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E-mail:xbywb@ouc.edu.cn

Review

Possible Use of Lumpfish to Control *Caligus elongatus* Infestation on Farmed Atlantic Salmon: A Mini Review

IMSLAND Albert K.[1), 2), *], REMEN Mette[3)], BLOCH-HANSEN Karin[4)], SAGERUP Kjetil[4)], MATHISEN Remi[5)], MYKLEBUST Elisabeth A.[6)], and REYNOLDS Patrick[7)]

1) *Akvaplan-niva Iceland Office*, *Akralind* 4, 201 *Kópavogur*, *Iceland*
2) *Department of Biological Sciences*, *University of Bergen*, *High Technology Center*, 5020 *Bergen*, *Norway*
3) *Akvaplan-niva*, *Trondheim Office*, *Havnegata* 9, 7010 *Trondheim*, *Norway*
4) *Akvaplan-niva*, *Framsenteret*, 9296 *Tromsø*, *Norway*
5) *Nordlaks Oppdrett AS*, *Post Box* 224, 8455 *Stokmarknes*, *Norway*
6) *Cermaq Finnmark AS*, *Markedsgata* 3, 9510 *Alta*, *Norway*
7) *GIFAS AS*, *Gildeskål*, 8140 *Inndyr*, *Norway*

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Abstract In this mini review, we systematized current knowledge about the number of *Caligus elongatus* on farmed Atlantic salmon in relation to the use of lumpfish as cleaner fish. The review was prompted by reports of an unusually large number of sea lice (*C. elongatus*) infestation of farmed salmon in northern Norway, Faroese Islands and Iceland and the urgent need to determine if common lumpfish can be used to reduce the number on farmed Atlantic salmon by actively grazing on sea lice. Available data from Norway clearly indicate that lumpfish grazes on *C. elongatus*, and it is possible to enhance this grazing with the assistances of live-feed conditioning prior to sea pen transfer and selective breeding. Observations from Iceland, Faroese Islands and Scotland also indicate that lumpfish can effectively lower infestations of *C. elongatus* on salmon. Overall, this mini review expresses that lumpfish can actively lower the number of *C. elongatus* on farmed Atlantic salmon.

Key words sea lice; *Caligus elongatus*; lumpfish; salmon farming; welfare

1 Introduction

The sea lice, *Lepeophtheirus salmonis* and various *Caligus* species, are ectoparasites of marine finfish (Copepoda: Caligidae). They have a major impact on salmonid aquaculture worldwide (Igboeli *et al*., 2012, 2014); they cause a loss of over €440 million in Norway annually (Abolofia *et al*., 2017). The lice live on the mucus and skin and in the blood of fish, resulting in wounds if not removed. The lice occur naturally on salmon in sea water and were described as early as in the middle of the 18th century (Torrissen *et al*., 2013). However, the problem has escalated with the commercial production of Atlantic salmon (*Salmo salar* L) and rainbow trout (*Oncorhynchus mykiss* Walbaum) in sea cages. The effectiveness of medicinal treatments by either bathing or oral administration may be affected by the development of reduced sensitivity, leading to reducing treatment efficacy. Therefore, more emphasis has being giving to mechanical treatments such as thermolicing and high pressure washing. Biological control using cleaner fish that pick the sea lice from salmonids is effective in reducing lice density and is adopted widely by salmon farming industry. As an alternative of cold-water cleaner fish, the common lumpfish, *Cyclopterus lumpus* L., is currently used to control sea lice infestation (Imsland *et al*., 2014a, b, c, 2015a, b).

The parasitic copepod family Caligidae comprises more than 30 genera (Kabata, 1979; Hemmingsen *et al*., 2020) and more than 450 species (Dojiri and Ho, 2013). Members of two of these genera, *Lepeophtheirus* and *Caligus*, have received notoriety; they have the greatest economic impact among parasite genera in salmonid fish mariculture (Costello, 2006) and have evolved collectively as so called 'sea lice'. Although this notoriety is mainly due to the serious impact of *L. salmonis*, the members of genus *Caligus* are also implicated. Johnson *et al*. (2004) estimated that 61% of copepod infestations in marine and brackish water fish culture are caused by the members of family Caligidae, 40% by the species of *Caligus* and 14% by the species of *Lepeophtheirus*. A major difference between *L. salmonis* and *Caligus* sp. is their host specificity. *L. salmonis* is an obligate parasite of salmonid fish (Ka-

* Corresponding author. E-mail: albert.imsland@akvaplan.niva.no

bata, 1979) whereas many *Caligus* sp. tend to be facultative (Kabata, 1979; Pike and Wadsworth, 1999) and have been found on > 80 fish species (Kabata, 1979). In the central and northern parts of Norway, a high *C. elongatus* Nordmann abundance on farmed fish frequently occurs in autumn (Øines *et al*., 2006). Infections have been assumed to connect to passing schools of pollock *(Pollachius pollachius* L.), saithe (*Pollachius virens* L.) and herring (*Clupea harengus* L.) (á Norði *et al*., 2015).

Mature *C. elongatus* is smaller than mature *L. salmonis* (Piasecki, 1996), and its two sexes are at an equal size (around 6 mm). *C. elongatus* is a much better swimmer than *L. salmonis* and can re-infect other fish species if being removed from its original host (Øines *et al*., 2006; Hemmingsen *et al*., 2020). Hence, determining if mature *C. elongatus* infects species like lumpfish and saithe should help to explain the rapid increase of *C. elongatus* in sea pens during certain periods of year (Heuch *et al*., 2007) in northern Norway. Lumpfish are now extensively used as cleaner fish in northern Norway (Imsland *et al*., 2018), Ireland (Bolton-Warberg, 2018), Scotland (Treasurer *et al*., 2018), Iceland (Steinarson and Árnason, 2018) and the Faroese Island (Eliasen *et al*., 2018). However, to this date, there exists no systematic knowledge and guiding line of the effect of lumpfish on *C. elongatus.* Earlier researches have clearly indicated that lumpfish prefer to graze the adult female *L. salmonis* (Imsland *et al*., 2014a, c, 2016, 2018). However, lumpfish in sea pens can be classified as the strongly opportunistic (Imsland *et al*., 2014c) and they do not restrict themselves to graze a single food source if others exist. They may readily graze on mature sea lice males as well as *C. elongatus.*

In this mini review, we summarized the findings from both small and large scale trials with lumpfish where grazing on *C. elongatus* has been reported in order to give recommendations on the possible use of lumpfish to combat *C. elongatus* on Atlantic salmon in sea pens.

2 Different Densities of Lumpfish: Effect on the Occurrence of *C. elongatus* on Atlantic Salmon in Small Scale Studies

Imsland *et al*. (2014a) investigated the efficacy of lumpfish grazing on attached *C. elongatus* on Atlantic salmon at two different lumpfish densities, 10% and 15%. *C. elongatus* were counted every two weeks during the trial (54 days). To investigate the stomach content of lumpfish, a gastric lavage was performed. The results showed that on day 38, 15% stocked cages had a significantly lower average number (0.72) of *C. elongatus* per salmon compared to that of control (1.18) and 10% stocked cages (1.37) (Tukey's multiple test, $P<0.05$, Fig.1). Similarly, on day 54, 15% stocked cages had a significantly lower average number per fish (0.78) compared to that of control cages (1.35) and 10% stocked cages (1.02) (Tukey's multiple test, $P<0.05$).

Both visual inspection and gastric lavage indicated the consumption of *C. elongatus* in the trial of Imsland *et al*. (2014a). The average number per fish varied throughout the trial although both 10% and 15% stocked cages had 25% and 42% fewer *C. elongatus* lice than controls on day 54. This finding strongly indicated that the presence of lumpfish has lowered the infestations of *C. elongatus* among Atlantic salmon. The results from the gastric lavage used to assess food choices in lumpfish displayed the presence of *C. elongatus* in the stomachs of several fish throughout the study. The proportion of lumpfish with sea lice (*L. salmonis* and *C. elongatus*) increased from 10% on day 11 to 28% on day 54. The number of lumpfish eating lice may in fact be much higher as these values were only determined from lavage fish every 14 days throughout the trial. The number of days between sample points allowed for lumpfish to consume sea lice and fully digest them, thus only giving a snapshot on lice eating. However, the relatively large increase in numbers of lumpfish found with ingested sea lice in their stomachs suggest that the level of grazing intensified throughout the study period. This may be an indicator of some forms of learning or habituation of lumpfish, which was investigated in a follow-up trial (see below).

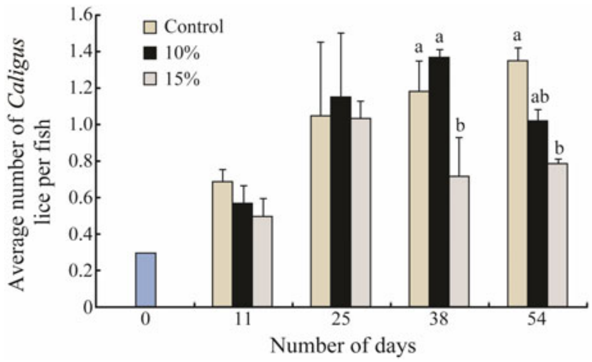

Fig.1 Total average number of *C. elongatus* per fish recorded for each duplicate treatment during each of the sampling dates in the trial of Imsland *et al*. (2014a). Values are presented as means ± S.D. Mean values which do not share a letter are significantly different (ANOVA, Tukey's multiple range test, $P<0.05$). The average number refers to the total number of fish individuals sampled from two cages at each sampling time.

3 Habituation of Lumpfish by Feeding Live Feeds Prior to Transfer to Atlantic Salmon Net Pens: Effect on the Occurrence of *C. elongatus*

Imsland *et al*. (2019) established two groups of individually tagged lumpfish in land-based tanks. One group received marine pelleted feed (MF group) whilst the other received a mix of pelleted feed, live adult Artemia and frozen sea lice (LF group). Sixty lumpfish each group were tagged and transferred to small scale sea pens with 300 Atlantic salmon each, and the occurrence of *C. elongatus* on the salmon was investigated for 62 days. They found that there were significantly less *C. elongatus* stage on salmon from both LF and MF groups on day 34 compared to the control (SNK post hoc test, $P<0.05$, Fig.2). On day 62, there was significantly less *C. elongatus* found on salmon from LF group compared to the control (SNK post hoc test, $P<0.05$) as there was 38% less *C. elongatus* found on salmon reared with LF lumpfish compared to MF lumpfish.

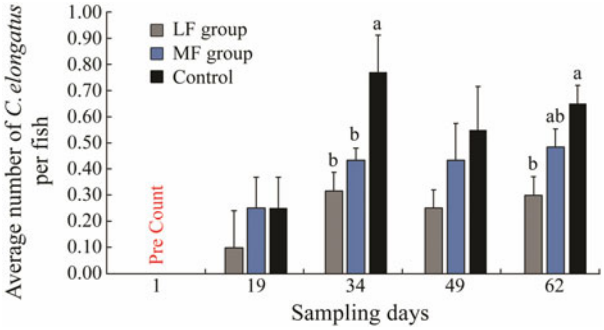

Fig.2 Total average number of *C. elongatus* per Atlantic salmon recorded for each duplicate treatment during each of the sampling dates of the sea pen study carried out by Imsland *et al.* (2019). Values are presented as means ± S.D. Mean values which do not share a letter are significantly different (ANOVA, SNK *post hoc* test, $P<0.05$). The average number refers to the total number of fish individuals sampled from two cages ($n=60$) each group each sampling time.

In the study of Imsland *et al.* (2019), the level of *C. elongatus* was significantly different between control and LF groups, indicating that the dietary treatment influenced the ability of lumpfish to effectively forage on *C. elongatus* as lumpfish conditioned prior to sea pen rearing were nearly 40% more efficient in grazing *C. elongatus* compared to controls. These results provided further supports to previous studies which reported that lumpfish do graze on *C. elongatus* (Imsland *et al.*, 2014a). *C. elongatus* is not included in Norwegian legislation, and there is therefore no legal limit to the level of infestation of *C. elongatus* at which a treatment should be initiated. However, the species has an economic impact in the production cycle of salmon (Boxaspen, 2006). There have been some concerns on using lumpfish as a cleaner fish; the fish is considered to be a preferred host of *C. elongatus* (Heuch *et al.*, 2007; Mitamura *et al.*, 2012), and lumpfish has the potential to act as a vector of *C. elongatus* that can infect salmon (Powell *et al.*, 2017). These concerns can be reduced if lumpfish graze indiscriminately on both species of lice and domesticated lumpfish free of *C. elongatus* were introduced into sea cages.

4 Lumpfish Grazing on *C. elongatus*: Possible Parental Control

Possible heritable component of *C. elongatus* grazing was investigated in two recent trials. Imsland *et al.* (2016) investigated possible parental control in grazing of *C. elongatus* in nine families of lumpfish distributed in duplicates among nine small sea cages stocked with 400 Atlantic salmon each. During the trial (78 d), gastric lavage was performed every two weeks to assess the feeding preference of individual lumpfish. Although *C. elongatus* infestation rate was found to be very low in the study (Fig.3), the percentage of lumpfish found to have consumed *C. elongatus* varied significantly between families, indicating a possible parental control of *C. elongatus* grazing.

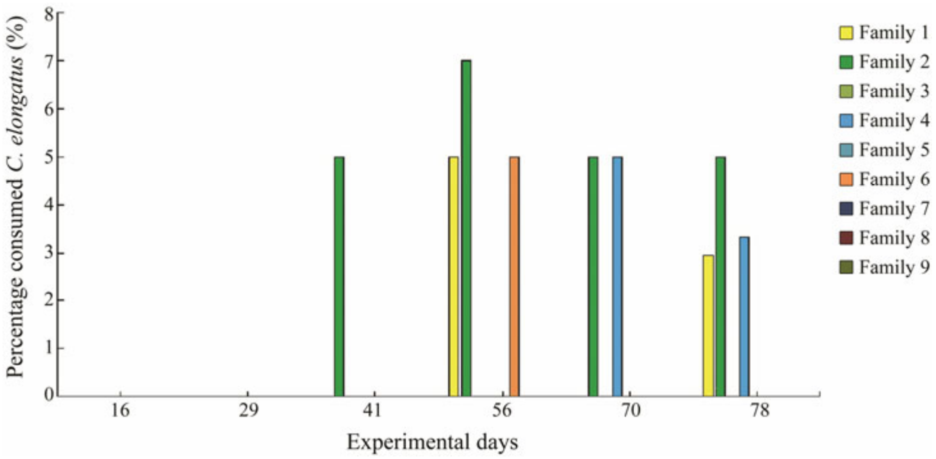

Fig.3 Mean percentage values of *C. elongatus* found in nine lumpfish families sampled at each sampling time point. Data are from Imsland *et al.* (2016).

In a study carried out by Imsland *et al.* (unpublished), 10 families of lumpfish, 480 individuals each, 46.5±4.3 g in mean weight, were distributed into ten sea cages (5 m × 5 m × 5 m) stocked with 400 Atlantic salmon each, 387.3 ± 10.3 g in mean weight. From each family, 20 lumpfish were stocked into one of 10 sea cages and 20 into another cage, thus establishing duplicate treatments each family, two families each cage. During the trial (73 d), gastric lavage was performed every two weeks to assess the feeding preference of lumpfish individuals.

Consumption of *C. elongatus* varied between families (Fig.4). Seven of the ten families were found to consume *C. elongatus* on day 18. Percentage of lumpfish that had consumed *C. elongatus* varied between 2% and 11% on day 18. On day 62, between 5% and 40% of lumpfish were found with *C. elongatus* in their stomach. Families 5 and 6 (half-siblings, same father) had the highest consumption of *C. elongatus* throughout the study (Fig.4).

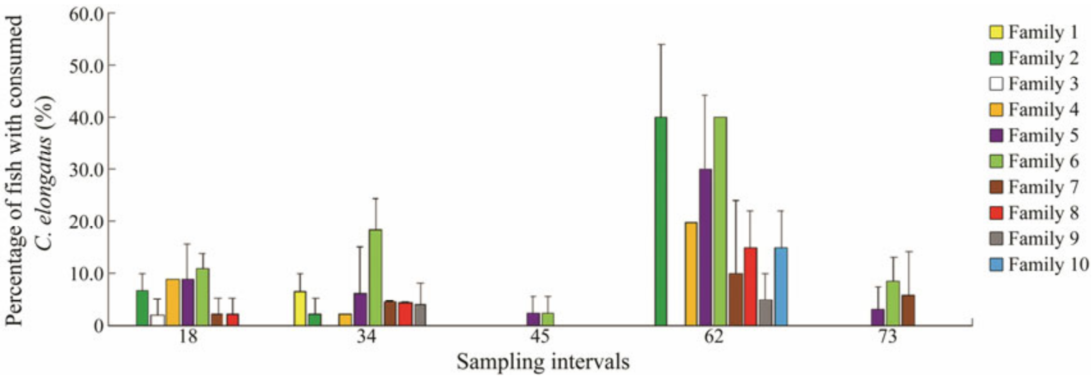

Fig.4 Percentage values of *C. elongatus* found in stomach of lumpfish of ten families sampled at each sampling time point. Values are presented as means ± S.D. Data are from Imsland *et al*. (unpublished).

Given the difference in consumption of *C. elongatus* in two family trials and other natural source (see Imsland *et al*., 2016 for details), it seems that some lumpfish may be more predisposed in actively seeking natural food sources, including that *C. elongatus* should have a genetic basis to underline such a behaviour. It is well known that behavioural traits respond to both natural and sexual selection. Fish from families 5 and 6 in the second trial where consumption of *C. elongatus* was much more pronounced shared the same father but had different mothers. Given that these differences had a degree of genetic influence, then it would appear more likely that this difference was passed through paternal rather than maternal lines. Recent studies have revealed both maternal (Royle *et al*., 2012) and paternal (McGhee and Bell, 2014) effects on offspring behaviour *via* epigenetic alterations of genome.

Results from two family trials indicated that consumption of *C. elongatus* varies among families. The families with the highest consumption of *C. elongatus* (trial 1, family 2; trial 2, families 5 and 6) were also those with the highest consumption of *L. salmonis*. Although energy rich salmon pellets were available, family 2 in trial 1 and families 5 and 6 in trial 2 preferred natural pray to a larger extent than other families did. This confirmed that the genetic influence of sea lice consumption can be strong (Imsland *et al*., 2016). Given the difference in the consumption of natural *C. elongatus* among families, we may speculate that these fish are more disposed to seek out natural food sources. If this behaviour has a genetic basis, it may be further enhanced through selection and targeted breeding.

5 The Effect of Lumpfish on *C. elongatus* Incidence on Atlantic Salmon: Large-Scale Observations

5.1 Large-Scale Trial at Lerøy Aurora, Troms, Norway

Imsland *et al*. (2018) performed a large-scale trial at a commercial Atlantic salmon sea farm (69.80˚N, 19.41˚E) (Lerøy Aurora, Troms County, Norway) from October 6, 2015 to May 17, 2016. The experiment was conducted in eight large sea cages (130 m in circumference, 37688 m^3 in volume) holding 193304±2089 smolts (Atlantic salmon) each, initial mean body weight 198 g±20 g. Lumpfish were stocked at densities 4%, 6% and 8%, respectively, in duplicate sea cages. During the trial, *C. elongatus* on 240 salmon were counted every two weeks.

The level of *C. elongatus* rose in all groups in autumn (Fig.5). Significantly, a lower level of *C. elongatus* was seen in lumpfish groups from late February to early April (Student-Newman-Keuls post hoc test, $P<0.05$, Fig.5). In April, *C. elongatus* level decreased in all experimental groups, and the final level in May was similar to the initial ones in October last year.

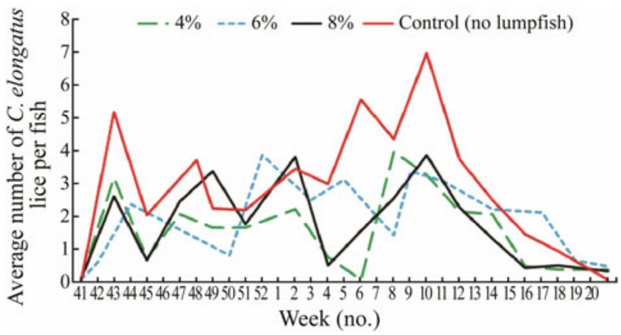

Fig.5 Occurrence of *C. elongatus* per salmon ($n=60$) each group each sampling point in large scale sea cages at Lerøy Aurora, northern Norway, with 0 (control), 4%, 6% and 8% of lumpfish recorded each duplicate treatment each sampling dates (bi-weekly).

5.2 Large-Scale Observations at Nordlaks, Nordland, Norway

A large-scale observation was performed at a commercial Atlantic salmon sea farm (68.40˚N, 15.11˚E) (Nordlaks, Nordland county, Norway) from July 1, 2017 to February 2, 2018. The observation was conducted in 12 large sea cages (160 m in circumference, 35000 m^3 in volume) holding smolts (Atlantic salmon). Two nearby locations, Finnkjerka and Mollgavlen, in the same seawater basin (10 km between them) were monitored. At Finnkjerka, there were six sea pens holding 198250±3200 salmon smolts each, initial mean body weight, 75 g±9 g, in October, 2016. In July 2017, about 12000 lumpfish each pen, mean weight, 32 g±3 g, were released to all these sea pens. In the nearby location of Mollgavlen, there were six sea pens holding 164724±8632 salmon smolts each, initial mean body weight, 76 g ± 12 g in October, 2016. Every other week during observation, thirty salmon each sea pen were sedated and weighted individually with lice on them recorded. After counting, the lice remaining in container if any were also recorded. Lice were registered in 4 categories: 1) *Lepeophtheirus salmonis*, adult female; 2) *L. salmonis*, pre-adult; 3) *L. salmonis*, Chalimus; 4) *Caligus elongatus*.

Overall, less *C. elongatus* were found on the salmon in pens with lumpfish (Finnkjerka location) compared with those without lumpfish (Fig.6). This effect was most evident in winter and spring and also in summer; *C. elongatus* increased at Mollgavlen (without lumpfish) from July whereas such increase was not seen at nearby Finnkjerka (with lumpfish in sea pens). Overall, there were more sea lice challenges at Mollgavlen, resulting in approximately 600 g loss of final slaughtering weight of salmon.

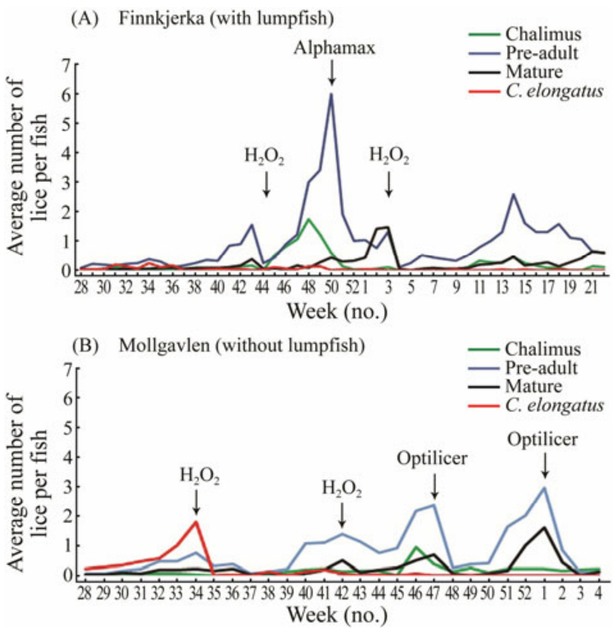

Fig.6 Sea lice development at two production sites of Nordlaks in northern Norway 2017–2018. Arrows indicate mechanical and chemotherapeutical delouse operations during the observation period.

The relatively high number of *C. elongatus* observed in two large-scale studies was the indicative of all production sites, as these lice are known to transfer easily between fish (Heuch *et al*., 2007). Despite the presence of lumpfish, there were a sufficiently high number of lice elsewhere at the site to allow continual re-colonization in the cages stocked with lumpfish. Nevertheless, the mean number of *C. elongatus* was lower in groups with lumpfish at both salmon farms. The added positive effect of lumpfish at the Nordlaks production sites was around 600 g gain of slaughter weight (3.82 kg *vs*. 3.18 kg) at the location with lumpfish and this is almost surely linked to less problems with sea lice (both *L. salmonis* and *C. elongatus*) at this location.

6 Summary

6.1 Lumpfish Efficacy for *C. elongatus* Removal

To summarize the relationship between the use of lumpfish and the occurrence of *C. elongatus* on Atlantic salmon, we have compiled current knowledge from the published literatures and reports and by interviewing fish health care persons in Atlantic salmon farming industry in Table 1. Available data clearly indicates that lumpfish grazes on *C. elongatus* and it is possible to enhance this grazing with the assistance of live-feed conditioning prior to sea-pen transfer and selective breeding. Grazing is observed in various size classes (25 g to 550 g), at temperatures ranging from 4 to 13℃ and in all seasons. Majority of published data come from northern Norway. There are also indications from the Faroe Islands, Scotland and Iceland that lumpfish grazes on *C. elongatus*. In the Faroe Islands, an investigation into 5511 lumpfish stomach (Eliasen *et al*., 2018) showed that *L. salmonis* was found in 13.5% of 743 individuals, of them, around 80% had also *C. elongatus* in their stomach (Kirsten Eliasen, Fiskaaling, Faroe Islands, pers. comm.). The consensus in the salmon farming industry in Faroe Islands is that lumpfish is effective in reducing the number of *C. elongatus*, but the infestation pattern is different from that of *L. salmonis*. Lumpfish is not systematically used as a biological delouser for *C. elongatus* (Kirsten Eliasen, Fiskaaling, Faroe Islands, pers. comm.). In Scotland, the *C. elongatus* number can be seasonally important, but the efficacy of cleaner fish to *C. elongatus* can be difficult to assess in summer as *C. elongatus* continue to re-infect from a range of wild fish species. Even after bath treatments, re-infestation of *C. elongatus* can be rapid (Jim Treasurer, FAI Aquaculture, Scotland, pers. comm.). In the Westfjords area of Iceland, *C. elongatus* infestations are presently considered a more severe problem than the *L. salmonis* and the number of *C. elongatus* on salmon can be high (> 10) in late autumn (October – November) (Eva D. Jóhannesdóttir, Arctic Sea Farm Ltd., pers. comm., Hjörtur Methúsalemsson, Arnarlax Ltd., pers. comm.). In this area, large scale trials have clearly shown that lumpfish is very effective in lowering the number of *C. elongatus*.

6.2 Lumpfish and *C. elongatus*: Survey from the Salmon Farming Industry

To investigate in more details the possible effect of lumpfish on *C. elongatus* on Atlantic salmon, we conducted a survey by interviewing fish health personnel and biological controllers working in salmon farming industry in Norway (n=18), Faroe Islands (n=5) and Iceland (n=2) (https://www.fhf.no/prosjekter/prosjektbasen/901539/). In Norway, we interviewed persons working in companies in northern Norway (*i.e*., from Production Areas (PA) 9–13, see Fig.1 in Overton *et al*., 2018). The survey findings showed that almost all participants agreed that lumpfish grazes on *C. elongatus* (Fig.7A), but the extent of grazing is unclear. On the Faroes Islands, all participants agreed that lumpfish grazes to a large extent on *C. elongatus* whereas in northern Norway the views can be divided into large extent and some extent (Fig.7A). The survey on whether the grazing of lumpfish leads to reduction of *C. elongatus* on Atlantic salmon showed different views on the extent of

Table 1 A summary of the current literature (peer-reviewed journal articles and scientific reports) and observations (including pers. comm.) on experiments with lumpfish and its effect on *C. elongatus* infestations on farmed Atlantic salmon

Citation	Experimental period and temperature	Experimental unit (number and size)	Experimental site/country	Size and density of lumpfish	Effect investigated	Effect on *C. elongatus* found?
Imsland *et al.* (2014a)	Jun.–Aug., 9.0–12.1℃	4 small sea cages 5 m×5 m×5 m (125 m^3)	Nordland county, Norway	53–182 g 10% and 15%	Different density of lumpfish	Yes, increased effect at 15% density
Imsland *et al.* (2019)	May–Jul., 7.2–13.3℃	4 small sea cages 5 m×5 m×5 m (125 m^3)	Nordland county, Norway	114–180 g 10%	Habitation of lumpfish	Yes, and habitation of lumpfish increased the effect
Imsland *et al.* (2016)	May–Aug., 7.1–13.2℃	9 small sea cages, 5 m×5 m×5 m (125 m^3)	Nordland county, Norway	169–549 g 10%	Different families, parental effect	Yes, and varied between families
Imsland *et al.* (unpublished data)	Sep.–Dec., 10.5–6.8℃	10 small sea cages, 5 m×5 m×5 m (125 m^3)	Nordland county, Norway	30–123 g, 12%	Different families	Yes, and varied between families
Imsland *et al.* (unpublished data)	July–Jan., 12.2–5.5℃	12 large sea cages (160 m circumference, 58900 m^3 volume)	Nordlaks AS, Nordland county, Norway	32–157 g, 6%	Large scale evaluation of sea lice grazing in lumpfish	Yes
Imsland *et al.* (2018)	Oct.–May, 8.3℃ in Oct., 3.6℃ in Mar., 6.8℃ in May	8 large sea cages (130 m circumference, 37688 m^3 volume)	Lerøy Aurora AS, Troms county, Norway	25–115 g, 4%, 6% and 8%	Different densities of lumpfish in large scale sea cages	Yes, similar at all densities
Eliasen *et al.* (2018), Kirsten Eliasen, Fiskaaling, Faroe Islands, pers. comm.)	Year round, 6–11℃	Large sea cages from 9 farming sites	Faroe Islands	13–545 g, Density not given	Cleaning efficacy of lumpfish in relation to size and season	Yes, around 80% of those that graze on *L. salmonis* graze on *C. elongatus*
Eva Dögg Jóhannesdóttir, Arctic Fish Ltd. (pers. comm.)	Jun.–Dec., 10.1–3.2℃	7 sea pens (160 m circumference)	Iceland, Dýrafjörður	20–255 g, 8%–10%	Comparison of sea lice at sites with and without lumpfish	Yes, significantly lower *C. elongatus* with lumpfish present
Hjörtur Methúsalemsson, Arnarlax Ltd. (pers. comm.)	Sep.–Sep. (one year) 1.9℃ (Feb.), 11.1℃ (early Sep.)	12 sea pens (160 m circumference)	Iceland, Arnarfjörður	32–340 g 8%–10%	Comparison of sea lice at sites with and without lumpfish	Yes, significantly lower *C. elongatus* with lumpfish present

Note: Data include experimental period and temperature, experimental unit, experimental site/country, stocking density of lumpfish, effect investigated and effect if any.

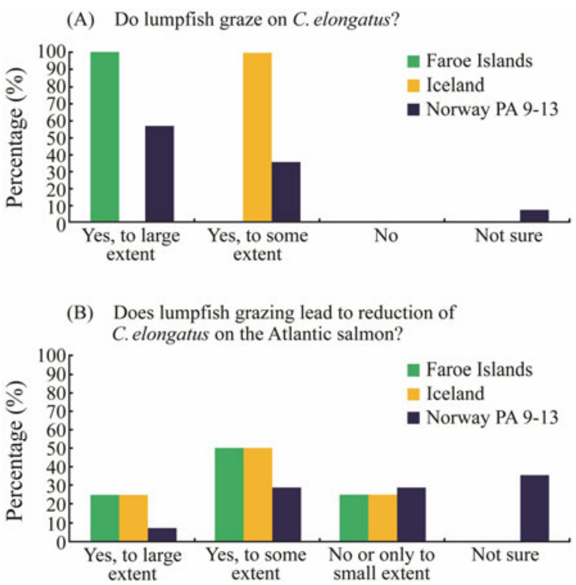

Fig.7 Results from interview survey of fish health care person and biological controllers working in the salmon farming industry in Norway ($n=18$), Faroe Islands ($n=4$) and Iceland ($n=2$).

C. elongatus reduction on salmon (Fig.7B). The majority in all three countries think that the grazing reduces *C. elongatus* on salmon to a large or some extent. In all three countries, it was commented that the lumpfish influences the number of *C. elongatus* if the number of *C. elongatus* on salmon is moderate or low.

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