 'lsmeans', R; Lenth 2016) generated adjusted p-values using least squares means to identify significant differences between groups.

3. RESULTS

3.1. Environmental conditions

Between 25 August (Day 1) and 11 October 2017 (Day 49), salinity (Fig. 2a) from 5 to 35 m depth ranged between 26 and 34 PSU. From 0 to 5 m, the first and last 3 wk were marked by periods of brackish water, reaching a minimum of 12 PSU. For most of this study period, there was a thermocline of warm water (>14°C) between 0 and 15 m and cooler water below 15 m (Fig. 2b). In the fifth week, the water temperature was uniformly warm (14–15°C) throughout the water column. During the last 2 wk of the study period, the mean temperature dropped to 12°C, with coldest temperatures occurring below 25 m and at the surface. Dissolved oxygen concentration generally remained above 85% in the first 5 m of water throughout the trial, while deeper waters experienced levels ~70% saturation (Fig. 2c). The third week was characterised by slightly lower dissolved oxygen levels (~60%) from 10 to 30 m. This was followed by 10 d of high oxygen saturation (>75%) across all depths, before levels once again stratified with high dissolved oxygen at the surface and ~70% at depth.

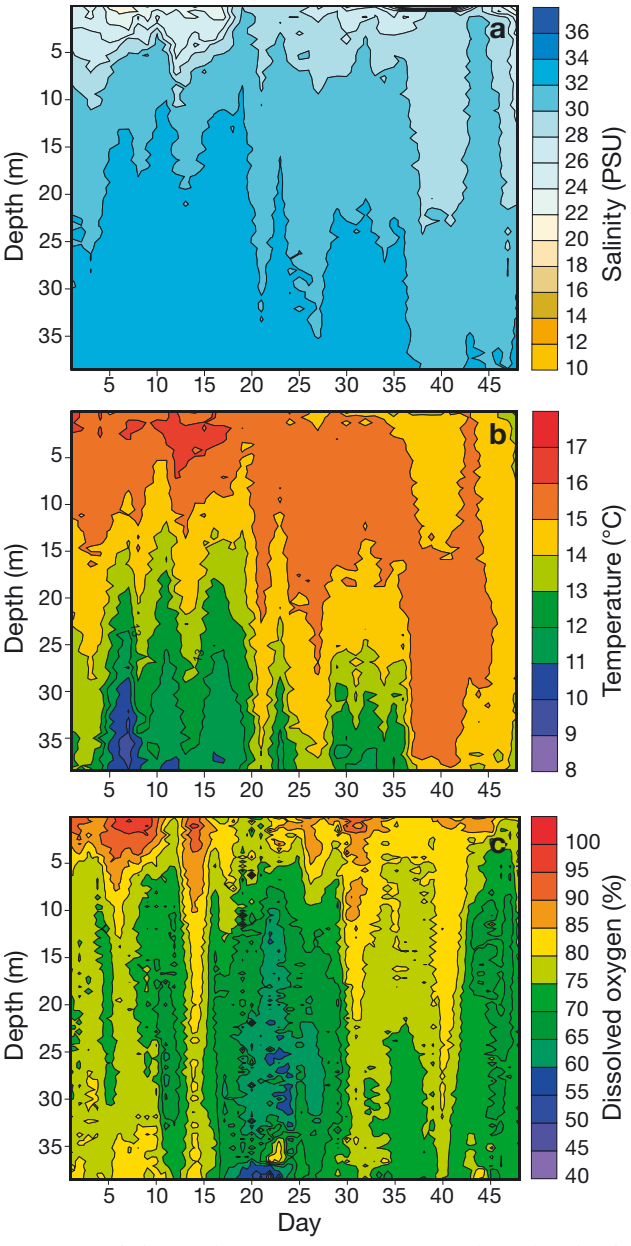

Fig. 2. (a) Salinity, (b) water temperature and (c) dissolved oxygen content (% saturation) from 0 to 35 m depth at Vindsvik CAC from 25 August (Day 1) to 11 October 2017 (Day 49)

3.2. Swimming depths of Atlantic salmon

An overall difference in the mean swimming depth of salmon between anti-lice strategies was observed throughout the experimental period (df = 3, $F = 6.4$, $p = 0.02$). On average, salmon swam far deeper in cages surrounded by lice skirts (mean ± SE: 11.1 ± 1.9 m) compared to CF (4.1 ± 0.04 m) and CF + FF (4.2 ± 0.5 m) cages (Fig. 3). Salmon subjected to deep lights but not lice skirts also swam shallower (6.6 ± 0.8 m) than those in skirt-encircled cages, but the post-hoc Tukey's test revealed there was no significant difference between these 2 cage types ($p = 0.1$).

3.3. Cleaner fish stocking levels and salmon lice levels among anti-lice strategies

At the start of Sample 1, an average of 6843 – 7920 cleaner fish were stocked onto the 4 anti-lice strategies (see Table S1 in the Supplement). Cleaner fish continued to be stocked throughout the experiment, so that numbers increased to 6843 – 9564 at Sample 3. Cleaner fish mortalities were recorded in each cage as the number of dead cleaner fish retrieved from the

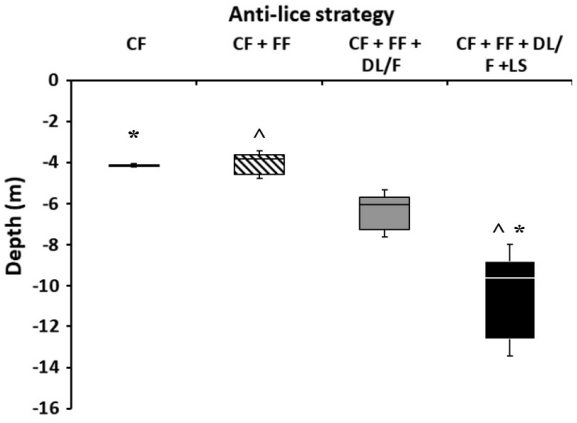

Fig. 3. Boxplots of mean salmon schooling depth for each anti-lice strategy, showing the third quartile (Q3) and first quartile (Q1) range of the data at the Vindsvik site from 30 August (Day 6) to 23 September (Day 30) 2017. CF: cleaner fish, FF: functional feed, DL/F: deep light and deep feeding, LS: lice skirts. Different symbols (* and ^) indicate significant differences revealed by Tukey's test

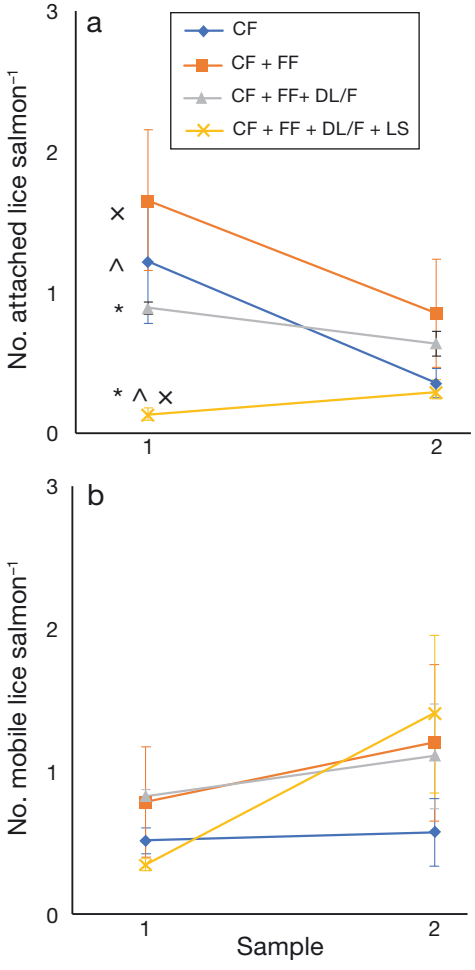

Fig. 4. Mean (± SE) number of (a) attached and (b) mobile lice per salmon in each treatment at Samples 1 and 2. See Fig. 3 for more details

salmon mortality net placed in the bottom of each cage (Fig. S1). At Sample 1, mobile lice per salmon averaged 0.57 to 1.4, increasing to 1.28 to 2.64 in Sample 2 (Fig. 4b). Attached salmon stages ranged from 0.1 to 1.7 in Sample 1 and from 0.3 to 0.9 in Sample 2 (Fig. 4a). Mean attached lice per salmon differed at Sample 1 only, with lice skirt treatments lower than all other treatments. Mean mobile lice per salmon did not differ among treatments at either time. Lice abundances were not estimated in Sample 3 due to lice-control treatments undertaken by the farm staff (Table S1).

3.4. Delousing efficacy of cleaner fish

Corkwing wrasse in lice skirt-encircled sea cages were the poorest delousers compared to other anti-lice strategies. This result was consistent over both measures of delousing efficacy: the number of lice in guts (Fig. 5) and CFE (Fig. 6).

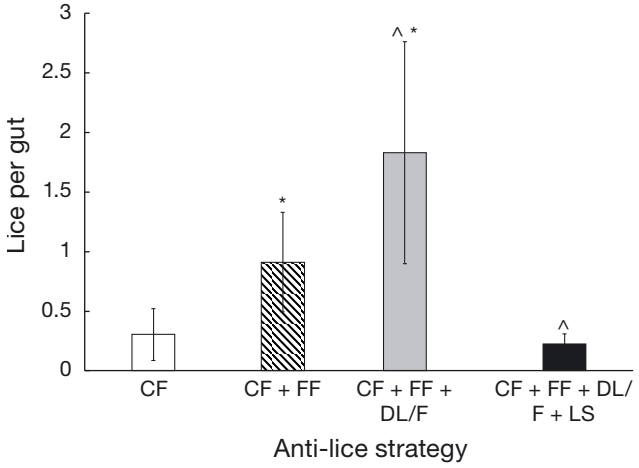

Fig. 5. Number of lice (mean ± SE) per corkwing wrasse gut for each anti-lice strategy. See Fig. 3 for more details

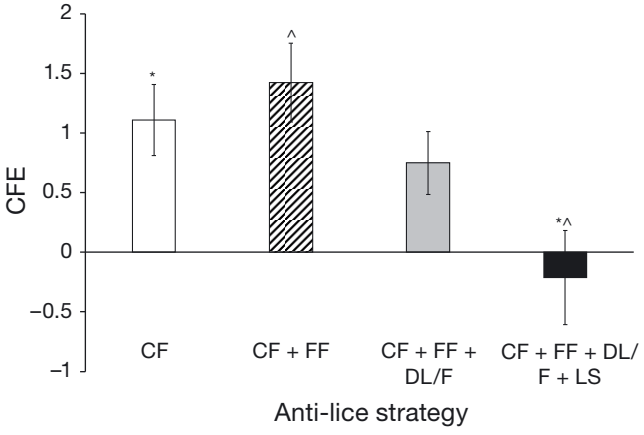

Fig. 6. Cleaner fish effect (mean ± SE), calculated as the mean predicted number of lice minus the mean actual number of lice for each anti-lice strategy. See Fig. 3 for more details

Of all fish sampled across treatment groups, 11% of corkwing wrasse consumed lice. The mean (±SE) number of lice per corkwing gut were 0.3 (± 0.2), 0.9 (±0.4), 1.8 (±0.9), and 0.2 (±0.0) for cleaner fish only, cleaner fish plus functional feed, cleaner fish plus functional feed plus deep lights/feeding, and cleaner fish plus functional feed plus deep lights/feeding plus lice skirt strategies, respectively (Fig. 5). The full model indicated a strong overall effect of anti-lice strategy on the number of lice in corkwing guts. Post-hoc pairwise testing revealed that this overall effect was driven by differences between the lice skirt treatment and both functional feed (z = 2.8, p = 0.001) and functional feed plus deep lights/feeding (z = 5.1, p < 0.0001) treatments.

Using the CFE estimate, the most effective cleaner fishes were in sea cages with cleaner fish plus functional feed (mean ±SE CFE = 1.4 ± 0.33). There was no difference between the cleaner fish only (CFE = 1.1 ± 0.30), cleaner fish plus functional feed plus deep lights/feeding (CFE = 0.8 ± 0.26), and lice skirt (CFE = –0.2 ± 0.40) strategies (Fig. 6). Comparison of full and reduced models indicated an effect of anti-lice strategy (F = 5.1, p = 0.009) and no effect of sampling time (F = 2.6, p = 0.12). Post-hoc pairwise comparison revealed the effect was driven by differences between the lice skirt-encircled cages and the cleaner fish only (p = 0.04) and between the lice skirt-encircled cages and cleaner fish plus functional feed (p = 0.008) treatments.

3.5. Physical condition of cleaner fish

The different anti-lice strategies had the same impact on corkwing wrasse physical condition. Condition (K-score: χ^2 = 5.9, p = 0.1, general model: [weight in g] = 0.014 [length in cm]^3, R^2 = 0.9), fin (χ^2 = 2.2, p = 0.5), skin (χ^2 = 1.6, p = 0.7), eye (χ^2 = 1.10, p = 0.8), and snout (χ^2 = 2.0, p = 0.6) damage were similar across anti-lice strategies. Gill and operculum damage were too rare (19 and 12 incidences respectively) to model reliably. Fin damage was commonly recorded on corkwing wrasse (75% prevalence), followed by skin (28%), eye (13%), operculum (4%), and snout (3%) damage (Fig. 7).

3.6. Gut contents of cleaner fish

The most common food in corkwing guts across cage groups was completely digested, unidentifiable matter (38%), followed by algae (29%), crustaceans

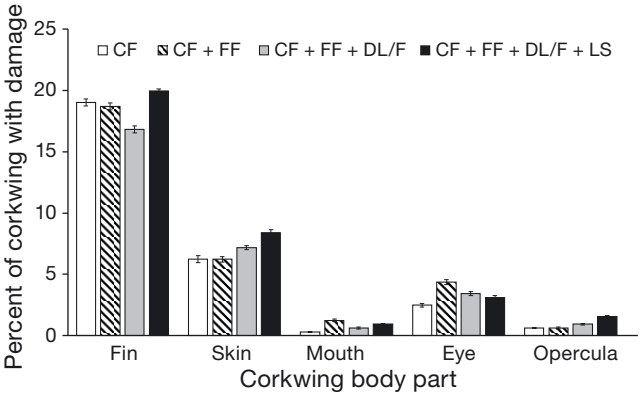

Fig. 7. Percent (mean ± SE) of corkwing wrasse with fin, jaw, skin, eye and opercula damage for each anti-lice strategy. See Fig. 1 for abbreviations

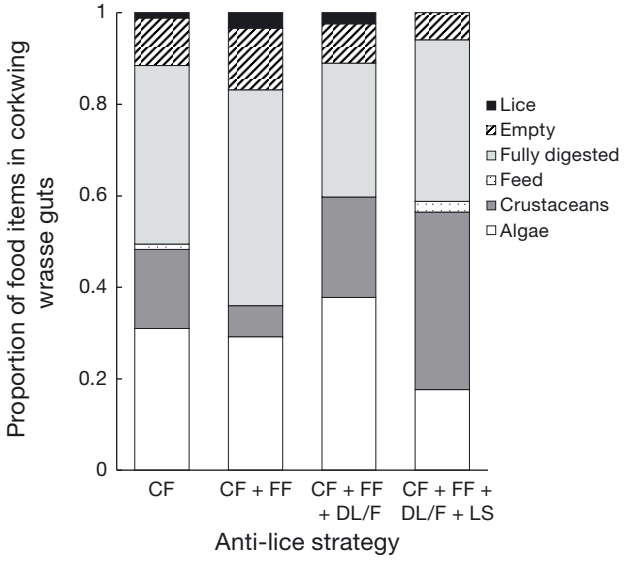

Fig. 8. Proportion of corkwing wrasse with primarily algae, crustacea, feed, lice, fully digested/unidentifiable content or nothing in their gut for different lice prevention strategies. See Fig. 1 for abbreviations

(21%), lice (1.8%), then feed (0.9%) (Fig. 8). Ten percent of guts were empty. Crustaceans, predominantly Caprellid amphipods, were 5-fold less important prey items for corkwing in the cleaner fish plus functional feed group compared to corkwing in the lice skirt-encircled group (z = –3.6, p = 0.001). There were no other differences between main food consumed and anti-lice regime. In total, 20% of corkwing wrasse had scales in their guts and anti-lice strategy did not affect prevalence of scales (χ^2 = 3.0, p = 0.4).

3.7. Behaviour of cleaner fish

We detected no differences in cleaner fish behaviours between anti-lice strategies (lice feeding: χ^2 = 3.6,

p = 0.3; inspection: χ^2 = 4.8, p = 0.2; swimming near salmon: χ^2 = 2.9, p = 0.4; alternate feeding: χ^2 = 1.9, p = 0.6; avoiding salmon: too infrequent to model reliably). The most commonly observed behaviour in sea cages across treatments over 10 observed hours was swimming near salmon (89 instances), followed by alternative feeding (45 instances), inspecting salmon for lice (25 instances), avoiding salmon (8 instances), and feeding on lice (6 instances).

4. DISCUSSION

In an experiment on a commercial-scale Atlantic salmon farm, we tested if cleaner fish delousing efficacy differed between anti-lice strategies. Based on stomach contents and estimations of CFE, cleaner fishes were least effective when used with anti-lice skirts. The experiment was conducted at 1 site in autumn over a 2 mo period and results relate to 1 species of cleaner fish. Our results do not mean that skirts will always reduce cleaner fish efficacy, as different circumstances may arise for other species and at other sites and times. However, given the broad use of cleaner fish (65% of farms use cleaner fishes: Norwegian Directorate of Fisheries 2018) and widespread use of skirts (Nodland 2017, Grøntvedt et al. 2018), the effects of different in-cage anti-lice strategies should be broadly tested in space, time, and across species to see if they alter lice consumption by cleaner fish.

Regardless of anti-lice strategy, measures of the physical condition of corkwing wrasse were similar. This means that farmers could implement new strategies for lice control — specifically those that involve unfamiliar structures and feed and light regimes — without high risk of harming corkwing wrasse any more than traditional sea cages.

4.1. Delousing efficacy

Delousing efficacy was measured in 2 ways: by counting lice in corkwing wrasse guts and estimating overall CFE for each anti-lice strategy. The poorest delousers (mean lice per gut = 0.23) and the lowest CFE (–0.21) were found in lice skirt-encircled sea cages, despite extended foraging hours permitted by 24 h deep lights. Several explanations for this result are possible. First, echosounder data showed that salmon swam ~5 m deeper when lice skirts were used, compared to other cages that also had deep lights and feed but no skirt; the deeper depth preference could possibly be to avoid patches of lower water quality caused by the skirt (Stien et al. 2012, Frank et al. 2015). Oldham et al. (2017) showed some avoidance of low dissolved oxygen conditions by salmon. However, it is likely that cleaner fishes mostly occupy the top 7 m of sea cages, to take advantage of shelter provided by hides and the observed warmest water (Brooker et al. 2018, Skiftesvik et al. 2014). Different depth distributions for salmon and cleaner fishes would lead to fewer interactions between the species and, subsequently, less lice-feeding opportunities. As different cleaner fish species demonstrate different habitat preferences (e.g. lumpsuckers avoid warm water), it may be possible to overcome the depth distribution problem by selecting species that will swim near salmon in a given farm's environment.

4.2. Physical condition

Corkwing wrasse physical condition was not affected by anti-lice strategies in this study, and *K*-score (1.39) was broadly similar to corkwing wrasse in previous studies (e.g. 1.34: Sayer et al. 1996, 1.43: Treasurer & Feledi 2014). Compared to other stressors that are negative for welfare, such as capture, transfer, net-raising, and stocking (Skiftesvik et al. 2014, Treasurer & Feledi 2014, European Union Reference Laboratory for Fish Diseases 2016), the physical modification inside a sea cage may have comparably little effect on wrasse welfare.

Across lice management strategies, 75% of corkwing had fin splits or erosion. The damage, along with skin and snout wounds, is probably due to fish capture (wild fishing and experimental) and contact with farm structures. Fin damage may also have occurred in hot water and mechanical delousing treatments (see Table S1), but further research is required to verify this observation. Severity of damage should be scored in a similar way as salmon welfare scoring (e.g. Stien et al. 2013) to study causes and development over time, environments, and treatments. Territorial fin nipping may also contribute to fin damage, although we did not observe fin nipping in video footage and nipping is rare when fish receive adequate feed (Moutou et al. 1998, Hatlen et al. 2006).

Previously, skin, eye, operculum, and snout damage in corkwing wrasse have been reported as negligible (Treasurer & Feledi 2014). This suggests that the damage rates recorded here, especially for skin (28%), are high. Veterinarians report 60 to 100% mortality of cleaner fishes in salmon farms (Olsen

2017), at least 33% of which occurs within 5 mo after stocking (Nilsen et al. 2014). It is likely that external injuries and disease cause high mortality, but methods to measure welfare, mortality, and escapees need to be improved. Nevertheless, by law, fish housed in sea cages must be treated to the same ethical standard as salmon (Norwegian Seafood Research Fund 2018). To meet this ethical requirement, and given the recent and rapid expansion in cleaner fish use (Norwegian Directorate of Fisheries 2018), there is an urgent need to advance, validate, and oversee implementation of industry recommendations (Norwegian Seafood Research Fund 2018) across Norway to secure cleaner fish welfare via a verified and measurable standard for 'healthy' cleaner fishes in sea cages. Without this benchmark, we cannot extrapolate our findings beyond this specific site, season, and species.

4.3. Gut contents

Across all anti-lice strategies, corkwing wrasse consumed similar but highly varied diets that included algae, crustaceans, fish feed, and salmon lice. The only difference was that wrasse in the cleaner fish plus functional feed treatment ate fewer crustaceans compared to all other strategies. Crustacea were generally Caprellid amphipods, which are abundant on salmon farm nets (Blöcher 2013), so corkwing wrasse would have many opportunities to reach optimal prey handling efficiency for this food item (Warburton 2003). Lice, however, are not reliable food sources on salmon farms as loads usually remain below 0.5 adult female lice per salmon. Thus, cleaner fishes would have had fewer opportunities to learn delousing behaviours and it would take longer to reach optimal prey handling efficiency of lice. The proportion of caprellids in gut contents was greatest in cages with skirts. Caprellids may have been more abundant in cages with lice skirts, as skirts create an area of reduced flow immediately behind the skirt wall, which may promote biofouling and Caprellid abundance.

4.4. Behaviour

In >600 min of video, lice feeding was recorded 6 times by 4 cleaner fish, indicating that cleaner fish–salmon interactions are rare and hard to capture on film in this setting. We detected no differences in the suite of behaviours performed by cleaner fishes among anti-lice strategies. Therefore, the differences observed in lice per gut and CFE likely reflect different encounter rates between salmon and cleaner fishes induced by the different anti-lice strategies.

4.5. Industry implications

Although focusing on only one species of cleaner fish in autumn, this work emphasises the importance of full-scale studies, where complex interactions between farm routines, environmental conditions, and cage technologies likely affect cleaner fish efficacy. A clear recommendation arising from this work is that aquaculture production managers should test new technologies across seasons and sites for interactions with cleaner fish performance. This will enable fine-tuning of cleaner fish deployment strategies to optimise their efficacy.

In this commercial-scale experiment, lice skirts impaired the delousing efficacy of corkwing wrasse. This likely occurred due to skirts creating a spatial mismatch between where corkwing (shallower at hide depths of 0 to 7 m) and salmon (deeper with an average swimming depth of 11 m) occurred in cages, which reduced the encounter rate between cleaners and salmon. If new technologies create a mismatch between cleanerfish swimming depths and salmon swimming depths, then strategies such as adjustments to hide depths could reduce this difference if cleaners follow hides deeper. Different species of cleaner fish may also have clear vertical preferences that can be used in co-management with prevention technologies. An example is the semi-pelagic nature of lumpsucker compared to the more bottom-dwelling wrasse, or the temperature tolerance of different species. If further research reveals that specific lice-prevention technologies consistently compromise the interactions of cleaner fish with salmon and reduce lice-feeding opportunities, periods of non-use of cleaner fish may prove the best strategy.

Salmon lice made up <2% of the diet of corkwing wrasse, and only 11% of guts contained salmon lice. Increasing the number of cleaners that engage in feeding behaviour and the frequency with which they engage should be a priority for cleaner-fish managers. If research can identify high-performing cleaner fish and transfer their behaviour to other individuals via selective breeding programs (e.g. lumpsuckers; Imsland et al. 2016) or through specific acclimation measures, such as pre-exposing cleaner fish to lice-infected salmon in small enclosures or providing live *Artemia* and frozen lice as feed before

they are stocked in full-scale sea cages (e.g. Gentry 2018, Imsland et al. 2019), then cleaner fishes could become more effective biological control agents. If corkwing wrasse welfare and efficacy cannot be improved, the industry may need to reconsider its use of this species as a biological control agent.

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