# Sea lice removal by cleaner fish in salmon aquaculture: a review of the evidence base

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Running head: Effectiveness of cleaner fish for lice control

**Abstract**

Stocking cleaner fish to control sea lice infestations in Atlantic salmon farms is widespread and viewed as a salmon welfare-friendly alternative to current delousing control treatments. The escalating demand for cleaner fish (~60 million stocked per year) coupled with evidence of poor welfare and high mortality in sea cages requires that the lice removal effect of cleaner fish be substantiated by robust evidence. Here, we systematically analysed (1) studies that tested the delousing efficacy of cleaner fish species in tanks or sea cages, and (2) studies of spatial overlap, and therefore likely encounter rate, between cleaner fish and salmon when stocked together in sea cages. Only 11 studies compared lice removal between tanks or cages with and without cleaner fish using a replicated experimental design. Most studies had insufficient replication (1 or 2 replicates) and were conducted in small-scale tanks or cages which do not reflect the large volume, deep cages in which they are deployed commercially. Reported efficacies varied across species and experimental scale, from a 28% increase to a 100% reduction in lice numbers when cleaner fish were used. Further, our review revealed that the interaction of cleaner fish and salmon in sea cages has rarely been documented. While much of the evidence is promising, there is a mismatch between the current evidence and the extent of use by the industry. We recommend replicated studies in nine key areas at full commercial scale across all species that are currently widely used. More targeted, evidence-based use of cleaner fish should increase their efficacy and help to alleviate economic, environmental, and ethical concerns.

**Keywords**

Biological control; cleanerfish; *Cyclopterus lumpus*; treatment; lumpfish; *Salmo salar*; *Lepeophtheirus salmonis*; wrasse

## 1. Introduction

Parasites are a key problem in Atlantic salmon (*Salmo salar*) aquaculture, with multiple strategies to either prevent infestations or remove pests. The industry continues to struggle with impacts arising from infestations from ectoparasitic sea lice, principally the salmon louse *Lepeophtheirus salmonis* and sea louse *Caligus elongatus* (Costello 2006, Costello 2009). Sea lice damage farmed stock directly by feeding on the skin, mucous, and blood of their hosts. Severe infestations can lead to skin erosion, physical damage, osmoregulatory failure, increased disease incidence, stress, and immunosuppression (Bowers et al. 2000, Grave et al. 2004, Hamre et al. 2009). Further, larvae produced by lice on farmed fish spill back to coastal waters where they infest wild salmonids; this process has been implicated in population declines of wild stocks (Krkošek et al. 2013, Vollset et al. 2017). Accordingly, minimising sea lice infestations in farms is one of the industry's key objectives.

Modern salmon farms typically hold hundreds of thousands to millions of fish, making effective parasite control at this scale complex. For the past four decades, the use of chemotherapeutants that remove salmon lice have dominated control efforts as they are practical at this scale (Overton et al. 2018). However, reliance on chemotherapeutants has resulted in widespread evolution of resistance to most active compounds (Aaen et al. 2015). Mechanical and thermal delousing methods have been recently introduced but are stressful and lead to elevated salmon mortality rates post-treatment (Overton et al. 2018). This has prompted investment in other control methods that have minimal welfare impacts upon salmon. Among the leading contenders are invertivorous 'cleaner fishes' that eat attached pre-adult and adult lice stages directly off salmon (Imsland et al. 2015, Powell et al. 2018). Five main cleaner fish species are now in use: lumpfish (*Cyclopterus lumpus*), corkwing wrasse (*Symphodus melops*), ballan wrasse (*Labrus bergylta*), goldsinny wrasse (*Ctenolabrus rupestris*), and cuckoo wrasse (*Labrus mixtus*).

The use of cleaner fishes as biological control agents of salmon lice began in the late 1980s (Bjordal 1991, Torrissen et al. 2013). In Norway, their use increased rapidly from 2012, coinciding with a phase-out of chemical delousing (Overton et al. 2018). In 2018, 49 million cleaner fish were stocked in Norway, with 65% of farms using them (wrasse: 18 million, lumpfish: 31 million; Norwegian Directorate of Fisheries 2019). Similarly, in Scotland, the use of lumpfish has recently increased sharply (2016: 2 million; 2017: 6 million; Munro & Wallace 2017, Munro & Wallace 2018). In contrast, wrasse use has recently decreased (2016: 2.2 million; 2017: 58000; Munro & Wallace 2017, Munro & Wallace 2018). In Ireland, cleaner fish use is also growing as a lice control strategy (lumpfish stocked: 2015: 105600, 2016: 245000; wrasse stocked: 2015: 275800, 2016: 320000; Bolton-Warberg 2017). Cleaner fish, principally lumpfish, are also used in the Faroe Islands (Eliasen et al. 2018).

Wrasse were first identified as potential cleaner fish via multiple experiments in tanks and cages in the 1980s and 1990s, building a foundation for industrial deployment (e.g. Bjordal 1991, Deady et al. 1995, Treasurer 1994, Tully et al. 1996). Lumpfish are a more recent addition, with the first studies to provide evidence for their efficacy at small and large commercial scale conducted in the last 5 years (e.g. Imsland et al. 2014a, 2014b, 2015, 2016, 2018). Wrasse are widely used in spring and summer but become inactive at temperatures below 6 °C (Imsland et al. 2014a, Powell et al. 2018). Lumpfish are better adapted to cold water, so are preferred in autumn and winter and at high latitudes (Imsland et al. 2014a, Eliasen et al. 2018). Both ballan wrasse and lumpfish are now farmed to keep up with the demand for cleaner fish in Norway. In 2018, controlled production based on wild-caught parents supplied 63% of all cleaner fish used, of which most were lumpfish (Norwegian Directorate of Fisheries 2019).

Cleaner fish are less expensive and less stressful to salmon than chemotherapeutants (Groner et al. 2013, Imsland et al. 2018, Powell et al. 2018) and are generally more acceptable to the public than chemotherapeutant use (Imsland et al. 2018). All cleaner fish species used in salmon aquaculture are opportunistic cleaners, unlike 'true' cleaner fishes that have dedicated symbiotic relationships with 'client' fishes (Vaughan et al. 2017). In salmon cages, the expression of cleaning behaviour by wrasse and lumpfish is likely learnt and context dependent (Vaughan et al. 2017). Once stocked in salmon cages, both wild-caught and cultured cleaners must adapt to the sea cage environment and learn to approach and clean salmon. Anecdotally, salmon farmers report variable success at commercial scale. Poor efficacy at certain places and times could be due to a range of factors. For example, access to feed pellets and biofouling may remove the need for cleaners to feed on lice and result in lower-than-expected lice removal rates (Imsland et al. 2015), while unsuitable environmental conditions may lead to inactivity or high mortality among cleaner fish.

The use of cleaner fishes also raises unique ethical considerations, as measures to secure the welfare of vertebrates are typically encoded within animal welfare legislation. Concerns have arisen recently after observations of high mortalities and disease loads of cleaner fish deployed on salmon farms (Nilsen et al. 2014, Treasurer & Feledi 2014), with considerable losses due to escapes, handling, predation or disease (Skiftesvik et al. 2014, Mo & Poppe 2018). Further, escapees of some cleaner fish (i.e. ballan wrasse: Quintela et al. 2016, corkwing wrasse: Gonzalez et al. 2016) can interact with local populations and alter their genetic structure (Faust et al. 2018). Cleanerfish can also introduce biosecurity risks for farmed salmon; for example, lumpfish are heavily parasitised by *Caligus elongatus,* and may provide a source population for infection of farmed salmon (see Powell et al. 2018 for summary). As most wrasses used are wild caught, high mortality rates result in continuous demand for more fish, driving fishing pressure on wrasse populations with impacts on their ecology and population dynamics (Skiftesvik et al. 2014, Halvorsen et al. 2017). Given evidence of poor welfare and high in-cage mortality rates, it is important that cleaner fish use in aquaculture is justified and guided by a strong evidence base.

Much of the production biology and health management issues of cleanerfish have been extensively addressed in two previous reviews (Brooker et al. 2018, Powell et al. 2018). However, there has been no comprehensive synthesis of studies that measured how effective cleanerfish are in reducing sea lice on salmon. Here, we assessed the current evidence base for cleaner fish efficacy and encounter rates with salmon by conducting a systematic review of the literature on (1) cleaner fish lice removal efficacy, and (2) the knowledge basis about interaction levels between salmon and cleaner fish in sea cages. Based on our findings, we highlight key areas that should be investigated to build a stronger evidence base regarding cleaner fish use by industry.

## 2. Methods

To discover all available literature surrounding cleaner fish use in salmon aquaculture, we searched the Web of Science and Google Scholar databases in March 2019 using the following search terms: ((salmon* or aquaculture*) AND (lump* or wrasse* or cleaner*)). Results were manually screened by title and abstract to identify articles or reports ('studies' herein) that were relevant to cleaner fish use in salmon aquaculture. For inclusion, studies needed to have addressed one or more of the five cleaner fish species currently used in salmon aquaculture. We then discovered additional studies by reading the reference lists of studies returned by the initial search. Within these search results, we conducted systematic reviews of: (1) studies that assessed the delousing efficacy of cleaner fish species in tanks or sea cages; and (2) studies of spatial overlap between cleaner fish and salmon when stocked together in sea cages (and therefore likely encounter rates between the two).

### 2.1 Cleaner fish efficacy

To be included in the systematic review of efficacy, studies must have measured lice removal by cleaner fish using either a before-after or control-treatment experimental design. Where a study provided multiple control-treatment comparisons, we treated these separately (referred to as "comparisons" herein). Comparisons that included two or more species of cleaner fish stocked together are referred to as "mixed". Where multiple studies presented data from the same trials, these were combined. We recorded the experimental period, sea water temperature, type and volume of the experimental unit, degree of site exposure, details on cleaner fish stocked (species, number, and stocking density), number of salmon and their size, whether a single species of cleaner fish was present in the cage or not, the number of control or 'before' replicates, the number of treatment or 'after' replicates, whether the experiment was conducted at multiple study sites, and the effect size (percentage change of lice numbers by cleaner fish relative to control or 'before' samples).

### 2.2 Spatial overlap between cleaner fish and salmon in sea cages

To be included in the systematic review of cleaner fish behaviour and swimming depth, studies must have provided data on cleaner fish swimming depth or other relevant behaviours when stocked in sea cages with salmon. Where a study provided results from multiple distinct comparisons at different experimental scales, these were treated separately. We recorded the study period, temperature, sea cage size, degree of site exposure, cleaner fish species, number of cleaner fish stocked and the stocking density, number and size of salmon, whether more than one cleaner fish species was stocked, number of replicate cages, whether experiments were conducted at multiple study sites, whether behaviour or swimming depth was recorded and if so, the observation method used.

## 3. Results

The literature search returned 141 studies on the topic of cleaner fish in salmon aquaculture. Early research focused on the 4 wrasse species most commonly used in salmon aquaculture (Fig. 1). From 2003 to 2011, little research was conducted on any cleaner fish species, perhaps due to reliance on chemotherapeutants for sea lice control. Research effort increased again after 2011, coinciding with concerns around chemotherapeutants and later reduction in their use (Aaen et al. 2015, Overton et al. 2018). However, this increase in research effort lagged behind the explosion in industrial use of cleaner fish and continues to do so. A total of 33 studies were published on cleaner fish in relation to salmon aquaculture in 2018, of which 67% concerned lumpfish, 24% wrasse, and 9% both wrasse and lumpfish (Fig. 1).

**3.1 Cleaner fish efficacy**

Experimental tests of cleaner fish efficacy (11 studies containing 46 comparisons of lice levels with and without cleaner fish) were conducted across a broad range of experimental scales, temperatures, locations, cleaner fish species, stocking densities, and with salmon of varying sizes (Fig. 2B; Table S1). The mean trial duration was 72 days (range: <1 to 335 days) (Fig. 2A). Seven studies reported water temperature, ranging from 8-16 °C for wrasse, and 4-16 °C for lumpfish (Table S1). Aside from Tully et al. (1996) and Treasurer (2013) at small commercial scale (2864-10742 $m^3$, 3 comparisons total) and Imsland et al. (2018) at large commercial scale (37688 $m^3$, 3 comparisons), experimental tests of cleaner fish efficacy have been performed in tank (1 $m^3$, 6 comparisons) and small cage (100-212 $m^3$: 34 comparisons) scale research settings (i.e. 87% of all comparisons conducted at tank and small cage scales; Fig. 2B). Stocking densities (number of cleaner fish per salmon, %) varied widely for each experimental scale and cleaner fish species, with larger ranges observed for wrasses (tank: 5-67%; small: 4-73%; small commercial: 1-4% stocking density) compared to lumpfish (small: 5-15%; large commercial: 4-8% stocking density) (Table S1). Of the 10 studies that conducted experiments in sea cages, 9 provided the study location; one study was in an inner fjord (i.e. Treasurer 1994), with the remainder at sites sheltered by at least one body of land. No studies were conducted at exposed coastal sites. There were 35 comparisons across 11 studies in which one species of cleaner fish was stocked within the treatment cage, allowing estimation of species-specific efficacy (Table S1). 9 studies (i.e. 23 comparisons, or 50% of all comparisons) had <3 replicates (Table S1).

Comparisons reported efficacies from a 28% increase to a 100% reduction in lice numbers (Fig. 2B). 98% of all comparisons (i.e. 45 out of 46 comparisons) estimated efficacy by comparing the number of lice in cages with and without cleaner fish, with one comparison using a before-after experimental design (i.e. Bjordal 1991; Table S1).One tank-based study and two small scale sea cage study stocked a single species of cleaner fish in isolation (such that the efficacy of a specific cleaner fish species could be assessed), had ≥3 replicate treatment cages, and reported a positive cleaner fish effect (tank-based: Leclercq et al. 2014, small scale: Skiftesvik et al. 2013, Skiftesvik et al. 2018; Fig. 2C). One small commercial scale cage study fulfilled the same criteria, but reported a negative effect of cleaner fish, in which salmon in cages with cleaner fish had 21% more lice than cages without cleaner fish (Tully et al. 1996; Fig. 2C). No studies examined cleaner fish efficacy at multiple sites (Table S1).

Studies reported high lice removal efficacy of ballan wrasse in experimental conditions, from tank scale (90-99% efficacy regardless of wrasse size or the presence of supplementary feeding: Leclercq et al. 2014) to small cage scale (91% efficacy: Skiftesvik et al. 2013;, 49% efficacy: Skiftesvik et al. 2018) and small commercial cage scale (100% efficacy in an unreplicated comparison; Treasurer 2013) (Table S1). Reported lumpfish lice removal efficacies are more variable, with lower efficacies at small scale (9-60% efficacy, but 97% for adult female lice: Imsland et al. 2014a, 2014b, 30-40% efficacy: Imsland et al. 2016, 10% efficacy: Skiftesvik et al. 2017, 30% efficacy: Skiftesvik et al. 2018) compared to large commercial scale (53-73% efficacy: Imsland et al. 2018). The efficacy of goldsinny wrasse has been tested at tank scale (0% efficacy: Tully et al. 1996), small cage scale (62% efficacy: Bjordal 1991, –14%

efficacy: Skiftesvik et al. 2017), and small commercial cage scale (–21% efficacy: Tully et al. 1996), with highly variable effects on lice density mean 30% reduction, range –21 to 77%: Table S1). Tests of rockcook wrasse efficacy at tank scale (96% efficacy: Tully et al 1996) and small cage scale (69% efficacy: Bjordal 1991) illustrate promising lice removal effects, although research has not been conducted in the last two decades or at larger scales (Table S1). Bjordal (1991) tested cuckoo wrasse efficacy at small cage scale with some success, (11% stocking density: 51% efficacy; 23% stocking density: 63% efficacy) (Table S1), although the authors reported that the wrasse did not become effective until after a delousing treatment. Finally, the efficacy of corkwing wrasse has been tested in two small cage scale studies, with mixed effects (–28% efficacy: Skiftesvik et al. 2017, 58% efficacy: Skiftesvik et al. 2018) (Table S1).

**3.2 Spatial overlap between cleaner fish and salmon in sea cages**

The literature search revealed several studies on cleaner fish behaviour when stocked with salmon, but it remains difficult to assess spatial overlap and likely encounter rates between cleaner fish and salmon in commercial settings. One study (Tully et al. 1996) recorded cleaner fish and salmon swimming depths simultaneously, via SCUBA diving observations (Table 1). However, the authors did not observe any cleaning behaviour during SCUBA diving observations between Oct-Dec and reported that goldsinny spent most of their time swimming close to the net and consuming biofouling organisms (Tully et al. 1996). A large proportion of wrasse were also observed resting in a torpid state during Nov-Dec (Tully et al. 1996).

Stocking densities varied for lumpfish (small: 4.7-40% stocking density; large commercial: 6% stocking density) and wrasse (small: 4.7-10% stocking density; small commercial: 0.3-7% stocking density) (Table 1). Of the 7 studies and 25 comparisons conducted, 17 comparisons were conducted with one species of cleaner fish within the treatment cage (Table 1). Four studies (i.e. 15 comparisons) had ≥3 replicates. Seven comparisons monitored cleaner fish behaviour and swimming depths within small commercial scale sea cages, but salmon swimming depths were only recorded in one. Salmon swimming depth was not monitored in any of the small-scale studies. Further, no studies were conducted at large commercial scale or across multiple study sites.

## 4. Discussion

### 4.1 Cleaner fish efficacy

Experiments that tested efficacy of cleaner fish for sea lice removal were typically unreplicated or had low replication, and our search returned just one study that assessed cleaner fish efficacy at large commercial scale (lumpfish: Imsland et al. 2018) and three comparisons at small commercial scale (ballan: Treasurer 2013, corkwing: Tully et al. 1996). Insufficient replication precludes the drawing of strong conclusions as confounding factors may contribute to the observed effects (Quinn & Keough 2002). A lack of studies at large commercial scale creates a mismatch between the small scale at which proof of concept has been tested and the cage volumes in which cleaner fish are now deployed. The design of experiments must pay attention to scale as results detected at small scale often do not match those detected at large scale (Wien 1989). Given the industrial use of ~60 million cleaner fishes per year by industry across multiple countries, the lack of well replicated experiments at commercial scale requires redress.

Most studies testing efficacy have been conducted in small cages with volumes between 100 and 125 $m^3$ (e.g. Bjordal 1991, Imsland et al. 2014a, 2014b), whereas circular commercial cages commonly have a 160 m circumference with a 15-35 m deep net that tapers to a cone shaped bottom in the last

5 m (cage volume: 20000-80000 $m^3$, e.g. Oppedal et al 2011). Therefore, cage volume is approximately 200-800× higher in commercial cages relative to the volumes and sizes used in most efficacy studies.

Evidence for effective sea lice removal for certain widely-used wrasse species is especially sparse. Efficacy of goldsinny wrasse has not been tested at large commercial scale (Table S1), while evidence for efficacy of corkwing wrasse is currently limited to 2 technical reports (Skiftesvik et al. 2017, 2018) (Table S1). Given that 5.9 million wild-caught corkwing wrasse and 7.9 million wild-caught goldsinny wrasse were stocked in sea cages in Norway in 2018 (Norwegian Directorate of Fisheries 2019), rigorous experimental assessments of the efficacy of goldsinny and corkwing wrasse at commercial scale should be prioritised to justify their ongoing use. In contrast, several studies assessed the cleaning efficacy of rock cook wrasse with promising results (Bjordal et al. 1991, Tully et al. 1996; Table S1), yet their use is negligible at present (Norwegian Directorate of Fisheries 2019). The most-used species, lumpfish, has the most robust evidence base among all cleaner fish species deployed, with several studies spanning small to large commercial scale. However, the evidence base for lumpfish requires further development as it does not span the range of farming conditions across which ~31 million lumpfish are deployed each year in Norway across all 13 production zones (Norwegian Directorate of Fisheries 2019).

Numerous studies that have examined cleaner fish stomach contents provide evidence that cleaner fish consume lice at various experimental scales (Deady et al. 1995, Treasurer 2002, Imsland et al. 2014a, Imsland et el. 2015, Skiftesvik et al. 2017, Eliasen et al. 2018). However, these studies typically occurred during the warmer months and at relatively sheltered sites. Only one large commercial scale experiment at a single inner fjord site tested the efficacy of lumpfish during winter (Imsland et al. 2018). However, the efficacy and survival of cleaner fish at exposed coastal sites has not yet been investigated.

Overall, there is a clear mismatch between the current evidence base for the efficacy of cleaner fish and the extent of their use by the industry. The current evidence base is derived from relatively few studies, in a narrow range of environmental settings, and largely in experimental units with small cage volumes and limited numbers of salmon that do not match the scale (volume and depth) of commercial cages, nor the large number of enclosed salmon. The use of cleaner fish in salmon aquaculture essentially trades-off the welfare of multiple fish species for that of another more commercially valuable species, and it is therefore important that a robust evidence base justifies their use from ethical, environmental, and economic perspectives.

### 4.2 Spatial overlap between cleaner fish and salmon in sea cages

The spatial overlap between cleaner fish and salmon in sea cages has been sparsely studied, with no research conducted in large commercial scale sea cages. Our systematic review has illustrated that studies mostly focus on cleaner fish behaviour and interactions with salmon in sea cages, however there has not been a substantial focus on salmon and cleaner fish swimming depths within sea cages as a measure of the likely interactions between the client and cleaner. As most proof-of concept scale studies have been done in shallow tanks or cages, this may not have been necessary at small scale. However, swimming depth preference is an important factor that should be measured at commercial scale as salmon and cleaner fish may have different preferences. Larger, deeper cages enable salmon to more readily express such preferences, which could result in fewer encounters between salmon and cleaner fish than expected, which could in turn reduce lice removal efficacy.

During the day when cleaner fish are most active (e.g. lumpfish: Powell et al. 2018, ballan: Brooker et al. 2018, goldsinny: Gonzalez & de Boer 2017), salmon typically move up into surface waters during feeding times before descending to preferred deeper swimming depths once satiated (Oppedal et al.

2011a). This general pattern may be altered by thermal stratification, with salmon choosing at the depth with the warmest water available (up to 16 °C). Typically, responses to temperature result in deeper swimming in winter when surface water is cold and avoiding surface waters that are too warm in late summer and during transitional periods from spring to autumn (Oppedal et al. 2011a).

While cleaner fish can control lice under certain conditions, their physiology and morphology are not suited for life in more exposed sea cage environments (Yuen et al. 2019). Wrasses are typically found in coastal rocky reefs and kelp beds where habitat structure provides the opportunity to shelter from sustained currents and wave surges (Leclercq et al. 2018, Pita & Freire 2011, Villegas-Ríos et al. 2013, Brooker et al. 2018). They are relatively poor swimmers compared to salmon: large ballan wrasse far larger than the size typically used as cleaner fish in aquaculture have a sustained swimming speed of only 27 cm $sec^{-1}$ at 25 °C (Yuen et al. 2019), which is considerably lower the sustained swimming speed of post-smolt salmon (75-93 cm $sec^{-1}$ at 3-18 °C, respectively; Hvas et al. 2017). Lumpfish also have poor prolonged swimming capacity (25-35 cm $sec^{-1}$ for 300 g fish at 3-15 °C and 13% lower in 75 g fish: Hvas et al. 2018) and given that cultured lumpfish are stocked at smaller sizes (Brooker et al. 2018), their welfare, survival and subsequent delousing performance could be severely compromised at sites with strong currents. Lumpfish are globiform teleosts native to the North Atlantic, where they are found in both pelagic waters and coastal regions (Blacker 1983, Daborn & Gregory 1983). While they cope well at cold temperatures, their mortality rate increases at temperatures above 16 °C (e.g. Hvas et al. 2018). In comparison to farmed ballan wrasse and lumpfish, the natural distribution of Atlantic salmon, while partially overlapping, extends much further north than wrasse and further south than lumpfish (Jensen et al. 2014) with a thermal niche overlapping lumpfish in the north and wrasse in the south. A better understanding of cleaner fish biology is needed to ensure that they are deployed at sites, seasons and sizes where good welfare and effective delousing is likely.

To date, cleaner fish and salmon swimming depths have not been mapped simultaneously using non-intrusive technologies. While Tully et al. (1996) recorded cleaner fish and salmon swimming depths simultaneously using SCUBA, evidence from other systems indicate that the presence of divers alters the behaviours of cleaner fish (Titus et al. 2015), and findings of SCUBA observations should be interpreted with caution. Non-intrusive echosounders have been used to record salmon swimming depths in experimental and commercial scale experiments for decades (see review by Oppedal et al. 2011a) and have been used to monitor salmon swimming depths when testing a range of new lice prevention technologies (Stien et al. 2016, Oppedal et al. 2017, Wright et al. 2017). This data has provided a fundamental understanding of salmon swimming depths, and how they can vary with light, temperature, salinity, oxygen, water currents, the entry of feed into cages, and the effects of lice prevention and control measures. In non-stratified conditions typical of coastal sites, salmon typically move to the surface to feed. Throughout the day, they can be found swimming deep within the cage, before ascending to swim in shallow surface layers at night time. Depth-based variations in temperature, salinity, and oxygen levels modulate this overall pattern. The consequence is that salmon rarely distribute evenly in a cage volume, instead packing into specific depth layers at swimming densities that exceed their stocking densities (typically 1.5-5× but up to 10-15×: Oppedal et al. 2011a, 2011b). If the swimming depths of salmon do not coincide with the preferred swimming depths of stocked cleaner fish, encounters will not occur, and lice cleaning efficacy will be diminished. This effect is likely to be exacerbated in larger cages.

As yet, echosounders have not been used in conjunction with technologies and techniques that could also monitor cleaner fish depth (e.g. PIT tagging, Nilsson et al. 2013, 3-D acoustic tagging, Leclercq et al. 2018) to provide quantitative data on depth distributions by cleaner fish and salmon. Understanding swimming depth preferences of both cleaner fish and salmon when stocked together is key to understanding the likelihood of encounters, as certain environment conditions (e.g. exposed coastal site or brackish water) and the presence of cage manipulations (e.g. control and preventative treatments) may curb cleaner fish efficacy.

### 4.3 Recommendations for experimental design and new measures to monitor and improve cleaner fish efficacy

Our systematic review revealed important gaps in the knowledge base underpinning the use of millions of cleaner fish. Here, we offer several recommendations and highlight areas of research that warrant further investigation to optimise cleaner fish use in commercial settings:

1. <u>Replicated studies</u>

Multiple replicate sea cages are needed to provide a rigorous estimate of cleaner fish efficacy. Regardless of experimental scale, studies should strive to have three replicates per treatment as an absolute minimum. More is preferable, as sea lice infestation pressure varies considerably between cages, both within and between farms. Given the scale of cleaner fish use in the salmon aquaculture industry, conducting studies that have enough replicates is essential when testing cleaner fish efficacy.

2. <u>Cage volume and depth</u>

There has been one published study that has documented the efficacy of one species of cleaner fish at large commercial scale (i.e. lumpfish: Imsland et al. 2018). Cleaner fish stocked in commercial farms are kept in cages that are much larger and deeper than cages commonly used for experimental trials. Working at commercial scale is expensive and logistically difficult in many cases, which may largely explain why small-scale studies have dominated in the proof-of-concept phase of developing cleaner fish as a biological control. However, the current mass use of cleaner fish by industry requires the promising proof-of-concept data to be benchmarked in experimental units that reflect modern commercial conditions. We recommend that researchers seek opportunities to partner with commercial actors that are already using cleanerfish in commercial-scale farms and apply logical, well-replicated experimental designs in these settings. Such partnerships are possible and have delivered full production-cycle data on the efficacy of other types of anti-lice technologies (e.g. Geitung et al. 2019).

### 3. Optimum stocking densities at commercial scale

Imsland et al. (2018) found that pre-adult and adult lice removal efficacy by lumpfish was highest at 8% stocking density. There is not yet any published literature on optimal stocking densities of wrasse species at commercial scale. Without clear guidelines that recommend stocking densities for wrasses, farmers may be stocking too little or too many, which could in turn compromise lice control within cages or drive unnecessarily high demand for cleaner fish.

### 4. Visual acuity of cleaner fish

Visual acuity, or the ability to perceive static spatial detail, is highly variable across fish species (Caves et al. 2017, Caves et al. 2018). Light spectrum sensitivity and contrast potential has been researched for ballan, corkwing and goldsinny wrasse, and lumpfish (Skiftesvik et al. 2017), but no research has determined the most relevant visual trait for their role in salmon aquaculture: the distance at which they can identify sea lice on fish. The ability of cleaner fishes to detect attached sea lice at distance, and the role of environmental factors that reduce visibility, will be key factors in their lice removal efficacy.

### 5. Efficacy and encounter rates under various environmental conditions

Environmental conditions and physiological traits of fish will influence encounter rates between cleaner fish and salmon. For example, lumpfish are slow swimmers with low critical swimming speeds and low thermal thresholds, especially in warm water (15% mortality over three weeks of acclimation at 18 °C: Hvas et al. 2018). Further research is needed to understand how environmental parameters such as current velocity, salinity, temperature, turbidity (particularly during spring algal blooms) and wave exposure affect cleaner fish performance.

## 6. Acclimation prior to stocking

As the use of cultured cleaner fish is expanding rapidly, many ‘naive’ fish will be stocked into salmon farms that have never experienced co-habitation with lice-infested salmon. This means all learning of cleaning behaviour must occur after stocking, and if lice numbers are low and interactions with salmon are few, many cleaner fish may never learn to consume lice. Accelerating this process by acclimating fish prior to stocking may therefore prove useful. Preliminary evidence at small cage scale suggests that lice cleaning behaviour can be learnt more rapidly if cleaner fish are exposed to lice-infested salmon or fed live feeds before stocking (Gentry 2018, Imsland et al. 2019); this should be tested further at commercial scale before implementation.

## 7. Interactions with lice preventative technologies

Increasing use of methods to prevent sea lice infestations such as skirts and snorkel cages (e.g. Stien et al. 2016, 2018, Grøntvedt et al. 2018) may alter encounter rates between cleaner fish and salmon. Skirts tend to cause salmon to swim deeper (Gentry 2018), but cleaner fish may prefer the sheltered conditions within the skirt. In a replicated experiment at commercial scale, Gentry (2018) found that corkwing wrasse ate 10× fewer lice when used in conjunction with skirts compared to cages without skirts. Increasing use of skirts and other barrier technologies will necessitate a greater understanding of this phenomenon to optimise cleaner fish hide deployments and other depth related management.

## 8. Interactions with lice control strategies

Despite stocking cleaner fish, many farms rely on other control strategies to reduce lice numbers when legislated limits are reached. Most cleaner fish are either captured and removed from cages prior to these treatments or held at the opposite side of the cage away from the pumping point, possibly for later re-stocking in the same or nearby cages. Some cleaner fish may go through the crowding, pumping and treatment processes along with the salmon. While chemotherapeutant-based treatments have declined in use, they are still important in some regions and there is some evidence that they can result in cleaner fish mortality. For example, Treasurer & Feledi (2014) recorded a 4% mortality rate of wrasse after a cage-based pyrethroid delousing treatment. Thermal and mechanical delousing measures are now the most common whole cage lice removal method applied in Norway (Overton et al. 2018), yet there is no data on their effects on cleaner fish efficacy in the weeks and months post-treatment. This is a clear area for experimental research to optimize their reuse and welfare.

## 9. Cleaner fish welfare

The high reported losses of cleaner fish and high incidence of diseases clearly indicate that the existing handling and treatment methods and environmental conditions do not fulfil the legal demands to secure fish welfare. While some studies have monitored or assessed aspects of cleaner fish welfare (e.g. Sayer & Reader 1996, Treasurer & Feledi 2014, Gentry 2018, Johannesen et al. 2018, Mo & Poppe 2018, Speare 2019), there is not yet a consistent welfare assessment used to document welfare within cages (as is done for salmon using the Salmon Welfare Index Model (SWIM): Stien et al. 2013). Further, while most cleaner fish experiments monitor cleaner fish mortality, there was no mandatory industry reporting of cleaner fish mortalities until July 2018 in Norway (Norwegian Ministry of Trade, Industry and Fisheries 2008). This contrasts to the long-term mandatory reporting of salmon mortality in place in many jurisdictions (e.g. Norway, Scotland), which has led to new insights into the outcomes of lice control practices (Overton et al. 2018). The future reporting of cleaner fish mortalities and stocking numbers within farms could lead to similar insights to improve their current management and identify favourable and unfavourable stocking conditions.

Other pathways to improve cleanerfish efficacy are possible, such as selective breeding to improve lice predation behaviour and developing production methodologies to ensure healthy, robust fish that survive well in sea-cage environments, as outlined by Brooker et al. (2018) and Powell et al. (2018).

## 5. Conclusion

The widespread use of cleaner fish (~60 million deployed per year) has outpaced the development of a robust evidence base to justify and guide their use. Current evidence, while clearly promising, is patchy in nature, and has been largely gathered in small volume experimental units that do not reflect the conditions within commercial cages where cleaner fish are used. Commercial scale experiments to ground-truth the promising results obtained at small scale are logistically difficult, expensive, and create ethical challenges due to the use of large numbers of experimental animals. However, the level of investment now placed by the industry into cleaner fish demands a more critical assessment of their benefits at commercial scale, and research effort should reflect their stature within the industry. Detailed research on a species by species basis is required to determine optimal stocking conditions that elicit high encounter rates and cleaning behaviours. The outcomes of this research will enable the industry to use cleaner fish judiciously and strengthen focus on creating cage conditions that optimize cleaner fish welfare and performance.

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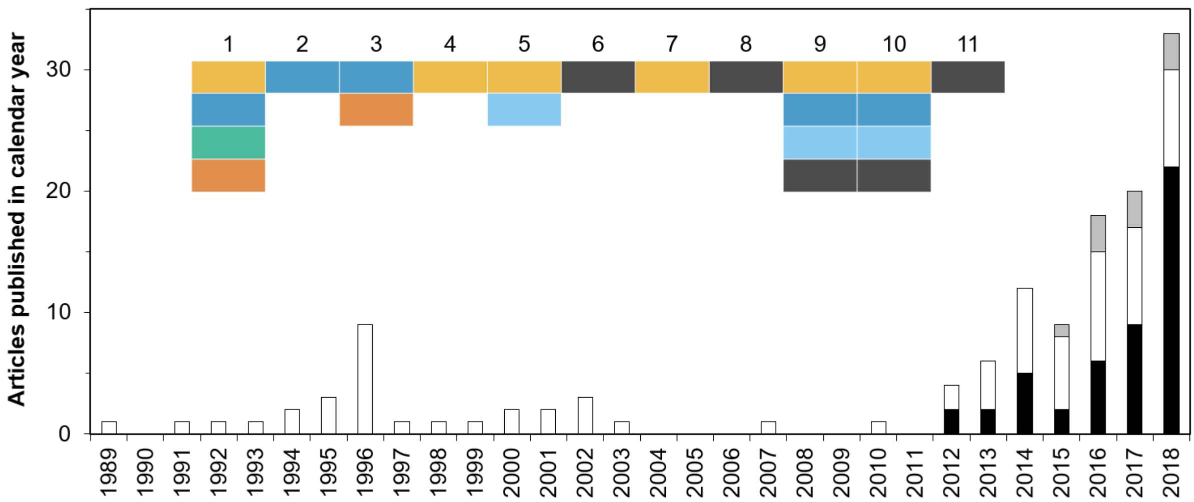
## Figure Captions

**Figure 1.** Research effort over time on cleaner fish use in salmon aquaculture, measured by the number of journal articles or technical reports published in each calendar year. Bars are colour-coded by the cleaner fish species studied: black = lumpfish, white = wrasse, grey = both. Studies that tested efficacy of cleaner fish for lice removal are also listed in chronological order (inset): 1 - Bjordal 1991; 2 - Treasurer 1994; 3 - Tully et al. 1996; 4 - Treasurer 2013; 5 - Skiftesvik et al. 2013; 6 - Imsland et al. 2014a, 2014b, 2015; 7 - Leclercq et al. 2014; 8 - Imsland et al. 2016; 9 - Skiftesvik et al. 2017; 10 - Skiftesvik et al. 2018; 11 - Imsland et al. 2018. The coloured blocks indicate the species used in each study: black = lumpfish, yellow = ballan wrasse, light blue = corkwing wrasse, green = cuckoo wrasse, dark blue = goldsinny wrasse, orange = rock cook wrasse.

**Figure 2.** Relationship between the volume of the experimental unit (tanks or cages) and the measured efficacy of cleaner fish, colour-coded by (A) the duration of the study, (B) the species of cleaner fish, or (C) the species of cleaner fish, excluding studies that did not have 3 or more replicates per treatment. For panel C: Where studies provided data for multiple treatment levels (e.g. stocking densities or cleaner fish body sizes) but did not meet the required replication within each treatment, we nonetheless included the study if taking the mean of treatment levels provided sufficient replication.

**Fig. 1**



Articles published in calendar year
0
10
20
30
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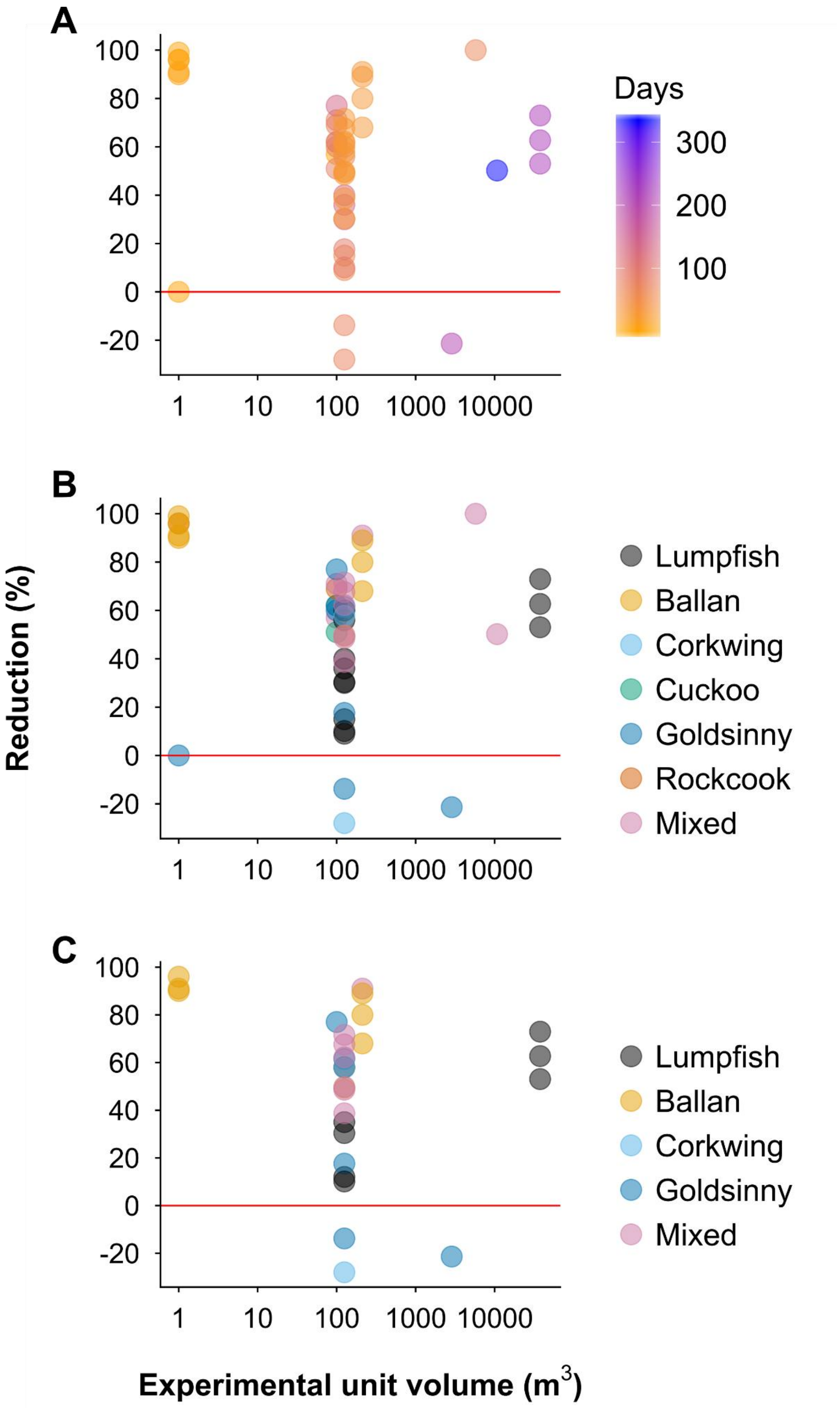
Fig. 2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B
C
Reduction (%)
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60
40
20
0
-20
1
10
100
1000
10000
Days
300
200
100
Lumpfish
Ballan
Corkwing
Cuckoo
Goldsinny
Rockcook
Mixed
Experimental unit volume ($m^3$)

**Table 1:** A summary of the current literature (peer-reviewed journal articles and scientific reports) on experiments recording cleaner fish encounters with salmon in small scale (100-250 $m^3$) and small commercial scale (1000-15000 $m^3$) sea cages. Experiment period and temperature, experiment unit size, degree of site exposure (where 1 = inner Loch or fjord, 2 = sheltered by at least one body of land, and 3 = exposed coastal site), the cleaner fish species, the number of cleaner fish stocked and the stocking density, number of salmon and their size, whether there was a single species in each cage, number of treatment replicates, number of control replicates, whether experiments were conducted at multiple study sites, behaviour and depth observation method, and whether cleaner fish depth and salmon depth were recorded.

| Experimental scale | Citation | Experiment period, temperature | Experiment unit size | Site exposure | Species, N, size, stocking density of cleaner fish | N salmon, size | Single cleaner fish species per cage? | N treatment replicates | N control replicates | Multiple study sites? | Observation method | Cleaner fish depth recorded? | Salmon depth recorded? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Small scale | Imsland et al. 2014 | 1 Dec 2011 to 25 Jan 2012, unspecified temperature | 5×5×5 m; 125 $m^3$ | 2 | Lumpfish (40 fish; 40% density) | 100 fish; 3500 g | Yes | 2 | 2 | No | Underwater camera | Yes | No |
| | Imsland et al. 2016 | 25 Jan to 5 July 2015, 4.5 °C to 10.8 °C | 5×5×5 m; 125 $m^3$ | 2 | Small lumpfish (23 g) (15 fish; 10% density)<br>Medium lumpfish (77 g) (15 fish; 10% density)<br>Large (114 g) lumpfish (15 fish; 10% density) | 150 fish; 538 g | Yes | 2 | 2 | No | Underwater camera | Yes | No |
| | Skiftesvik et al. 2017 | 8 Dec 2015 to 9 Mar 2016, 7°C | 5×5×5 m; 125 $m^3$ | 2 | Cultured ballan wrasse (25 fish; 5% density)<br>Wild goldsinny wrasse (25 fish; 5% density)<br>Cultured lumpfish (25 fish; 5% density) | 500 fish; 130 g | Yes | 3 | 3 | No | PIT tag and GoPro | Yes | No |
| | Skiftesvik et al. 2017 | 1 Nov 2016 to 12 Jan 2017, 8.5°C | 5×5×5 m; 125 $m^3$ | 2 | Wild goldsinny wrasse (30 fish; 10% density)<br>Wild corkwing wrasse (30 fish; 10% density) | 300 fish; 2922 g | Yes | 3 | 3 | No | PIT tag and GoPro | Yes | No |
| | Skiftesvik et al. 2018 | 3 to 30 Sept 2014, 15.9°C | 5×5×5 m; 125 $m^3$ | 2 | Goldsinny wrasse (24 fish; 4.7% density)<br>Corkwing wrasse (24 fish; 4.7% density) | 510 fish; 435 g | Yes | 3 | 3 | No | PIT tag and GoPro | Yes | No |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Ballan wrasse (24 fish; 4.7% density) | | | | | | | | |
| | | | | | Lumpfish (24 fish; 4.7% density) Goldsinny and ballan wrasse (12 fish of each; 4.7% density) | | No | | | | | | |
| | | | | | Goldsinny and corkwing wrasse (12 fish of each; 4.7% density) | | | | | | | | |
| | | | | | Goldsinny wrasse and lumpfish (12 fish of each; 4.7% density) | | | | | | | | |
| | | | | | Corkwing and ballan wrasse (12 fish of each; 4.7% density) | | | | | | | | |
| | | | | | Corkwing wrasse and lumpfish (12 fish of each; 4.7% density) | | | | | | | | |
| | | | | | Ballan wrasse and lumpfish (12 fish of each; 4.7% density) | | | | | | | | |
| **Small commercial scale** | Deady et al. 1995 | Oct 1992, 11-13 °C | 10×10×10 m; 1000 $m^3$ | Unspecified | Goldsinny wrasse (24 fish; 0.6% density) | 4200 fish; unspecified size | Yes | 1 | 0 | No | SCUBA diving | Yes | No |
| | | | | | Corkwing wrasse (14 fish; 0.3% density) | | | | | | | | |
| | Deady et al. 1995 | Sept 1992, 16 °C | 10×10×10 m; 1000 $m^3$ | Unspecified | Goldsinny wrasse (50 fish; 0.9% density) | 5500 fish; unspecified size | Yes | 1 | 0 | No | SCUBA diving | Yes | No |
| | Deady et al. 1995 | Mid-Sept 1992, unspecified temperature | 10×10×10 m; 1000 $m^3$ | Unspecified | Corkwing and goldsinny wrasse (1% density) | Unspecified* | No | 1 | 0 | No | SCUBA diving | Yes | No |
| | Tully et al. 1996 | 28 June 1992 to 18 Jan 1993, unspecified temperature | 50-70 m circumference, 10 m depth; 2864 $m^3$ | 2 | Goldsinny wrasse (~166 $cage^{-1}$; ~1% density) | Treatment: 13000-24000 fish; unspecified size<br><br>Control: 20000 fish; unspecified size | Yes | 3 | 3 | No | SCUBA diving | Yes | Yes |
| | Leclercq et al. 2018 | 24 Mar to 1 June 2015, 8.1 ± 0.7°C | 24×24 m square cages, 15-20 m depth inverted pyramid; 8640-11520 $m^3$ | 1 | Farmed lumpfish (2396 fish; 6% density) | 43529 fish; 2059 g | Yes | 1 | N/A | No | 3-D acoustic tag | Yes | No |
| | | | | | Wild caught ballan (58%; 78 g), goldsinny (30%), corkwing (8%), rock cook (4%) and cuckoo (0.9%) wrasse (total 3200 fish; 7% density) | | No | | | | | | |

*While the number of fish was not specified, cage size suggests that the study was conducted at small commercial scale.