Complexities in commercial scale use of non-invasive controls against parasites in aquaculture

Lena Geitung

Thesis for the degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
2021

UNIVERSITY OF BERGEN

Complexities in commercial scale use of non-invasive controls against parasites in aquaculture

Lena Geitung

Thesis for the degree of Philosophiae Doctor (PhD)
at the University of Bergen

Date of defense: 30.06.2021

Year: 2021

Title: Complexities in commercial scale use of non-invasive controls against parasites in aquaculture

Name: Lena Geitung

Print: Skipnes Kommunikasjon / University of Bergen

Scientific environment

The present thesis and experiments were conducted at Bremnes Seashore AS commercial salmon sea cage farm sites Låva and Prestholmane (Stavanger, Norway) and sea cages of Institute of Marine Research (IMR), Matre/Solheim site (Masfjorden, Norway) and Austevoll/Sauganeset (Norway) in cooperation with the Department of Biology, University of Bergen (UiB). The experiments were funded by Bremnes Seashore AS through time-restricted research and development licenses and SkatteFUNN #279226, Research council of Norway project #256318 and #267800 (Future Welfare) and Ministry of Trade, Industry and Fisheries through IMR's Surveillance of Fish Welfare #14930.

The work has been carried out under the supervision of Dr Daniel William Wright (IMR), Dr Frode Oppedal (IMR), Dr Lars Helge Stien (IMR) and Prof. Egil Karlsbakk (UiB) with input from Geir Magne Knudsen (R&D manager at Bremnes Seashore AS) in the period 2017–2021.

BREMNES SEASHORE

Acknowledgements

This thesis would not have been possible without the help of a number of people. First, I would like to thank Bremnes Seashore, with a special thanks to Geir Magne Knutsen and Frode Oppedal, for giving me the opportunity and the funding to pursue this industrial Ph.D. I would also like to thank UiB and IMR for letting me use their facilities and resources.

A magnitude of thanks goes to my four supervisors, Daniel William Wright, Frode Oppedal, Lars Helge Stien and Egil Karlsbakk, for all your support, guidance and patience throughout these years. Thank you for always making time for me, for sharing your knowledge and giving input during planning and execution of experiments, revisions of manuscripts and guiding throughout the publication process. I could not have asked for a better supervisor team! A special thanks here goes to Danny for always being available and helping out with every aspect of this thesis coming together. I would also like to thank all of my co-authors for making the papers in this thesis possible, as well as the anonymous reviewers for their feedback to the manuscripts.

I am extremely grateful for all the fantastic colleagues at both Bremnes Seashore and IMR, for their contributions to the work and for making this an enjoyable experience. I would also like to thank everybody at Bremnes Seashore sites Låva and Prestholmane and IMR sites Sauganeset and Solheim for a helping hand during sample collection, with a special mention to site managers Jarle Langvik and Hanne Grytdal for always accommodating for us and having a solution to every problem. Thank you to all the great people at Matre Research station for always making me feel welcome when I was visiting. Last, but definitely not least, I would like to thank Velimir Nola, for immense help in executing experiments, invaluable technical assistance and for making lice counting an enjoyable affair.

Finally, I wish to thank my brilliant family for their never-ending encouragement and support, the much needed “pull yourself together’s” and for always letting me shoot almonds at you. A million thanks to my amazing friends for always being there with when I needed a break and listening to all my fish-rambling. You are the absolute best!

Contents

Abstract

Salmon lice (*Lepeophtheirus salmonis*) are one of the major challenges faced by the Atlantic salmon (*Salmo salar*) aquaculture industry. Due to the risk of poor welfare outcomes and high mortality during treatments against salmon lice, as well as increasing resistance towards many of the available chemical therapeutants, prophylactic measures that mismatch host and parasite environments are emerging. For salmon lice, these depth-based strategies exploit the positioning of free-living lice larvae in the upper part of the water column before they attach to salmon skin. They work by uncoupling salmon from mostly surface-dwelling lice larvae while still providing surface air access required for salmon swim bladder reinflation, buoyancy control and optimal welfare.

One of the most extensively studied depth-prevention technologies is the snorkel sea cage. It consists of a standard cage fitted with a roof net to keep fish deeper and an enclosed tarpaulin tube (a snorkel), where salmon have access to the surface air used for filling their swimbladder while still avoiding surface waters where lice larvae are most abundant. Previous work show they can reduce salmon lice infestation levels in sea cages without major impacts on salmon welfare. However, long full-scale studies, which are crucial to understand the real-world consequences of these technologies on salmon lice infestation, are lacking. Knowledge is also needed on i) how additional lice removal strategies might work in combination with lice prevention technologies and ii) the effects of these controls on other co-occurring salmon parasites.

The purpose of this thesis was to examine the impact of commercial-scale snorkel sea cages on external (*L. salmonis* and *Paramoeba perurans*) and internal parasites (*Eubothrium* sp.) of Atlantic salmon and investigate possible *in situ* control methods (cleaner fish and optical laser) for reducing remaining salmon lice infestations that develop. This knowledge will help reveal the successes, challenges, and solutions in managing parasites with snorkel sea cages in salmon farming and will provide insights on the ramifications of other lice barrier technologies combating the salmon lice problem.

In a study observing the long-term effects of depth-based technology at commercial scale, salmon lice infestations on Atlantic salmon were examined in triplicate snorkel and standard sea cages over a 12–month production cycle. Snorkel sea cages reduced newly settling lice on Atlantic salmon by 75 % and salmon lice treatments by nearly half throughout the study, confirming that snorkel sea cages can effectively control lice over commercial production cycles. Lice reductions depended on an environment free of layering with surface brackish water (salinities < 28 ppt) and warm water (temperatures > 16 °C), highlighting the importance of considering local environment conditions when applying depth-based prevention technologies.

With the potential for depth-based technologies to influence salmon parasites other than lice, we document that snorkel sea cages reduced both prevalence and abundance of marine tapeworms (*Eubothrium* sp.) in salmon guts. In a study comparing commercial snorkel and standard sea cages, tapeworm prevalence was 3–5 times lower and tapeworm abundance 10–20 times lower in snorkel sea cages. In separate studies, there are indications that the presence of snorkels might increase the risk and intensity of infestation by the marine amoeba *P. perurans*, the causative agent of amoebic gill disease (AGD). This problem seems to increase with shielding depth. However, creating a low salinity surface layer inside the snorkel may limit these infestations if salmon enter for sufficient time to reduce AGD levels.

Continuous deployment of lice-eating cleaner fish and lice-shooting optical lasers are increasingly used to remove lice from farmed salmon. However, information about their effects are lacking. This is especially important in depth-based prevention cages, in which adding efficient lice reducing controls could prevent the need for removing the prevention technology to perform other de-licing procedures, saving time and effort for salmon farmers. In this thesis we document that using optical lasers in combination with 16 m deep snorkel sea cages during winter did not lower the infestation density of mobile salmon lice compared to cages without laser nodes installed. Additionally, based on high mortalities and minimal feeding by ballan wrasse and a possible mismatch between lumpfish and salmon swimming depths in standard salmon cages, which may be even more pronounced in depth-based

prevention cages, it was suggested that cleaner fish may have low effectiveness against salmon lice over autumn-winter.

The main conclusions from this thesis is that snorkel sea cages have the potential to reduce both salmon lice and marine tapeworm infestations in commercial scale sea cages, while the risk and intensity of AGD seem to be increased compared to standard cages. However, freshwater filling inside the snorkel show promise as an *in situ* control method for the amoeba. On the other hand, even with several options available (e.g. cleaner fish and optical delousing), none of the *in situ* lice control methods stood out as a clear lice removal method to be used in combination with preventive technology. Optical lasers did not reduce lice compared to cages without lasers and cleaner fish experience high mortality, poor welfare and possible opposing depth distribution to salmon. More work focussing on depth distribution for both cleaner fish and salmon is needed to improve the efficiency of the lice removal options for depth-based prevention technologies.

List of Publications

I. Geitung, L., Oppedal, F., Stien, L.H., Dempster, T., Karlsbakk, E., Nola, V., Wright, D.W. (2019) Snorkel sea cage technology decreases salmon louse infestation by 75% in a full-cycle commercial test. *International Journal for Parasitology* 49, 843–846. https://doi.org/10.1016/j.ijpara.2019.06.003

II. Geitung, L., Wright, D. W., Stien, L. H., Oppedal, F., Karlsbakk, E. (*under review*) - Tapeworm (*Eubothrium* sp.) infestation in sea caged Atlantic salmon decreased by lice barrier snorkels during a commercial-scale study.

III. Wright, D. W.*, Geitung, L.*, Karlsbakk, E., Stien, L. H., Dempster, T., Oldham, T., Nola, V., Oppedal, F. (2018) – Surface environment modification in Atlantic salmon sea-cages: effects on amoebic gill disease, salmon lice, growth and welfare. *Aquaculture Environment Interactions* 10, 255–265. https://doi.org/10.3354/aei00269

*Joint first author

IV. Bui, S., Geitung, L., Oppedal, F., Barrett, L. T. (2020) – Salmon lice survive the straight shooter: a commercial scale sea cage trial of laser delousing. *Preventive Veterinary Medicine* 181, 105063. https://doi.org/10.1016/j.prevetmed.2020.105063

V. Geitung, L., Wright, D. W., Oppedal, F., Stien, L. H., Vågseth, T., Madaro, A. (2020) – Cleaner fish growth, welfare and survival in Atlantic salmon sea cages during an autumn-winter production. *Aquaculture* 528, 735623. https://doi.org/10.1016/j.aquaculture.2020.735623

1. Introduction

1.1 General introduction

Today aquaculture is one of the fastest growing food sectors in the world and is predicted to become the main source of marine food for humans by 2050 (FAO, 2018; Stentiford et al., 2020). As the global human population increases, so too is the demand for aquatic food products. With capture fisheries yield stagnating since the 1980s and reports that >30 % of marine fish stocks are overfished (FAO, 2018), aquaculture is now considered to be the best option for meeting these growing demands.

One of the most successful aquaculture species is the Atlantic salmon (*Salmo salar*). Since production started in Norway in the 1970s it has grown from a few thousand tonnes per year to about 2.4 million tonnes per year in 2018 and is now a key industry in several countries; Norway, Chile, Tasmania, Canada, Scotland, Faroe Islands and Iceland (FAO, 2020). Norway is the leading country in salmon aquaculture producing >50% of the global production, with approximately 1.3 million tonnes sold at a value of 64.5 billion NOK in 2018 (Norwegian Directorate of Fisheries, 2020b). Presuming that it is environmentally sustainable, the Norwegian government has ambitions to increase production with 5 million tonnes by 2050 (Sandvik et al., 2020). However, concerns around environmental impacts have halted salmon farming growth over recent years (**Fig. 1**).

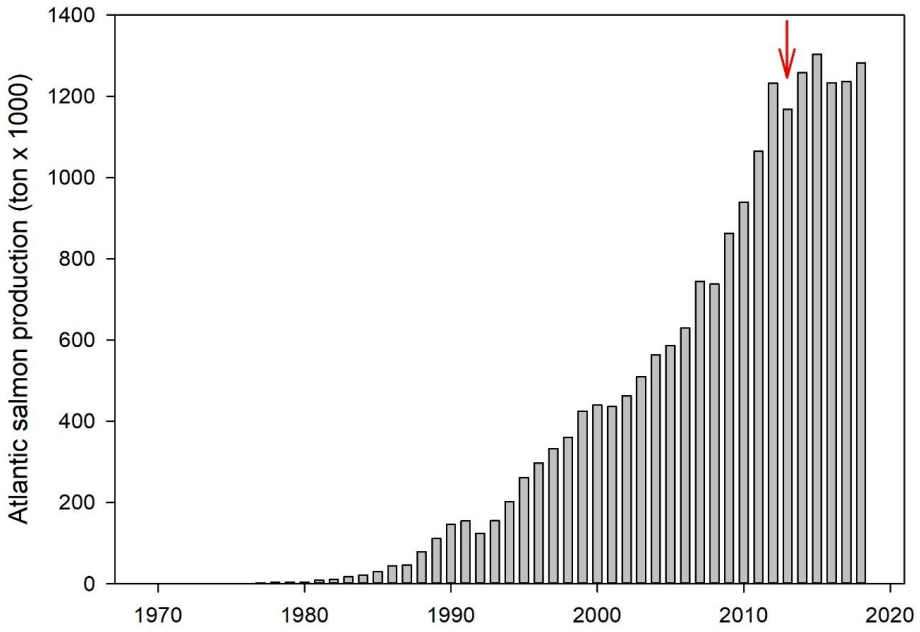


Figure 1. *Salmon production (tonnes) in Norway from 1970–2018 (data taken from FAO, 2020). Arrow represents when salmon lice regulation was introduced by the Norwegian government.*

Intensive animal farming systems can often experience problems with parasite proliferation, causing production and profit losses and poor animal welfare (Barber, 2007; Jansen et al., 2012; Overton et al., 2018a) This often results from abnormally high host densities increasing the risk of infections (Arneberg et al., 1998; Krkošek, 2010) and constraints placed on the natural anti-parasite behaviours of hosts which normally avoid or minimise contact with parasites (Hart, 1990; Moore, 2002; Barber, 2007). Understanding these behaviours has created opportunities to spatially separate parasite and host to reduce parasite encounters and infections (Bui et al., 2019). Such methods may be difficult to apply in marine animal farming systems where animal enclosures are open to the surrounding environment allowing parasite entry and their rapid spread over broad geographical scales (McCallum et al., 2003; McCallum et al., 2004). However, success has come in the form of novel sea-cage designs or host

behaviour manipulations aiming to mismatch depths of farmed fish hosts from those of parasites in free-living stages (Bui et al., 2019). These preventive methods appear fruitful against the primary parasite issue in salmon farming, the salmon lice *Lepeophtheirus salmonis*.

1.2 Salmon lice

Sea lice is the common name for several marine ectoparasitic copepods belonging to the family Caligidae. Among these is the salmon louse (*Lepeophtheirus salmonis*) which naturally occurs in the northern hemisphere and is divided into two subspecies, the Pacific *L. salmonis oncorhynchi* and the Atlantic *L. salmonis salmonis* (Skern-Mauritzen et al. 2014). Both subspecies live as specialized parasites of salmonids.

The life cycle of salmon lice comprises of 8 life stages, with both free-swimming (two planktonic nauplii stages, one infective copepodid stage) and host-attached stages (two attached chalimus stages, two mobile preadult stages, one mobile adult stage) (Hamre et al., 2013). The nauplius larvae hatch directly from eggs carried in a pair of eggstrings that are extruded from the abdomen of the adult female. There are two non-feeding planktonic nauplius stages before moulting into free-living copepodids, the infective stage. The hatched larvae live entirely on energy reserves and need to find and attach to a host before these reserves are depleted (Tully, 1992). Lice development is temperature dependent (Hamre et al., 2019) with energy reserves lasting longer at colder temperatures and enabling dispersal long distances during winter compared to summer (Samsing et al., 2017).

The free-swimming stages of salmon lice are dispersed by water currents. The lice larvae are not able to swim against this current, but they are able to adjust their vertical depth to some degree. Infective copepodids can vertically migrate into surface waters using positive phototaxis and possibly geotaxis (Bron et al., 1993; Heuch et al., 1995) at average swimming speeds of 1.55 mm s^{-1} (Heuch et al., 1995). Since migrating salmon are observed swimming at shallow depths (LaBar et al., 1978; Rikardsen et al., 2007; Plantalech Manella et al., 2009; Strøm et al., 2018) this

could be an adaptation to improve host encounter rates (Johannessen, 1977; Heuch et al., 1995). Salinity also alters the vertical distribution of salmon lice, with both nauplii and copepods displaying a preference for full salinity water (Heuch et al., 1995; Crosbie et al., 2019) as low salinities can be lethal (Bricknell et al., 2006; Sievers et al., 2019). They are therefore often found to aggregate just below the halocline (Crosbie et al., 2019). Nauplii show greater avoidance of low salinities than copepodids, which can still be found at salinities down to 16 ppt (Crosbie et al., 2019). The copepodid is the infective stage and possibly need a higher tolerance toward low salinities than nauplii as salmon often swim at shallow depths where brackish waters often occur. Copepodids showed no obvious temperature preference, while nauplii showed a preference towards low temperatures (Crosbie et al., 2020). Coates et al. (2020) demonstrated that lice respond strongly to hydrostatic pressure; an increase in pressure, equivalent of 5 and 10 m depth, doubled the number of lice that migrated to the top of vertical columns. The distribution of salmon lice copepodids is therefore assumed to be the result of hydrodynamic forces and copepodids swimming towards the surface or avoiding unfavourable salinities.

After successful infestation the copepodid and the remaining life stages feed and develop on the fish host (Hamre et al., 2013). There are two sessile stages (chalimus) that are attached to the fish by protein filaments and three mobile stages (pre-adult and adult) that are able to move around on the host using their cephalothorax region as a suction cup if in danger of being detached (Kabata, 1982). All stages feed on the skin, blood and mucus of the salmon (Costello, 2006), but pathology has mainly been associated with the larger mobile stages (Jones et al., 1990; Jónsdóttir et al., 1992; Grimnes and Jakobsen, 1996). Lice infestation negatively affects the welfare of hosts as it can lead to physical damage, skin lesions, osmoregulatory constraints, secondary infections, immunosuppression, chronic stress, decreased growth and worst-case scenario death for the host (Grimnes and Jakobsen, 1996; Tully and Nolan, 2002; Costello, 2006; Torrissen et al., 2013; Bui et al., 2016; Fjelldal et al., 2020). In addition to pathology on the host, lice can reduce harvest quality for salmon farmers.

Infestations by salmon lice have been a persistent problem in Norway for over 50 years, with the first outbreaks soon after salmon cage culture began (Braaten, 1975; Brandal and Egidius, 1977). Salmon lice are a natural part of the environment and wild salmon returning to rivers are usually infested, with several accounts of this occuring prior to salmon farming and in areas with few farms (Torrissen et al., 2013). However, the amplification and constant availability of hosts due to industry growth has drastically increased salmon lice densities, posing a serious risk for wild salmon populations (Krkošek et al., 2011; Krkošek et al., 2013; Kristoffersen et al., 2018). Since the 1970s, total abundance of wild Atlantic salmon populations has declined (Chaput, 2012; ICES, 2020) and the proportion of salmon returning to rivers has more than halved (Anon., 2019). The decline has mainly been attributed to escaped farmed salmon and the proliferation of salmon lice from fish farms (Forseth et al., 2017).

To reduce the environmental impact of salmon lice infestations in aquaculture, the Norwegian government has implemented strict regulations. When lice infestations exceed an average of 0.5 adult females per fish (0.2 adult females during the out-migration of wild salmon, weeks 16–21) farmers are required to intervene and delouse (Lovdata, 2012). In addition, the Norwegian lice surveillance program require farms to develop a plan for management of salmon lice, which include describing regional routines for delousing operations, evaluating treatment efficacy and fallowing (Lovdata, 2012). Furthermore, Norwegian authorities have introduced production volume limits in 13 defined production zones along the coast with a new "traffic light system" (Vollset et al., 2017; Myksvoll et al., 2018). In essense this regulation determines whether production in a zone is allowed to grow, keep its current production or must decrease production. It is based on the percentage of wild salmon estimated to die due to salmon lice in each production zone (<10 % = increased production (green), 10–30 % = no change in production (yellow), >30 % = reduced production (red)). In order to meet these regulations, the Norwegian salmon industry spent more than 5 billion NOK in 2015 in attempts to control the salmon lice

(Brooker et al., 2018). Consequently, there has been considerable research and investment into new prevention and treatment methods.

1.3 Sea lice treatments

When managing salmon lice in commercial salmon sea cages, the primary approach is to monitor lice abundance on farmed fish through weekly lice counts and delouse when allowable limits are approached or exceeded. Since the introduction of chemotherapeutants in the 1970s, the industry has relied on chemicals to treat against salmon lice (Burka et al., 1997; Aaen et al., 2015). Chemical treatments can be divided into two main categories: a) bath treatments with neurotoxins (organophosphates and pyrethroids) and hydrogen peroxide or b) oral treatment with medicated feed (emamectinbenzoate, diflubenzuron and teflubenzuon) (Burridge et al., 2010; Aaen et al., 2015). Bath treatments are performed either by adding chemotherapeutants directly into sea cages by lining a tarpaulin around the cage or by pumping fish into a well-boat, where the chemoterapeutant is then added (Overton et al., 2018a). After treatments chemotherapeutants are traditionally discarded into the surrounding water, although new technology development is currently underway to filter treatment water before it is released to the sea (Moore, 2021). For oral treatments the chemotherapeutant is added to the feed which is then administered as normal for a recommended treatment time. Waste feed and some active ingredient from feaces will spread to surrounding waters (Burridge et al., 2010). Oral treatments are less time-consuming and resource-intensive than bath treatments, but fish often require longer retention time before harvest to ensure product is chemical-free and treatment success can be variable due to differences in appetite and size of fish in sea cages.

In recent years, salmon lice have begun developing a resistance towards many of the chemotherapeutants used (Grøntvedt et al., 2013; Aaen et al., 2015; Helgesen et al., 2015) rendering most less effective. In addition, some chemoterapeutants may have an environmental impact, and there are concerns about both bioaccumulation in the surrounding environment and possible negative effects on non-target species

(Burridge et al., 2010; Escobar-Lux et al., 2019; Samuelsen et al., 2020). Furthermore, the treatments are often costly and, as fish are usually treated repeatedly during a production cycle, and with bath treatments there is an increased risk of poor welfare outcomes (Overton et al., 2018a; Overton et al., 2018b).

In response to this, several new chemical-free parasite controls have been developed, including mechanical and thermal delousing (Grøntvedt et al., 2015; Roth, 2016; Gismervik et al., 2017). Three types of mechanical delousing technologies (Flatsund (FLS) engineering AS, SkaMik AS and the Hydrolicer®) and two types of thermal delousing systems (Thermolicer® and Optilicer®) have been developed in the last few years (Overton et al., 2018a). All technologies require salmon to be crowded and pumped into a delousing system. Mechanical delousing uses pressure washers, brushes or vaccums to mechanically remove lice from fish (Overton et al., 2018a). Thermal delousing exposes fish briefly to warm seawater (<34°C) to detach lice from the host (Overton et al., 2018a). Both delousing methods have proven to be effective at removing mobile lice from salmon and have little to no impact on the environment or non-target species (Grøntvedt et al., 2015; Roth, 2016; Gismervik et al., 2017). However, as with the use of chemical treatments, they appear stressful for fish (Poppe et al., 2018; Gismervik et al., 2019; Nilsson et al., 2019) and can lead to high post-treatment mortalities in certain circumstances (Overton et al., 2018a). Hence, for the Norwegian salmon aquaculture to be able to reach the goal of producing 5 million tonnes by 2050 there is a need for new technologies and strategies that can mitigate parasite infestations without negatively impacting the surrounding environment or fish welfare.

1.4 Preventative methods

Most research and development efforts on salmon lice control have focused on removing host-attached stages. However, the ideal situation would be to limit or prevent infestations from occurring. Preventative controls might invoke less resistance evolution in parasite populations and reduce the need for farms to delouse (Bui et al., 2019). Possible approaches include reducing encounter rates between

hosts and parasites (e.g. barriers, behavioural manipulations, spatiotemporal management) and reducing the infestation success of the parasite (e.g. vaccines, functional feed, breeding) (Barrett et al., 2020b).

Several new preventive methods which manipulate the host-parasite relationship by mismatching their environments are emerging. In salmon farming, prevention strategies that exploit lice copepodids positioning in the upper part of the water column (Heuch et al., 1995; Hevrøy et al., 2003) have been developed in recent years. As Atlantic salmon typically spend extensive periods in the surface waters (Oppedal et al., 2011) prevention strategies that shield or move them away from surface-dwelling salmon lice copepodid can be powerful controls. Technologies based on this strategy include barrier cages (skirt or snorkel tarpaulin wrapped around the upper part of the cage), submerged sea-cages (repeatedly submerged or submerged with an air dome), semi-enclosed cages (water pumped in from the deep) and submerged lighting and feeding (motivating salmon to swim at deeper depths) (**Table 1**). Several trials and case studies into these technologies mainly demonstrate that avoiding fish contact with surface waters reduces salmon lice infestations on salmon (**Table 1**). However, these new technologies are typically sub-optimally tested at i) research scale, ii) for short time periods or iii) using imperfect study designs (**Table 1**). Because of this, there remains some uncertainty around their performance in commercial settings.

Testing new technology at commercial scale is essential when determining the effectiveness of the technology as it mimicks the normal conditions that salmon encounter. Only testing over a limited timeframe during a production cycle does not cover the full extent of season variations that occur. For example, seasonal changes in environmental variables, such as temperature, affect lice larvae development (Samsing et al., 2016b; Hamre et al., 2019), dispersal and connectivity between farms (Samsing et al., 2017). Periodic fluctuations in depth profiles of environmental variables, such as brackish surface layers, affecting vertical distributions in lice larvae (Heuch et al., 1995; Samsing et al., 2016a) may also not be captured over short periods of testing. In addition, there are a range of potential negative side effects of

technologies that could be missed without thorough testing at commercial scale. These include impact on fish welfare and behaviour, co-occuring parasites and the daily routine of salmon farmers. Full-production cycle investigations will therefore be vital to definitively elucidating overall performance of preventive technologies and identifying wider challenges.

Depth-based preventive cage	**Study**	**Commercial scale**	**≥3 replicates**	**All seasons covered**	**Effect of in-situ lice control**	**Other parasites**
Snorkel	Stien et al. (2016)		x			
	Oppedal et al. (2017)		[a]			
	Wright et al. (2017b)	x				x
	Paper III		**x**			**x**
	Paper I	**x**	**x**	**x**		
	Oppedal et al. (2019)		*x*			
	Paper IV	***x***	***x*[b]**		***x***	
	Paper II	***x***	[a]	***x***		***x***
Skirt	Stien et al. (2018)	x	x			
	Grøntvedt et al. (2018)	x	x			
Floating enclosed	Nilsen et al. (2017)	x	x[c]	x[d]		
Deep light	Hevrøy et al. (2003)					
Deep feed and light	Frenzl et al. (2014)	x				
Skirt, deep feeding and light	*Bui et al. (2020)*	*x*	*x*	*x*		
	Gentry et al. (2020)	*x*	*x*		*x*	
Submerged	Korsøen et al. (2009)		x			
	Sievers et al. (2018)		x			
	Glaropoulos et al. (2019)		x			
Dome	*Warren-Myers et al. (unpublished)*		*x*	*x*		

[a]Regression design
[b]No standard sea cages
[c]Different sites used, with different lice infection pressures
[d]Cages stocked over inconsistent periods using different fish cohorts with variable lice infection dynamics

Table 1. *The scale, replication and seasonal coverage of studies assessing salmon lice infections in preventive depth-based cage designs versus standard cages (modified from* ***paper I****, with new additions written in italic).*

1.5 Snorkel technology and salmon lice

The snorkel sea cage is one of the most exstensively studied salmon lice prevention technologies (**Table 1**). It consists of a standard cage fitted with a roof net to keep fish deeper and an enclosed tarpaulin tube (a snorkel) where it a) allows salmon to access the surface air for filling their swim bladder to maintain buoyancy regulation (Fahlén, 1971; Dempster et al., 2011) and b) it serves as a barrier to surface waters where lice larvae are most abundant (Heuch et al., 1995; Hevrøy et al., 2003) (**Fig. 2**). Several experiments and case studies have been conducted on the performance of snorkel sea cages from a research scale for proof of concept (Stien et al., 2016; Oppedal et al., 2017) to a commercial scale at salmon farm sites (Wright et al., 2017b). These demonstrate that sea lice infestation levels can be reduced in snorkel compared to control cages with negligible impacts on salmon welfare (Stien et al., 2016; Wright et al., 2017b) and that effectiveness increases with increasing snorkel depth (Oppedal et al., 2017). However, the use of snorkel sea cage technology does not come without challenges. In a recent study, a brackish surface layer penetrating down to the snorkel depth negatively affected the performance of the snorkel (Oppedal et al., 2019). Lice copepodids, which avoid low salinity levels (Heuch et al., 1995; Bricknell et al., 2006; Crosbie et al., 2019), most likely remained below the snorkel and were able to infect fish. Prior to 2019, studies had failed to address how this technology would perform during a whole production cycle with different seasons and environmental fluctuations and in fully replicated studies at commercial scale (**Table 1**).

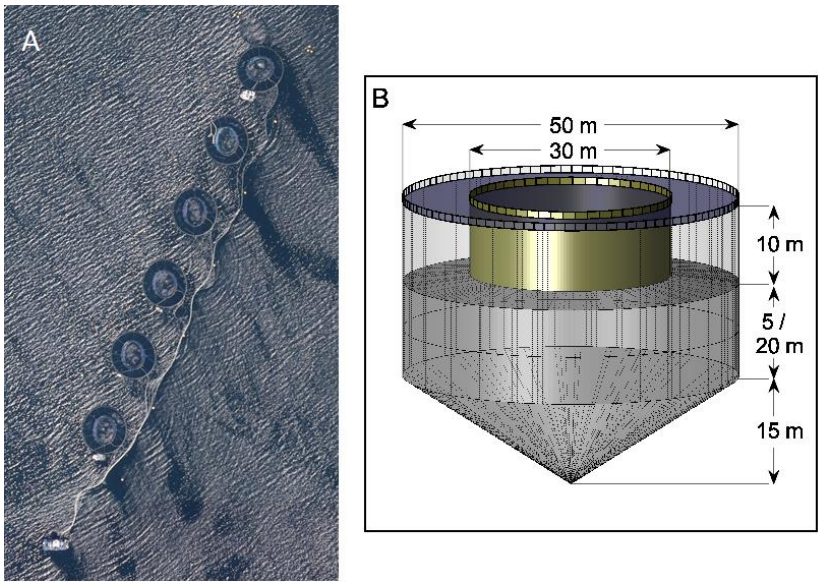


Figure 2. *Overview of a) fish farm (Låva, 2019) with 6 snorkel sea cages (screenshot from norgeibilder.no) and b) schematic of a commercial snorkel sea cage (modified from* ***paper I****).*

1.6 Snorkel technology and general parasite management

Salmon farm management often extends beyond a single pathogen. Several pathogens can be transmitted through the same hydrodynamic pathways or infection of one pathogen can increase the susceptibility of another. Therefore, coordinated management of multiple pathogens may be advantageous for farmers. The use of new prevention technology and modified cages could have unknown implications on other salmon parasites. While depth-based prevention technologies can reduce salmon lice infestations (Stien et al., 2016; Wright et al., 2017b; Stien et al., 2018), minimal research has focused on how these techniques affect other salmon parasites (**Table 1**). If parasites display similar depth-related infestation patterns to salmon lice, snorkel sea cages and other depth-based prevention technologies could be effective. On the other hand, increased fish crowding inside the snorkel may intensify infestation of parasites relying on host proximity.

A common parasite in salmon aquaculture which potentially displays the same depth-

related infestation pattern as salmon lice are marine tapeworms (*Eubothrium* sp.). While marine tapeworms are of less concern than salmon lice for salmon farmers reports of infestations are increasing in Norway (Hjeltnes et al., 2019). Tapeworm infestations can significantly reduce salmon growth (Bristow and Berland, 1991; Saksvik et al., 2001a) and lead to production and profit losses, which in one study was estimated to equate to a 10% growth loss when reaching market size (Bristow and Berland, 1991). As tapeworm infestations primarily occur when salmon ingest intermediate copepod hosts, and these copepods are often associated with the surface layers, depth-based technologies aimed at salmon lice prevention could also work against marine tapeworm infestations.

Respiratory diseases are a huge cause of loss in farmed Atlantic salmon in Norway (Herrero et al., 2018; Rozas-Serri, 2019) and the marine amoeba *Paramoeba perurans*, which is responsible for amoebic gill disease (AGD) (Young et al., 2007), is one of the culprits of this rising concern (Oldham et al., 2016; Marcos-López and Rodger, 2020). As these amoebae seem to be distributed throughout the water column (Wright et al., 2017a), depth-based prevention techniques are not expected to shield salmon from AGD outbreaks. On the contrary, salmon residing in snorkel sea cages appear more prone to AGD outbreaks (Wright et al., 2017b). Freshwater has long been used as a treatment against *Paramoeba perurans* (Nowak, 2012). Therefore, an in-situ treatment option with a freshwater layer inside the snorkel sea cage has been proposed to mitigate the impact of both parasites (Wright et al., 2017b). This method still lacks research attention in terms of effects on parasites, fish welfare (both salmon and cleaner fish) before adoption by the salmon industry. There is also concern that lice could develop a resistance towards freshwater (Ljungfeldt et al., 2017; Groner et al., 2019) which could have catastrophic consequences for wild salmon and sea trout.

1.7 Snorkel technology and *in situ* control options

While lice prevention effects of depth-based technologies can be significant, there are instances, as mentioned, that copepodids are deep enough to bypass these barriers and infest salmon (Oppedal et al., 2017; Wright et al., 2017b; Oppedal et al., 2019). This

likely occurs due to combinations of hydrodynamic processes pushing lice deeper in the water column via vertical mixing (Samsing et al., 2016a), lice sinking deeper to avoid brackish surface layers (Crosbie et al., 2019) or variation in depth preference between lice families (Coates et al., 2020). Hence, through a whole production cycle, additional control methods may have to be resorted to. While chemical, mechanical and thermal treatments are all options for lice removal, these require snorkels to be removed before treatment. Removing snorkels is a process requiring significant resources and time and periods without snorkels risk exposing fish to surface layers laden with salmon lice larvae. To avoid the latter concern, fish can be pumped into a second cage already fitted with a snorkel, but this requires an extra empty cage at a farm location. Thus, finding *in situ* control options that are effective in snorkel sea cages would be optimal from a farm management and cost perspective over controls that require dismantling or creating new snorkel cages.

The main approaches currently aimed at continuously removing louse within salmon sea cages are lice-eating cleaner fish and optical lasers. Both methods can be used while keeping the snorkels in place. However, information about cleaner fish welfare and behaviour, and optical laser effects on salmon lice in commercial scale settings is needed before these methods can be widely used.

Cleaning activity, where one species seeks out another to remove ectoparasites from their body, is a well-known phenomenon among marine species (Vaughan et al., 2017). In salmon aquaculture, cleaner fish comprise several species of wrasse (ballan, corckwing and goldsinny wrasse) and in recent years also lumpfish (Skiftesvik et al., 2013; Powell et al., 2017). First used in the 1980s, deployment of cleaner fish to remove salmon lice has increased considerably over the last few years (Norwegian Directorate of Fisheries, 2020a). Small-scale research studies have shown them to be effective in removing mobile lice from salmon with no negative effects on salmon welfare (Deady et al., 1995; Treasurer et al., 2002; Skiftesvik et al., 2013; Imsland et al., 2014). However, variable effects on lice have been reported from commercial salmon farms using cleaner fish (Barrett et al., 2020a) and few studies have been performed at commercial scale to back up the findings from smaller scale trials

(Overton et al., 2020). Additionally, concerns about cleaner fish welfare and mortality in commercial salmon sea cages have raised an ethical dilemma about their continued use (Mo and Poppe, 2018; Hvas and Oppedal, 2019; Yuen et al., 2019; Stien et al., 2020).

Differences in environmental preferences and swimming depths between salmon and cleaner fish, which are more easily expressed in larger scale cages, could be a factor explaining the reduced efficiency and welfare at commercial scale. This is an increasingly important factor when using depth-based prevention techniques which could affect both cleaner fish and salmon behaviour and welfare (Gentry et al., 2020). Cleaner fish vary greatly in their biology and life history, with ballan wrasse being a temperate species inhabiting shallow reefs and kelp beds, while lumpfish is a cold-water, semi-pelagic species. Based on their environmental preferences they might occupy different depths than salmon, thereby reducing lice-eating events. Therefore, understanding environemental preferences and swimming depth for both cleaner fish and salmon could be key to predicting encounter rates and potential lice-eating events and optimising cleaner fish deployment in both standard sea cages and cages using depth-based prevention techniques.

As an alternative to cleaner fish, optical lasers are now in use at several locations in Norway (Overton et al., 2018a). This method aims to control salmon lice infestations using underwater lasers to beam and kill lice on fish. It consists of a vertically movable submerged node attached to a horisontally movable floating buoy inside the sea cages. The node contains an automated camera system that scans passing fish for potential lice and beam at them with a pulse of light when a suspected lice is detected (**paper IV**). The system is trained to identify and not beam salmon eyes and does not harm the skin of the salmon (Brown, 2016; Frenzl, 2017). Therefore, lasers do not appear to have negative impacts on either the environment or fish welfare. However, the delousing effects from this technology still requires scientific validation. Laser deployment in snorkel sea cages could improve beams on lice as salmon can be closely packed inside the snorkel. However, as snorkel sea cages are often deeper than standard cages, to account for restricted space caused by the snorkel, there is a

possibility that the salmon school could extend beyond the maximum operating depth (25 m due to restricted cable length) of the laser nodes.

Therefore, to be able to efficiently manage salmon when using depth-based prevention technologies, more information is needed on their ramifications on general parasite management under commercial production conditions. This information is vital for farmers to accurately and cost-effectively choose which control options to use in the landscape of parasites they need to manage, salmon production they need to maximise, and fish welfare they need to ensure.

.

2. Aims of study

The study aimed to, more comprehensively, evaluate production of Atlantic salmon (*S. salar*) in commercial-scale snorkel sea cages in terms of effects on lice, effects on other parasites and the suitability of simultaneously deploying *in situ* control methods. Specifically, it aimed to determine (paper 1–5):

1. long-term effects of snorkel sea cage technology performance on salmon lice through a whole production cycle at commercial scale,

2. long-term effects of snorkel barrier technology on marine tapeworm (*Eubothrium* sp.) infestations at commercial scale,

3. short term effects of snorkel sea cage technology on marine amoebae (*Paramoeba perurans)* causing amoebic gill disease and *in situ* freshwater-filling of snorkels for treatment at semi-commercial scale,

4. short-term effects of *in situ* optical lasers in snorkel sea cages on salmon lice at commercial scale,

5. baseline information on how salmon sea cage culture affects cleaner fish welfare, behaviour and survival at semi-commercial scale (a front-running *in situ* control with snorkel cages).

3. Abstract of papers

Paper I

Snorkel sea-cage technology decreases salmon lice infestation by 75% in a full-cycle commercial test

Lena Geitung, Frode Oppedal, Lars Helge Stien, Tim Dempster, Egil Karlsbakk, Velimir Nola, Daniel W. Wright

Methods to prevent parasite infestations in farmed fish are becoming widespread, yet tests of their effects often lack commercial relevance and statistical power, which may lead to technology misuse. Here, we examined salmon lice infestations on Atlantic salmon in triplicate commercial snorkel lice barrier and standard cages over a 12-month production cycle. Barrier cages reduced newly-settling lice on Atlantic salmon by 75%, with variability of parasite reduction through time depending upon environmental variables. The commercial, triplicate, long-term study design serves as a template to validate performance and detect weaknesses of anti-parasite techniques in fish mariculture.

Paper II

Tapeworm (Eubothrium sp.) infestation in sea caged Atlantic salmon decreased by lice barrier snorkels during a commercial-scale study

Lena Geitung, Daniel W. Wright, Lars Helge Stien, Frode Oppedal, Egil Karlsbakk

Reports of infestation by marine parasitic tapeworms (*Eubothrium* sp.) and an associated growth reduction in Norwegian farmed salmon are on the rise. With few acceptable treatment options available, due to drug resistance evolution in tapeworms or negative drug impacts on fish, alternative controls against the parasite are in demand. In a 10-month commercial-scale study involving standard sea cages and lice barrier snorkel sea cages of different depths (4, 8, 12 and 16 m), we examined if this depth-based preventive technology primarily used against salmon lice (*Lepeophtheirus salmonis*) also reduced tapeworm infestation. A submerged net roof opening to a central barrier tube (snorkel) was added to standard cages to move salmon deeper but retain surface access; a cage manipulation that avoids contact with mostly surface-dwelling salmon lice larvae and may also separate fish from calanoid copepods, the intermediate hosts of *Eubothrium* sp. Salmon populations in unmodified standard cages had higher tapeworm prevalence (63–93 %) and abundances (4.6–5.7 *Eubothrium* sp. fish^{-1}) than those in snorkel cages (20–36 % and 0.2–0.6 *Eubothrium* sp. fish^{-1}). Based on these observations, tapeworm prevention could be another beneficial parasite management outcome of snorkel cage technology or other depth-based prevention techniques against salmon lice.

Paper III

Surface environment modification in Atlantic salmon sea-cages: effects on amoebic gill disease, salmon lice, growth and welfare

Daniel W. Wright*, Lena Geitung*, Egil Karlsbakk, Lars Helge Stien, Tim Dempster, Tina Oldham, Velimir Nola, Frode Oppedal

*Joint first authors

Surface environment modification is a potential parasite control strategy in Atlantic salmon sea-cage farming. For instance, a temporary low salinity surface layer in commercial-scale snorkel sea-cages has coincided with reduced amoebic gill disease (AGD) levels after an outbreak. We tested if a permanent freshwater (FW) surface layer in snorkel sea-cages would lower AGD and salmon lice levels of stock relative to snorkel cages with seawater (SW) only and standard production cages with no snorkels. Triplicate cages of each type with 2000 post-smolts were monitored in autumn to winter for 8 wk and sampled 4 times. Lower proportions of individuals with elevated AGD-related gill scores were registered in SW and FW snorkel cages compared to standard cages; however, these proportions did not differ between SW and FW snorkel cages. Individuals positive for AGD-causing *Paramoeba perurans* were reduced by 65% in FW snorkel relative to standard cages, but values were similar between SW snorkel cages and other types. While total lice burdens were reduced by 38% in SW snorkel compared to standard cages, they were unchanged between FW snorkel and other cage types. Fish welfare and growth were unaffected by cage type. Surface activity was detected in all cages; however, more surface jumps were recorded in standard than snorkel cages. Overall, fish in FW snorkel cages appeared to reside too little in freshwater to consistently reduce AGD levels and salmon lice compared to SW snorkel cages. Further work should test behavioural and environmental manipulations aimed at increasing freshwater or low salinity surface layer use.

Paper IV

Salmon lice survive the straight shooter: A commercial scale sea cage trial of laser delousing

Samantha Bui, Lena Geitung, Frode Oppedal, Luke T. Barrett

Ectoparasitic salmon louse (*Lepeophtheirus salmonis*) infestations are costly for Atlantic salmon (*Salmo salar*) farmers in Norway. As a result, there is a strong desire for solutions to prevent and control infestations, and new technologies are typically developed and commercialised rapidly, without rigorous validation. Here, we tested the efficacy of a new commercially available control measure—delousing by underwater lasers—using a replicated design at full commercial scale. Laser delousing was used in combination with a preventive method (snorkel cages), with laser nodes deployed in 3 of the 6 sea cages at the site. The trial ran for 54 days, after which time there was no difference in infestation density of mobile salmon louse stages (pre-adult, adult male or adult female) in cages with or without laser nodes installed. By the end of the trial, adult female lice numbers in all cages were close to the legislated trigger for mandatory delousing (0.5 adult female lice per fish). The laser nodes delivered a large number of pulses relative to the number of lice in the cages, indicating that a lack of lethality rather than a lack of target detection was the limiting factor. If all pulses had been effective, they should have removed between 4–38 % of mobile lice each day. There was no effect on salmon welfare indicators such as skin condition or eye status. Our results highlight the importance of rigorous validation of new technologies across a range of conditions before widespread implementation by industry.

.

Paper V

Cleaner fish growth, welfare and survival in Atlantic salmon sea cages during an autumn-winter production

Lena Geitung, Daniel William Wright, Frode Oppedal, Lars Helge Stien, Tone Vågseth, Angelico Madaro

Cleaner fish used as a biological control agent against salmon lice is rapidly increasing in Atlantic salmon aquaculture. However, concerns have been raised about the welfare and mortality of cleaner fish in salmon cage systems, which could in turn affect their performance in controlling salmon lice. In a 4-month autumn-winter study, we monitored growth, welfare, mortality and daytime depth distribution of the most commonly used cleaner fish, farmed ballan wrasse and lumpfish, in six salmon production sea cages where thermo- and haloclines were present. Ballan wrasse did not grow (SGR: small: -0.01 % day^{-1}, large: -0.06 % day^{-1}), while lumpfish significantly doubled in size (SGR: 0.87 % day^{-1}) during the study. High losses (registered mortality + unregistered loss) were observed in both species (57 and 27 % of ballan wrasse and lumpfish, respectively). The welfare status of remaining individuals generally improved over the study period, regardless of species. Brief daytime camera observations at hides found ballan wrasse were typically deeper at warmer (median 12.4 °C) more saline (median 31.7 ppt) depths, where salmon were expected to reside during day periods, compared to lumpfish generally occupying colder (median 7.3 °C), brackish (median 18.9 ppt) water in surface layers. Considerable mortalities, minimal feeding (inferred from ceased growth) by ballan wrasse and a possible mismatch in lumpfish and salmon depths (inferred from limited daytime camera observations) suggest that cleaner fish may have low long-term effectiveness against salmon lice in stratified salmon sea cages over autumn-winter. Similar studies across seasons, locations and cage types (e.g. depth-based cage technologies) are vital to understand the extent of these issues in salmon aquaculture more broadly.

4. Methodological considerations

4.1 Experimental conditions

With the aim of providing salmon farmers with knowledge on depth-based prevention technology performance, two semi-commercial scale studies and three commercial scale studies were carried out, leading to a mixture of study designs and methodological approaches.

Research scale studies in smaller sea cages have the benefit of being more controlled, with a greater ability of to regulate sources of bias. On the other hand, these studies could suffer from scale-dependent differences linked to fish numbers and cage volumes that may mean results are not transferable to commercial-scale settings. In the semi-commercial trials performed for this thesis, experimental conditions were kept as close to commercial sea cage conditions as possible. Nonetheless, 12 m × 12 m, 12–14 m deep cages were used instead of 160 m circumference commercial cages, 25–50 m deep. This meant, for instance, that 4 m deep snorkels used in one of the research scale trials (**paper III**) were 179 times smaller in volume than regular 10 m deep snorkels used in commercial scale trials, giving fish much less space to reside in. It was concluded that salmon spent much less time in the snorkel than was observed in a previous commercial scale study, which possibly explained the difference freshwater-filling of snorkels had on controlling amoebic gill disease between the two studies. The study results were still valuable information, as it suggested that manipulations may be required to attract salmon into a freshwater layer for sufficient time to treat the gill disease. Additionally, the smaller sea cages used to observe depth distribution for cleaner fish (**paper V**) could have altered their behaviour, as larger deeper cages can enable both salmon and cleaner fish to express depth preferences more readily. It is therefore important to also commence such tests in larger commercial scale sea cages.

Commercial scale studies are highly relevant to the salmon industry as they mimick the conditions that salmon are expected to encounter in everyday situations but are

often influenced by unexpected and uncontrollable factors. As an example, in a continuation of the trial with optical lasers (**paper IV**), cleaner fish were added to cages without lasers to document if there was a difference in lice reductions between cleaner fish or lasers when used in snorkel cages. Several unforeseen events transpired such as a lumpfish mortality event due to disease and fire in a cage, which altered cage setups (**Fig. 3**). In addition, delousing events due to commercial regulations were only performed in some of the cages (**Fig. 3**). To account for effects such as periodic delousing and variable use of cleaner fish, sessile lice (copepods, chalimus I, II) were used when assessing the snorkel cage effect on salmon lice (**paper I**). On occasions hydrogen peroxide and thermolicer treatments used could have reduced numbers of chalimus to some extent, but there were usually opportunities for chalimus to develop after a treatment and before the next sampling event. However, as control cages had more treatments during the trial, the amount of chalimus reduced due to treatments would have been higher in the standard cages compared to the snorkel cages. Therefore, the results presented were conservative (**paper I**). In addition, to minimize the time fish were exposed to surface waters the snorkels were always scheduled to be deployed the day after treatment. For marine tapeworm infestations (**paper II**), we decided to terminate the experiment when the first snorkel cage was removed, as exposure to surface waters could have influenced their subsequent tapeworm infestations.

Commercial scale studies can also be problematic due to farm logistical issues. Fish batches and stocking time are often dependent on the salmon farm hatcheries and well-boat availability and delivering identical batches of high numbers of fish to a farm site at the same time can prove difficult. In **paper I,** Atlantic salmon came from two different strains and were stocked at two different times (Salmobreed strain in 4 cages stocked in mid-June and Mowi strain in 2 cages stocked in mid-September). This created a growth difference between groups of fish possibly interfering with behavior and infestation pressure of salmon lice, AGD and tapeworms. To account for this the strains were split evenly between cage types. Nevertheless, even as they are more logistically difficult to perform than smaller scale studies, long term

commercial scale studies are the ultimate test of effectiveness and feasibility for new technologies.

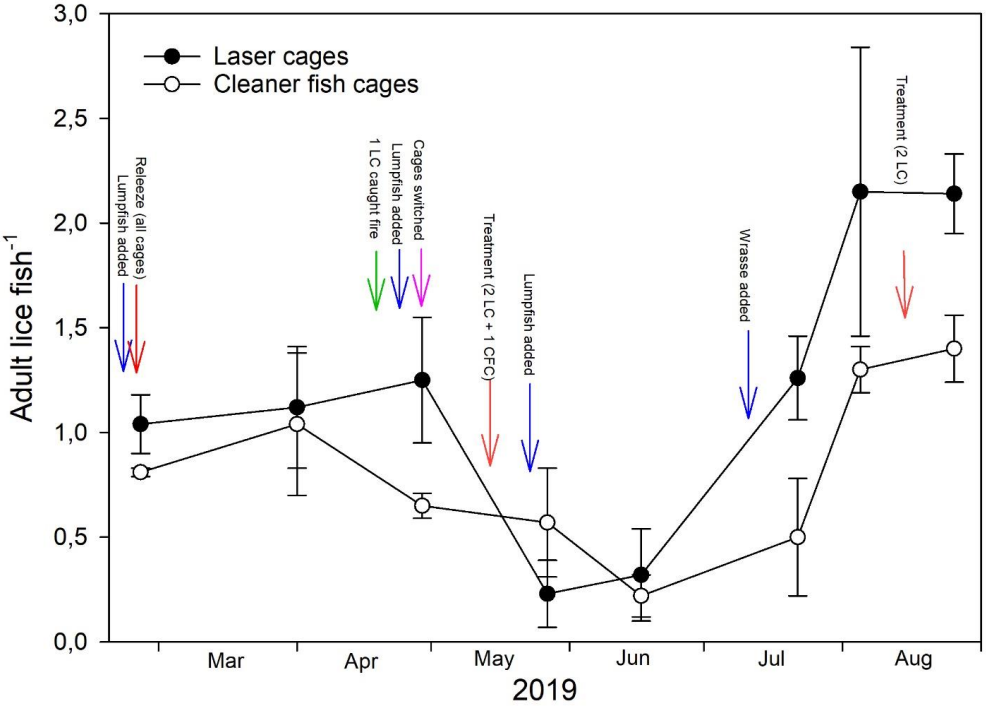


***Figure 3**. Mean (± SE) adult lice (male and female) in triplicate 16 m deep snorkel sea cages with two optical lasers and triplicate cages with cleaner fish (lumpfish and wild caught wrasse) at Prestholmane farm site. The trial was a continuation of **paper IV**. Arrows represents different events (ex. blue = cleaner fish stocking, red = treatment).*

4.2 Fish sampling

An important consideration when sampling salmon in large sea cages is how to meet the assumption of random sampling. However, this can seldom be guaranteed or even expected in real situations (Nilsson and Folkedal, 2019). Several factors could contribute to sampling bias such as sampling method, sampling time and numbers of

fish sampled. We will try and cover all of these aspects and how we tried to deal with them.

When sampling fish in larger sea cages there are various sampling techniques to choose from (Folkedal et al., 2016; Nilsson and Folkedal, 2019). In all the commercial scale trials we used sweep nets (10 m × 5 m), which are weighted in the bottom line and have floats attached on the surface line. To catch fish, the net is set around an area of the cage and fish are lured to the surface by hand feeding. The bottom net is then hauled in at both sides to crowd the fish and random individuals are netted out from the sweep net. This was chosen as it is the preferred method for farmers and was therefore well incorporated into farm operations and easy to perform. However, sampling fish with manual netting near the surface could create sampling bias as individual salmon occupy different cage areas depending on factors such as size, (i.e. smaller fish tend to swim near the surface, Folkedal et al., 2012), hunger (i.e. hungry fish tend to swim near the surface, Juell et al., 1994), physiological state (i.e. loser fish tend to swim near the surface, Vindas et al., 2016), and parasite infections (i.e. fish with higher lice loads swim deeper at night, Bui et al., 2016). The presence of snorkel cages could make this sampling bias larger, as fish caught shallower than the snorkel depths were most likely not individuals from larger schools swimming below the snorkel edge. To compensate for this, feeders were stopped prior to sampling in each cage. As the fish were actively feeding throughout the day, withholding feed and hand feeding pellets to draw them towards the surface near the sweep net was expected to improve the chances of sampling fish representative of caged populations.

In addition, the fact that cages were sampled at different times during the day could also create a sampling bias as individual fish occupy different areas of the cage during the course of one day (Juell et al., 1994; Oppedal et al., 2011). To compensate for this farm personell randomly chose the order the cages were sampled each time, however sampling in the order cage 1–6 or cage 6–1 are possibly overrepresented as this was the order they used when counting lice the weeks we were not present.

In most trials a sample size of 20 fish per cage were used. This protocol was chosen as it is recommended for lice counting in commercial aquaculture (Lovdata, 2012), standard among several previous papers (Stien et al., 2016; Oppedal et al., 2017; Wright et al., 2017b; Stien et al., 2018) and was manageable for personnel to perform. However, small sample sizes may lead to poor accuracy in results with sea lice counts on salmon farms known to vary significantly both between and within cages (Revie et al., 2005; Revie et al., 2007). Although, Revie et al. (2005) also highlighted that sampling smaller numbers of fish from larger numbers of pens typically results in a more accurate estimate of abundance than sampling "many fish from few pens". For a majority of the trials, we sampled three replicate cages at several sampling times thereby accounting for some of the variance in lice counts. When comparing lice levels in cages with or without optical lasers (**paper IV)** 50 fish from each cage were counted at one sampling point, but the variance did not seemingly improve as the majority of lice ended up falling off and being counted in the sampling bucket for division equally across the cage. When sampling for marine tapeworms (**paper II**) the sample size were increased from 20 fish to 30 fish per cage to be able to pick up differences between cages as the abundance of tapeworm were quite low at a few sample times and cage types were not replicated.

5. General discussion

The studies presented in the papers describe the production of Atlantic salmon in snorkel sea cages with emphasis on: (1) prevention effects against salmon lice (**paper I, III**), (2) prevention effects against co-ocurring parasites (**paper II, III**) and (3) *in situ* lice control methods (**paper IV, V**).

5.1 Prevention effect against salmon lice

5.1.1 Lice

The concept of mismatching farmed salmon and salmon lice by using depth-based prevention techniques have proven to be successful. Based on several case studies it is clear that snorkel sea cage technology provides prophylaxis against salmon lice infestations (**paper I, III**) (Stien et al., 2016; Wright et al., 2017b; Oppedal et al., 2019). A snorkel sea cage reduces encounter rates between farmed salmon and free-living infective salmon lice larvae abundant in surface waters by forcing salmon to swim mostly below a net roof and inside a snorkel tube to the surface, and preventing parasite penetration into the snorkel space via semi-impermeable material. Its efficiency has been experimentally documented in shorter research and commercial scale trials where reduction in new lice infestation have ranged from 24–65 % (Stien et al., 2016), 33–47 % (**paper III**) and 76 % (Oppedal et al., 2019) using 4 m deep snorkels and 84 % lice reduction using 10 m deep snorkels (Wright et al., 2017b). In this thesis the prophylactic effect was further confirmed in a study of considerably more relevance to salmon farmers (**Table 1**), where using 10 m deep snorkels through an entire production cycle gave an average lice reduction of 75% in snorkel sea cages compared to standard cages (**paper I**). This is comparable with other barrier technologies. Skirts have been documented to reduce salmon lice by 30 % (5 m deep skirts, Grøntvedt et al., 2018) to 80 % (10 m deep skirts, Stien et al., 2018), and submerged cage fitted with a underwater dome filled with air reduced new lice infestation by up to 91 % compared to standard sea cages (submerged to 15 m depth, Warren-Myers et al. unpublished). Within the group of preventive barrier cage

technologies, skirts are considered to be moderately effective, snorkels highly effective, while closed containment systems almost entirely avoid lice infestations (Barrett et al., 2020b). In comparison with other preventive technologies, techniques utilising a constant physical barrier shielding or separating salmon from surface waters (e.g. skirt, snorkel, submerged cage with airdome, closed-contained cage) show a more consistent lice reducing effect than approaches focussing on manipulation of salmon swimming depth (e.g. deep feeding and lights) (Barrett et al., 2020b). This adds to the theory that limiting exposure to surface waters is the main driver for obtaining persistent effects. The importance of shielding salmon from infective copepodids in surface water was further supported with results from Oppedal et al. (2017) where lice infestation rates decreased exponentially with increasing barrier depth, as salmon kept in snorkel cages near the surface (0–4 m) had 10–20 times more lice than salmon kept in deeper snorkel cages (12–16 m).

5.1.2 Environment

The lice reducing effect of depth-based prevention technologies are dependent on environmental conditions. During a long-term study of commercial snorkel sea cages, effects on lice varied considerably throughout a production cycle, ranging from a 35% increase to 100% reduction of new lice in snorkel compared to standard sea cages (**paper I**). It was determined that the snorkel effect were weakest when surface brackish water (salinities < 28 ppt) and warm surface waters (temperatures > 16 °C) occurred (**paper I**). Similarly, other studies report the presence of a strong vertical salinity gradient (Oppedal et al., 2019) and similar swimming depths by fish in both snorkel and standard cages (Stien et al., 2016) causing little difference in lice infestation between snorkel and standard sea cages. Infective copepodids are positively phototactic, but have reduced survival at salinities < 29 ppt, and are assumed to avoid brackish water and aggregate just below the halocline (Heuch et al., 1995; Crosbie et al., 2019). In commercial sea cages, peaks of infestations have often occurred when salmon swim within 5 m of the halocline (Bui et al., 2020). The depth of the halocline is therefore an important factor in relation to infestation risk of salmon lice. As such, when the brackish surface layer extends close to, or below the

snorkel bottom edge, salmon lice are pushed deeper than the snorkel, thereby threatening parasite encounters for fish in these cage types (Oppedal et al., 2019). Warm surface temperatures also affect the lice reduction efficiency of snorkels. Caged Atlantic salmon prefer depths nearest 16 °C for thermoregulation (review by: Oppedal et al., 2011), clearly avoid warmer waters (Johansson et al., 2006; Stehfest et al., 2017) and likely swim deeper in snorkel and standard sea cages when surface temperatures are above this threshold. During these times fish in both snorkel and standard sea cages probably swim at similar depths and experience similar infestation pressures. Additionally, periods of turbulence could explain times of low lice reduction efficiency, where vertical mixing could transport lice larvae below the bottom of the snorkel (Johnsen et al., 2016). However, mixing of the water column seems to improve dissolved oxygen levels inside lice skirts and is worsened by the prescence of a strong pycnocline (Jónsdóttir et al., 2020). In a recent study more planktonic nauplii were found inside lice skirts than directly outside the cages, and the same was not seen for standard cages (Øverlid, 2017). From this it could be suggested that lice might not disperse as normal and develop and re-infect fish inside lice skirts (Oppedal et al. unpublished). This might also be true for snorkel sea cages, but the smaller volume makes water exchange easier and might therefore create less of a problem in these cages. There is also a theoretical risk of contamination of salmon lice from waves and rough weather, but this is unlikely as lice barriers often extend around one meter above sea level. Swimming depth of fish in standard sea cages, halocline prescence and depth and vertical turbulence are likely crucial in understanding and predicting variations in lice reduction effects from depth-based prevention technologies (Samsing et al., 2016a).

With this information farmers can assess local environmental conditions and make informed decisions on whether to choose a prevention technology and how it might be optimised. For instance, farm sites near freshwater run-off with periodic or constant brackish surface waters (ex. fjord site) might not require snorkels or require deeper snorkels to obtain optimal results. As an option, lice skirts could be lowered deeper to shield salmon from the halocline while letting potential lice-free brackish

water in from the surface (Bui et al., 2020). Contrastingly, sites with similar salinity levels throughout the water column (coastal site) are locations where snorkels will be effective and farmers may see similar shielding effects between a range of snorkel depths, potentially leading to shallower snorkels in some instances. As deeper snorkels and skirts can be harder to operate, the use of barrier cages is most recommended for areas with more homogenous salinity depth profiles. It is important to note that other prevention technologies such as submerged light and feeding that encourage salmon to swim at depths of lower infestation risk (Hevrøy et al., 2003; Frenzl et al., 2014; Bui et al., 2020), could be used either on their own or together with barrier cages to cover the full range of environmental conditions that salmon farms experience.

5.1.3 Fish welfare

When implementing new technologies, it is vital to investigate any potential risks to fish welfare. With barrier technologies such as skirt and snorkel cages, occasions of low dissolved oxygen levels have occurred (Stien et al., 2012; Wright et al., 2017b; Stien et al., 2018) and may be an increasing issue as deeper snorkels are used with less water exchange (Oppedal et al., 2017). For salmon, periods of low oxygen levels, also seen in standard cages (e.g. Oldham et al., 2018; Solstorm et al., 2018; Burke et al., 2021), may cause poor appetite (reviewed by Remen et al., 2016), reduced growth (Remen et al., 2012) and in extreme cases death (Nilsson and Östlund-Nilsson, 2008; Remen et al., 2012). Increased mortality in a deep snorkel (16 m) was attributed to stress and low oxygen concentrations due to fish aggregation inside the snorkel, but with improved water exchange mortality rates were lowered (Oppedal et al., 2017). Previous research has so far not revealed any differences in growth of salmon between snorkel and standard sea cages (Stien et al., 2016; Oppedal et al., 2017; Wright et al., 2018; Oppedal et al., 2019). In commercial scale studies lasting between 6–12 months, measures of fish welfare (fin and snout damage) as well as condition factor did not differ in snorkel compared to standard cages (**Table 2**) (Wright et al., 2017b). In shorter studies at research scale (**paper III**) (Stien et al., 2016; Oppedal et al., 2017; Oppedal et al., 2019), snout damage from possible

collisions with net roof and snorkel structures has occurred (Stien et al., 2016; Oppedal et al., 2019), and this may be avoided in larger commercial cages with much greater snorkel and cage volume. At commercial scale the volume to physical equipment ratio will also be much higher, thus reducing the potential for collisions considerably. Maintaining decent water flow and ensuring sufficient snorkel volume, therefore, appear a management key in upholding good fish welfare.

Sampling time	Mean (± SE) fin damage (1–4)		Mean (± SE) snout damage (1–3)		Mean (± SE) condition factor	
	Standard	Snorkel	Standard	Snorkel	Standard	Snorkel
2	3.1 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	1.13 ± 0.04	1.08 ± 0.06
4	3.1 ± 0.0	3.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.24 ± 0.03	1.16 ± 0.03
6	3.0 ± 0.0	3.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.26 ± 0.06	1.17 ± 0.08
8	3.2 ± 0.1	3.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	1.22 ± 0.08	1.19 ± 0.05
10	3.1 ± 0.1	3.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.23 ± 0.06	1.19 ± 0.04
12	3.2 ± 0.2	3.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.0	1.28 ± 0.09	1.22 ± 0.05
14	3.1 ± 0.1	3.1 ± 0.1	1.6 ± 0.3	1.8 ± 0.2	1.22 ± 0.10	1.20 ± 0.04
16	3.3 ± 0.1	3.3 ± 0.1	1.7 ± 0.1	1.6 ± 0.2	1.14 ± 0.09	1.13 ± 0.07
18	3.2 ± 0.1	3.3 ± 0.1	2.3 ± 0.1	2.0 ± 0.1	1.20 ± 0.09	1.18 ± 0.09
20	3.2 ± 0.1	3.5 ± 0.1	2.0 ± 0.3	2.0 ± 0.2	1.28 ± 0.18	1.17 ± 0.09
22	3.2 ± 0.1	3.2 ± 0.0	1.9 ± 0.4	1.9 ± 0.3	1.17 ± 0.14	1.21 ± 0.09
24	3.2 ± 0.1	3.3 ± 0.1	1.6 ± 0.3	1.8 ± 0.3	1.07 ± 0.12	1.21 ± 0.08
26	3.1 ± 0.1	3.0 ± 0.0	1.4 ± 0.3	1.5 ± 0.3	1.03 ± 0.12	1.25 ± 0.06
28	3.2 ± 0.1	3.3 ± 0.1	1.5 ± 0.3	1.4 ± 0.2	0.93 ± 0.17	1.16 ± 0.06

***Table 2**. Mean (±SE) of fin damage (1–4), snout damage (1–3, higher values represent worse fin and snout scores) and condition factor (higher values specify better condition) of 20 fish per cage in three snorkel and three standard sea cages at every second sampling time (once a month) throughout the study period at Låva 2016–2017 (additional data to **paper I**).*

Additionally, there has been concern that constricted cage surface area could prove troublesome for salmon re-filling their swim bladder with air. Without surface access, salmon become negatively buoyant and experience welfare issues often within 6 weeks (Dempster et al., 2009; Korsøen et al., 2009). However, even though previous studies reported slightly faster swimming and lower level of breaching behaviour in salmon snorkel sea cages compared to standard cages (Oppedal et al., 2017; Oppedal et al., 2019), they are unlikely to affect welfare status as salmon seem to use the snorkel sufficiently to maintain neutral buoyancy (Stien et al., 2016; Oppedal et al., 2019). It is noteworthy that salmon lice create physical damage to their host and decrease fish welfare (Costello, 2006), and the lice treatments they necessitate often have poor fish welfare outcomes (Overton et al., 2018a). Thus, reducing lice loads and treatments through depth-based prevention technologies such as snorkel cages (**paper I**), has significant potential to improve fish welfare.

5.1.4 Resistance building

Most methods used for lice treatment can drive treatment selection pressure in louse populations (Aaen et al., 2015; Gallardo-Escárate et al., 2019). It is currently unclear if implementing depth-based prevention methods in commercial production could result in the evolution of resistance against prevention methods in lice. There is, however, the possibility that shielding salmon from surface layers could select for deeper swimming lice that are able to circumvent prevention barriers (Coates et al., 2020). To slow down this potential selection pressure Barrett et al. (2020b) suggested salmon farmers should use several management strategies, such as combining multiple prevention and treatment methods, using spatial “firebreaks” or fallowing to minimise connectivity of louse populations and performing selective breeding for louse-resistant salmon lineages. At the same time, some evolutionary trajectories could have benefits. For instance, selection pressure for deeper swimming lice might reduce infestation pressures on wild salmon (Barrett et al., 2020b; Coates et al., 2020) which often swim at shallow depths (LaBar et al., 1978; Rikardsen et al., 2007; Strøm et al., 2018). However, further studies and modelling efforts are required to test this hypothesis.

5.2 Effect on co-occuring parasites

Depth-based prevention techniques have the potential to influence infestation rates of salmon parasites other than salmon louse. Snorkel sea cages were shown to have the potential to reduce infestation rates of marine tapeworm (*Eubothrium* sp.) (**paper II**) and increase the risk and intensity of infection by the amoeba *P. perurans* (Wright et al., 2017b), the causative agent for AGD (Young et al., 2007). This was also observed in two commercial studies where fish in snorkel sea cages had higher gill scores than standard cages (**Fig. 4a**), which also worsened with deeper snorkels (**Fig. 4b**). However, creating a freshwater surface layer inside the snorkel has the possibility of mitigating infection by amoebae (Wright et al., 2017b) if salinity in the freshwater layer is low enough and salmon reside within it for sufficient time (**paper III**).

5.2.1 Marine tapeworm

In a recent study at commercial scale, salmon populations in standard cages were observed to have 3–5 times as many fish infected with 10–20 times more marine tapeworms than salmon residing in snorkel sea cages (**paper II**). Due to the apparent effectiveness of the depth-based technology, but with no clear relationship between tapeworm infestation and snorkel depth, it was suggested that marine tapeworm transmission most likely occurs in surface waters. Atlantic salmon are infected when they ingest intermediate copepod hosts (Hodneland and Solberg, 1995; Saksvik et al., 2001b) which are often associated with the surface layers. As salmon are usually fed from the surface in commercial sea cages this might lead to increased risk of voluntary or accidental ingestion of intermediate copepods and explain why infestations are reduced in snorkel sea cages. If this principle works, there is also the possibility that using other lice barrier cages or simply using deep feeders might prove to be similarly efficient at reducing tapeworm infestations. However, further studies using different depth-based and deep feeding technologies are needed to ascertain the consistency of these effects on tapeworm infestations in salmon aquaculture.

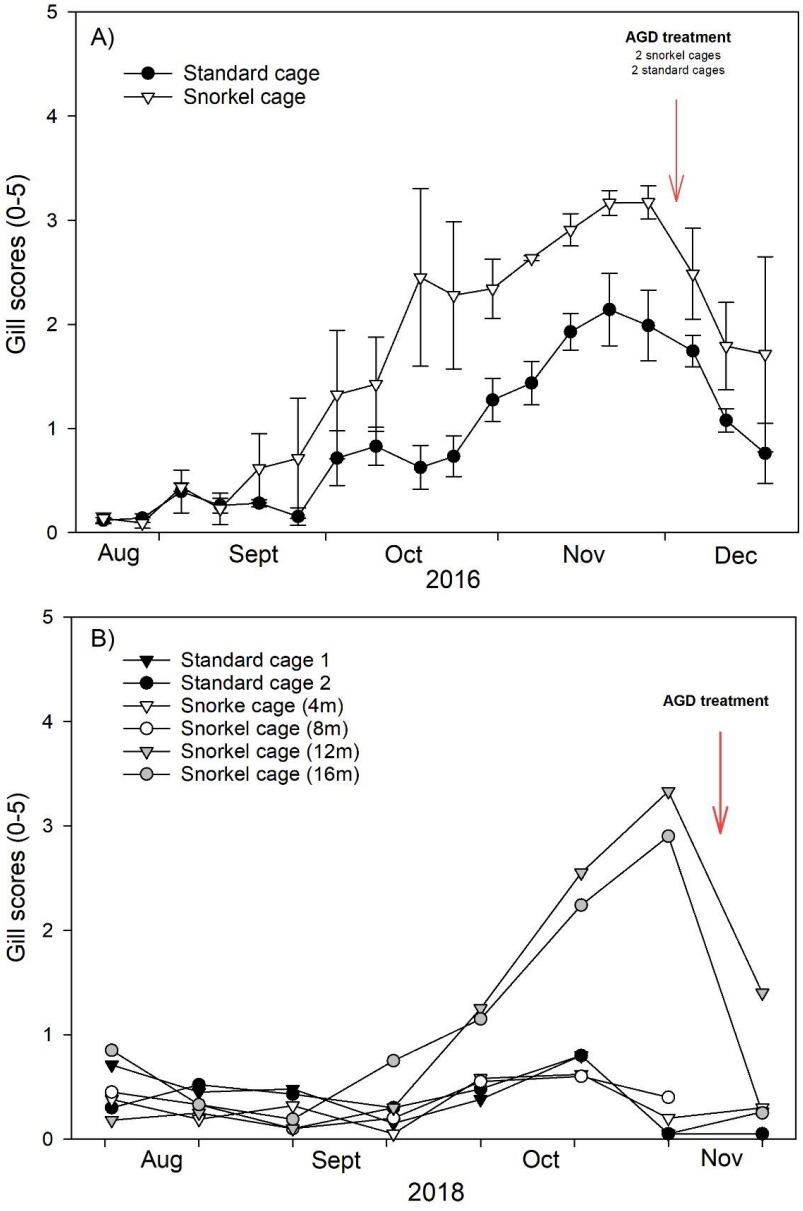


Figure 4. *Mean gill-score (± SE) from 20 fish per cage a) in triplicate snorkel and standard sea cages at Låva in 2016 (additional data to* ***paper I****) and b) two standard cages and four snorkel cages with different shielding depths (4, 8, 12, 16 m) at Prestholmane in 2017 (additional data to* ***paper II****).*

5.2.2 Amoebic gill disease

In contrast, outbreaks of the amoeba *Paramoeba perurans* causing amoebic gill disease (AGD) have been a persistent problem when using and testing snorkel sea cages (**Fig. 4**) (Wright et al., 2017b). There is seemingly both a higher risk for AGD outbreaks (e.g. occurring earlier) and increased intensity of infestation in snorkel sea cages than in standard cages (e.g. higher AGD-related gill scores) (**Fig. 4**), which appear to increase with deeper snorkels (**Fig. 4b**). According to Wright et al. (2017b), a reason for this could be increased crowding of fish inside the snorkel cages, resulting in increased host-to-host transmission of *P. perurans* which relies on host proximity (Crosbie et al., 2010; Nowak, 2012), while another reason could be that the outbreaks were usually preceded by periods of brackish surface water which might have lowered AGD levels in fish held in standard cages by affecting the freshwater-sensitive amoeba *P. perurans* (Oldham et al., 2016). However, by continuously adding freshwater into the snorkels, creating a low salinity layer, AGD outbreaks appear to be mitigated in commercial scale cages (Wright et al., 2017b) without having to remove the snorkels to treat (e.g. hydrogen peroxide or freshwater bath treatments). Though, creating a constant low salinity layer in large sea cages requires huge amounts of freshwater which might be difficult at some sites without adding large expenditures. Additionally, to maintain oxygen levels in larger sea cages it might be necessary to add oxygen to the freshwater, as creating the low salinity layer prevents for constant water exchange in the snorkel and could therefore cause low dissolved oxygen levels and poor fish welfare.

When treating for AGD with freshwater the common exposure length is 3–4 hours (Powell et al., 2015). However, the theory behind the freshwater layer in the snorkel is that salmon will self-treat as they swim through the low salinity layer on their way to re-fill their swim bladder (Dempster et al., 2011) and that these small bursts of freshwater on the gills will delay and mitigate the AGD outbreaks. However, in a smaller scale trial, snorkel sea cages with a constant freshwater layer did not reduce AGD-related gill scores compare to fish in standard or seawater snorkels (**paper III**). It was argued that the lack of effect during this study was because salmon did not

have enough contact with the freshwater layer for it to effectively remove the amoeba. Therefore, for this to be an effective method it may be important to increase attraction to the freshwater layer, so salmon use it long enough to reliably treat against AGD. This could be achieved using i) submerged night lights in the freshwater to attract salmon (Juell and Fosseidengen, 2004; Wright et al., 2015), ii) freshwater temperatures more preferred by salmon (e.g. around 16 °C, Johansson et al., 2006), iii) a more gradual salinity gradient in the freshwater and iv) making sure oxygen levels are within the preferred range for salmon (**paper III**). However, more work is needed to uncover the effects of these fish behaviour manipulations between standard, freshwater snorkel and saltwater snorkel cages, ideally at commercial scale.

5.2.3 Multi-parasite management

Collectively, the findings suggest that commercial use of snorkel technology could control both salmon lice, AGD and marine tapeworm when implemented strategically. Using the prevention technology at the start of a production cycle is likely important for both salmon lice and tapeworm prevention. Newly-transferred post-smolts often show a preference to swim near the halocline for the first couple of months in sea (Oppedal et al., 2011) placing fish in standard cages at high risk for infestation. Additionally, small salmon are more likely to ingest copepods (Ruud, 2019), the intermediate host of tapeworm and using depth-based prevention technology when salmon are small might decrease the infestation of marine tapeworm. As it has been demonstrated that there are weak or no effects on lice infestations from snorkel cages during pycnoclines with warm brackish upper layers, depth-based prevention technology could be abandoned over late summer and early autumn when these situations often occur. Such a strategy could also be beneficial for infections of the amoeba, *P. perurans*, as this is often a problem during autumn. However, based on infestation dynamics, for optimal tapeworm prevention, depth-based technologies should ideally be deployed from May-September. Yet, as snorkel sea cages at 4 m depth were as effective in preventing tapeworm infestations as deeper snorkels (**paper II**) and AGD outbreaks were less intense in shallower snorkels (**Fig. 4b**), pulling or moving up snorkels or other depth-based prevention

technologies during these periods might be beneficial. Deep feeding might also be an option if snorkels are not preferred at this time for lice and amoeba, as feeding could seem a crucial time to avoid surface waters for salmon not to obtain tapeworm. All these studies highlight the importance of monitoring a range of common parasites when testing the performance of a new parasite control strategy and adapting multiple controls in the fight against these parasites.

5.3 *In situ* control methods

Finding an approach to continuously treat against lice that can be used in combination with preventive depth-based technologies is exceedingly important in farm management. This is because lice infestation still occurs in these cage types (**paper I**), and if treatment is required disassembly of the snorkel sea cages is often needed which adds to labour and costs for the farmers. The main methods currently used for continuous lice removal within salmon sea cages are lice-eating cleaner fish and lice-shooting optical lasers. They can both be combined with different preventive approaches, but it is important that the different combinations are trialled first to determine their lice removing efficiency (**paper IV**) (Gentry et al., 2020). Furthermore, in the case of cleaner fish, it is essential that the welfare of this biological agent is not unacceptably compromised during use (**paper V**).

5.3.1 Cleaner fish

Poor welfare and high mortality were observed in different cleaner fish species held in small salmon sea cages over autumn-winter (**paper V**). This relates to reports from the industry of mortalities ranging from 18–48 %, with individual farms experiencing up to 100 % mortality of cleaner fish (Nilsen et al., 2014; Stien et al., 2020). This suggests that fish welfare is of serious concern when using this lice control method and that high mortalities will either lead to weak effects on lice or require multiple introductions of cleaner fish to cages (**paper V**). High unregistered losses were also reported, creating concern for a weakening of genetic composition and local population structures of wild cleaner fish (Faust et al., 2018) if these losses are due to escaped cleaner fish (**paper V**). Therefore, to ensure their welfare and efficiency,

tailored cleaner fish husbandry will be required for the continued use of cleaner fish in salmon aquaculture. If this is achieved, cleaner fish may prove a suitable *in situ* control method, providing further reductions in lice infestations to snorkel cage technology (**paper I**).

However, prevention technology may also alter cleaner fish welfare. Although lice skirts were not found to impact the welfare of corkwing wrasse in a recent study (Gentry et al., 2020). Using cleaner fish in depth-based prevention cages may improve cleaner fish welfare due to reduced delousing events (e.g. in snorkel sea cages) (**paper I**). Additionally, neither wrasse or lumpfish are capable of fast swimming speeds like Atlantic salmon (Hvas et al., 2018; Yuen et al., 2019), and could therefore experience challenges at sites with strong currents (Jónsdóttir et al., 2019; Hvas et al., 2020). As depth-based prevention cages reduce surface current (Frank et al., 2015; Klebert and Su, 2020; Jónsdóttir et al., 2021a; 2021b) they may create a more sheltered environment for cleaner fish compared to standard sea cages, thereby improving cleaner fish welfare. However, this could reduce encounter rates between them and the salmon they clean for parasites, as cleaner fish may prefer to stay in the low-flow water conditions inside the snorkel or skirt (Skiftesvik et al., 2015; Yuen et al., 2019), which are often avoided by salmon (Oldham et al., 2017). Furthermore, low dissolved oxygen events occurring in prevention cages (Stien et al., 2012; Wright et al., 2017b; Stien et al., 2018) may negatively impact both cleaner fish welfare and their depth distributions compared to salmon. In events of low dissolved oxygen, salmon have been observed to swim deeper in cages below the hypoxic water (Oppedal et al., 2011; Oldham et al., 2017), while cleaner fish seem to have a higher physiological tolerance to similar levels of hypoxia (Hvas and Oppedal, 2019). This could create a mismatch between cleaner fish and salmon depth, where salmon swim below the snorkel to avoid the hypoxic water while the cleaner fish continue to stay inside the snorkel or skirt. However, hypoxia avoidance in salmon is not always observed (Stien et al., 2012). Even if depths do match under these conditions, lumpfish activity levels may be reduced at low levels of dissolved oxygen and negatively impact lice-eating performance (Hvas and Oppedal, 2019).

Differences in environmental preferences beyond dissolved oxygen levels and the need for shelter could also affect the depth distribution of cleaner fish and salmon. In a semi-commercial study during autumn-winter, we observed ballan wrasse deep in the cage at warmer, more saline depths while lumpfish were seen to occupy the colder, brackish surface layers (**paper V**). During these conditons salmon would be expected to reside at the same depths as ballan wrasse, preferring warmer temperatures up to 16 °C (Oppedal et al., 2011), thereby creating a mismatch in lumpfish and salmon depths. Depth-based prevention cages could increase this spatial mismatch between salmon and cleaner fish, leading to fewer interactions and lice-feeding opportunities, as was observed in a commercial scale study where corckwing wrasse ate 10 times fewer lice when used in combination with skirts around cages (Gentry et al., 2020). With the increasing use of depth-based prevention cages a greater understanding of swimming depth and preferences of both cleaner fish and salmon across seasons, locations and cage types is vital to assess of encounter probabilities and optimize cleaner fish management.

Behavioural manipulations could prove useful to close the potential gap between depth-distribution of cleaner fish and salmon. Cleaner fish depend on hiding places inside cages (Deady et al., 1995; Imsland et al., 2015), partly due to their need to rest and attach during night (Imsland et al., 2015). In addition, hiding places are often used to create a "cleaning station" for salmon inside cages. In standard cages hides are often placed at the surface extending 10 m deep (Imsland et al., 2018a; 2018b). In depth-based prevention cages however, hides should preferably extend the whole length of the cage to improve the chance of interactions between cleaner fish and salmon while still providing suitable conditions for optimal cleaner fish welfare. Deep feeding and deep lights are used to attract salmon deeper in cages (Frenzl et al., 2014); concepts that could be applied to attract cleaner fish deeper in cages. Additionally, cleaner fish clean salmon during the day (Deady et al. 1995), when lice are visually detected. As light intensity is reduced with depth, the depth of snorkel may affect cleaner fish efficiency by reduced light and prey recognition. Adding deep

lights may therefore also have a positive impact on lice-eating efficiency of cleaner fish.

5.3.2 Optical delousing

Another commercially available control method suitable for deployment in preventive depth-based cages is optical delousing, using laser nodes to continuously target and beam lice off the fish. However, using this method in combination with 10 m deep snorkel sea cages over the course of 54 days during winter did not lower the infestation density of mobile salmon lice compared to cages without laser nodes installed (**paper IV**). One possibility for why the lasers were ineffective could be salmon behaviour, with different environmental conditions and salmon hunger levels changing their depth preference and swimming patterns and influencing encounter rates with laser nodes (**paper IV**). It is therefore important to use available information on salmon behaviour to find the optimal placement for laser nodes throughout the day. In snorkel sea cages, salmon are confined to much smaller volumes than salmon in standard sea cages, which could increase the number of fish passing within the effective range of the node (~ 1.5 m). Fish in snorkel sea cages often swim off-centre and directly below the snorkel edge, possibly making it easier to predict where salmon reside and place laser nodes accordingly. However, the smaller volume of the snorkel might also restrict the horizontal movement of nodes, making it difficult to optimally place the laser. Using lasers in combination with other depth-based technologies such as skirts and deep feeding and lights would mitigate this issue. Snorkel cages are also normally deeper than standard cages to account for the volume lost by the snorkel. As the cable length for the laser nodes is currently restricted to 25 m, deep snorkels may push fish to depths outside the range of the laser. Even so, our study found laser nodes to deliver large numbers of pulses relative to the number of lice in the cage, indicating that a lack of lethality rather than target detection was the limiting factor (**paper IV**). There are no other scientific studies using optical lasers in standard cages making it difficult to compare and conclude if the low effect observed was due to snorkel cage construction or if the effect is the same when used in standard cages. There is therefore a need for more long-term

studies evaluating the effectiveness of optical lasers in different cage types and with different numbers and positions of laser nodes. Full-scale studies elucidating interactions between farm routines, environmental conditions and cage technologies will also be invaluably.

6. Conclusion and future perspectives

The use of depth-based preventive technologies to reduce or limit salmon lice infestations are increasing in salmon aquaculture. These technologies can be successfully used without causing environmental or fish welfare issues. Based on the results from the present studies it is evident that using snorkel sea cages in commercial scale salmon farming provides prophylaxis against salmon lice (*L. salmonis*) and marine tapeworm (*Eubothrium* sp.) infestations throughout a production cycle with the potential to diminish the need treatments for both parasites (**paper I, II**). However, it was also seen that, under specific environmental conditions (i.e. deep halocline and warm surface waters), snorkel sea cages became less effective in preventing salmon lice infestations (**paper I**). It is therefore important to understand the environmental conditions at each aquaculture site when deciding which preventive method to deploy.

Furthermore, salmon in snorkel sea cages may be more prone to AGD outbreaks. However, freshwater filling in snorkels show promise as an *in situ* therapeutic or prophylactic control method towards the freshwater sensitive amoebae, *P. perurans*, if salmon enter for sufficient time to affect amoebal survival (**paper III**). Because snorkel cages increase labour intensity and cost during lice treatments, as they need to be dismanteled and reassembled before and after treatment, *in situ* lice controls will be preferred in conjunction with this technology. We identified problems with two current options for *in situ* lice control within snorkel sea cages. Optical lasers did not significantly reduce lice compared to cages without lasers (**paper IV**). In addition, cleaner fish were found to experience high mortality and poor welfare, in addition to potentially having opposing depth distributions to the salmon they clean (**paper V**). Optimisation of optical laser and cleaner fish deployments is recommended before use as supplementary *in situ* lice controls for depth-based cage technologies.

In order to provide salmon farmers with a comprehensive knowledge base for decision-making, it is necessary to test whether results from small scale trials are relevant to commercial scale farms. It is vital that control strategies used in salmon

sea cages are thoroughly tested before widespread deployment to i) confirm whether they are effective against a parasite, and ii) assess the potential welfare concerns for salmon and any biological control agents used. In that aspect, improving cleaner fish husbandry and lice-eating efficiency in commercial sea cages is an important consideration for future trials.

Further monitoring of fish behaviour in snorkel cages and other depth-prevention cages is also key for improved farm management. Therefore, future trials on how to operate laser nodes more effectively based on salmon behaviour could help improve encounter rates with salmon and lasers. Large gains in lice reduction efficiency and fish welfare could also be achieved by understanding salmon and cleaner fish depth distributions in commercial snorkel sea cages and applying appropriate behavioural manipulations. More focus is therefore needed on the spatial overlap between cleaner fish and salmon in sea cages as a measure of likely interaction, where research conducted in large commercial scale sea cages is imperative as these effects are likely exacerbated in larger cages. With the increasing use of depth-based prevention technology it is also vital that the effects and welfare of cleaner fish and other *in situ* lice controls are tested in combination with the preventive measure.

These studies also highlight the importance of monitoring a range of common parasites when testing the performance of a new parasite control strategy, to ensure the success of overall parasite management of farms. More research into co-occuring parasites is needed to conclusively determine the effect of depth-based prevention cages on marine tapeworms and improve *in situ* freshwater filling as a continuous treatment against AGD. However, research on the potential impact of using depth-based prevention cages on other parasites should also be included, such as the sea lice *Caligus elongatus*, which seemingly increases in snorkel sea cages (pers. obs.). The *Caligus* lice larvae do not show the same aggregation towards the surface as salmon lice larvae (á Norði et al., 2015), and depth-based prevention technologies might therefore impact the infestation rates of *C. elongatus* differently (Stien et al., 2018). However, further studies are required to test this hypothesis.

In the future it will be important to use multiple controls in the fight against these parasites so that resistance evolution is not driven in a single direction. It is also imperative that future studies focus on whether implementing depth-based prevention technology impact resistance building or selection pressure of lice.

References

á Norði, G., Simonsen, K., Danielsen, E., Eliasen, K., Mols-Mortensen, A., Christiansen, D.H., Steingrund, P., Galbraith, M., Patursson, Ø., 2015. Abundance and distribution of planktonic *Lepeophtheirus salmonis* and *Caligus elongatus* in a fish farming region in the Faroe Islands. Aquac. Environ. Interac. 7, 15-27

Aaen, S.M., Helgesen, K.O., Bakke, M.J., Kaur, K., Horsberg, T.E., 2015. Drug resistance in sea lice: a threat to salmonid aquaculture. Trends Parasitol. 31, 72-81

Anon., 2019. Status for norske laksebestander i 2019 [Status for Norwegian salmon stocks in 2019]. Report from Vitenskapelig råd for lakseforvaltning nr 12, 126 s.

Arneberg, P., Skorping, A., Grenfell, B., Read, A.F., 1998. Host densities as determinants of abundance in parasite communities. Proc. R. Soc. Lond. B. Biol. Sci. 265, 1283-1289

Barber, I., 2007. Parasites, behaviour and welfare in fish. Appl. Anim. Behav. Sci. 104, 251-264

Barrett, L.T., Overton, K., Stien, L.H., Oppedal, F., Dempster, T., 2020a. Effect of cleaner fish on sea lice in Norwegian salmon aquaculture: a national scale data analysis. Int. J. Parasitol. 50, 787-796

Barrett, L.T., Oppedal, F., Robinson, N., Dempster, T., 2020b. Prevention not cure: a review of methods to avoid sea lice infestations in salmon aquaculture. Rev. Aquac. 12, 2527-2543

Braaten, B.R., 1975. Recent Norwegian experience in fish farming. Oceanology International, 168-172

Brandal, P.O., Egidius, E., 1977. Preliminary report on oral treatment against salmon lice, *Lepeophtheirus salmonis*, with Neguvon. Aquaculture. 16, 177-178

Bricknell, I.R., Dalesman, S.J., O'Shea, B., Pert, C.C., Luntz, A.J.M., 2006. Effect of environmental salinity on sea lice *Lepeophtheirus salmonis* settlement success. Dis. Aquat. Org. 71, 201

Bristow, G.A., Berland, B., 1991. The effect of long term, low level *Eubothrium* sp. (Cestoda: Pseudophyllidea) infection on growth in farmed salmon (*Salmo salar* L.). Aquaculture. 98, 325-330

Bron, J., Sommerville, C., Rae, G., 1993. Aspects of the behaviour of copepodid larvae of the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837). In: Pathogens of wild and farmed fish: Sea lice. Boxshall, G.A., Defaye, D. (Eds.), Ellis Horwood, Chichester, pp. 125-142.

Brooker, A.J., Skern-Mauritzen, R., Bron, J.E., 2018. Production, mortality, and infectivity of planktonic larval sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837): current knowledge and implications for epidemiological modelling. ICES J. Mar. Sci. 75, 1214-1234

Brown, J.H., 2016. Worth a shot. Fish Farmer Magazine. Edinburgh.

Bui, S., Oppedal, F., Stien, L., Dempster, T., 2016. Sea lice infestation level alters salmon swimming depth in sea-cages. Aquac. Environ. Interac. 8, 429-435

Bui, S., Oppedal, F., Sievers, M., Dempster, T., 2019. Behaviour in the toolbox to outsmart parasites and improve fish welfare in aquaculture. Rev. Aquac. 11, 168-186

Bui, S., Stien, L.H., Nilsson, J., Trengereid, H., Oppedal, F., 2020. Efficiency and welfare impact of long-term simultaneous in situ management strategies for salmon louse reduction in commercial sea cages. Aquaculture. 520, 734934

Burka, J.F., Hammell, K.L., Horsberg, T., Johnson, G.R., Rainnie, D., Speare, D.J., 1997. Drugs in salmonid aquaculture–a review. J. Vet. Pharmacol. Ther. 20, 333-349

Burke, M., Grant, J., Filgueira, R., Stone, T., 2021. Oceanographic processes control dissolved oxygen variability at a commercial Atlantic salmon farm: Application of a real-time sensor network. Aquaculture. 533, 736143

Burridge, L., Weis, J.S., Cabello, F., Pizarro, J., Bostick, K., 2010. Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. Aquaculture. 306, 7-23

Chaput, G., 2012. Overview of the status of Atlantic salmon (*Salmo salar*) in the North Atlantic and trends in marine mortality. ICES J. Mar. Sci. 69, 1538-1548

Coates, A., Phillips, B.L., Oppedal, F., Bui, S., Overton, K., Dempster, T., 2020. Parasites under pressure: salmon lice have the capacity to adapt to depth-based preventions in aquaculture. Int. J. Parasitol. 50, 865-872

Costello, M.J., 2006. Ecology of sea lice parasitic on farmed and wild fish. Trends Parasitol. 22, 475-483

Crosbie, P.B.B., Bridle, A.R., Leef, M.J., Nowak, B.F., 2010. Effects of different batches of *Neoparamoeba perurans* and fish stocking densities on the severity of amoebic gill disease in experimental infection of Atlantic salmon, *Salmo salar* L. Aquacult. Res. 41, 505-516

Crosbie, T., Wright, D.W., Oppedal, F., Johnsen, I.A., Samsing, F., Dempster, T., 2019. Effects of step salinity gradients on salmon lice larvae behaviour and dispersal. Aquac. Environ. Interac. 11, 181-190

Crosbie, T., Wright, D.W., Oppedal, F., Dalvin, S., Myksvoll, M.S., Dempster, T., 2020. Impact of thermoclines on the vertical distribution of salmon lice larvae. Aquac. Environ. Interac. 12, 1-10

Deady, S., Varian, S.J.A., Fives, J.M., 1995. The use of cleaner-fish to control sea lice on two Irish salmon (*Salmo salar*) farms with particular reference to wrasse behaviour in salmon cages. Aquaculture. 131, 73-90

Dempster, T., Korsøen, Ø., Folkedal, O., Juell, J.-E., Oppedal, F., 2009. Submergence of Atlantic salmon (*Salmo salar* L.) in commercial scale sea-cages: A potential short-term solution to poor surface conditions. Aquaculture. 288, 254-263

Dempster, T., Kristiansen, T.S., Korsøen, Ø.J., Fosseidengen, J.E., Oppedal, F., 2011. Technical note: Modifying Atlantic salmon (*Salmo salar*) jumping behavior to facilitate innovation of parasitic sea lice control techniques. J. Anim. Sci. 89, 4281

Escobar-Lux, R.H., Fields, D.M., Browman, H.I., Shema, S.D., Bjelland, R.M., Agnalt, A.-L., Skiftesvik, A.B., Samuelsen, O.B., Durif, C.M.F., 2019. The

effects of hydrogen peroxide on mortality, escape response, and oxygen consumption of *Calanus* spp. FACETS. 4, 626-637
Fahlén, G., 1971. The functional morphology of the gas bladder of the genus Salmo. Cells Tissues Organs. 78, 161-184
FAO, 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.
FAO, 2020. Global Aquaculture Production Dataset 1950–2018. Food and Agriculture Organisation of the United Nations. Available at: http://www.fao.org/fishery/statistics/global-aquaculture-production/en (Accessed 01 February 2021).
Faust, E., Halvorsen, K.T., Andersen, P., Knutsen, H., André, C., 2018. Cleaner fish escape salmon farms and hybridize with local wrasse populations. Royal Society Open Science. 5, 171752
Fjelldal, P.G., Hansen, T.J., Karlsen, Ø., 2020. Effects of laboratory salmon louse infection on osmoregulation, growth and survival in Atlantic salmon. Conservation Physiology. 8
Folkedal, O., Stien, L.H., Nilsson, J., Torgersen, T., Fosseidengen, J.E., Oppedal, F., 2012. Sea caged Atlantic salmon display size-dependent swimming depth. Aquat. Living Resour. 25, 143-149
Folkedal, O., Pettersen, J., Bracke, M., Stien, L., Nilsson, J., Martins, C., Breck, O., Midtlyng, P., Kristiansen, T., 2016. On-farm evaluation of the Salmon Welfare Index Model (SWIM 1.0): theoretical and practical considerations. Anim. Welfare. 25, 135-149
Forseth, T., Barlaup, B.T., Finstad, B., Fiske, P., Gjøsæter, H., Falkegård, M., Hindar, A., Mo, T.A., Rikardsen, A.H., Thorstad, E.B., Vøllestad, L.A., Wennevik, V., 2017. The major threats to Atlantic salmon in Norway. ICES J. Mar. Sci. 74, 1496-1513
Frank, K., Gansel, L., Lien, A., Birkevold, J., 2015. Effects of a shielding skirt for prevention of sea lice on the flow past stocked salmon fish cages. Journal of Offshore Mechanics and Arctic Engineering. 137, 011201
Frenzl, B., Stien, L.H., Cockerill, D., Oppedal, F., Richards, R.H., Shinn, A.P., Bron, J.E., Migaud, H., 2014. Manipulation of farmed Atlantic salmon swimming behaviour through the adjustment of lighting and feeding regimes as a tool for salmon lice control. Aquaculture. 424-425, 183-188
Frenzl, B., 2017. Lasers against parasites: revolutionizing the control of sea louse in Atlantic farmed salmon. . Journal of Ocean Technology. 12, 92-93
Gallardo-Escárate, C., Arriagada, G., Carrera, C., Gonçalves, A.T., Nuñez-Acuña, G., Valenzuela-Miranda, D., Valenzuela-Muñoz, V., 2019. The race between host and sea lice in the Chilean salmon farming: a genomic approach. Rev. Aquac. 11, 325-339
Gentry, K., Bui, S., Oppedal, F., Dempster, T., 2020. Sea lice prevention strategies affect cleaner fish delousing efficacy in commercial Atlantic salmon sea cages. Aquac. Environ. Interac. 12, 67-80
Gismervik, K., Nielsen, K.V., Lind, M.B., Vijugrein, H., 2017. Mekanisk avulsing med FLS-avlusersystem = dokumentasjon av fiskevelferd og effekt mot lus. . Norwegian Veterinary Institute's Report Series: 6-2017

Gismervik, K., Gåsnes, S.K., Gu, J., Stien, L.H., Madaro, A., Nilsson, J., 2019. Thermal injuries in Atlantic salmon in a pilot laboratory trial. Veterinary and Animal Science. 8, 100081

Glaropoulos, A., Stien, L.H., Folkedal, O., Dempster, T., Oppedal, F., 2019. Welfare, behaviour and feasibility of farming Atlantic salmon in submerged cages with weekly surface access to refill their swim bladders. Aquaculture. 502, 332-337

Grimnes, A., Jakobsen, P.J., 1996. The physiological effects of salmon lice infection on post-smolt of Atlantic salmon. J. Fish Biol. 48, 1179-1194

Groner, M.L., Laurin, E., Stormoen, M., Sanchez, J., Fast, M.D., Revie, C.W., 2019. Evaluating the potential for sea lice to evolve freshwater tolerance as a consequence of freshwater treatments in salmon aquaculture. Aquac. Environ. Interac. 11, 507-519

Grøntvedt, R., Jansen, P., Horsberg, T., Helgesen, K., Tarpai, A., 2013. The surveillance programme for resistance to chemotherapeutants in *L. salmonis* in Norway. Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report

Grøntvedt, R.N., Nerbøvik, I.-K.G., Viljugrein, H., Lillehaug, A., Nilsen, H., Gjevre, A.-G., 2015. Thermal de-licing of salmonid fish–documentation of fish welfare and effect. Norwegian Veterinary Institute's Report series 13-2015

Grøntvedt, R.N., Kristoffersen, A.B., Jansen, P.A., 2018. Reduced exposure of farmed salmon to salmon louse (*Lepeophtheirus salmonis* L.) infestation by use of plankton nets: Estimating the shielding effect. Aquaculture. 495, 865-872

Hamre, L.A., Eichner, C., Caipang, C.M.A., Dalvin, S.T., Bron, J.E., Nilsen, F., Boxshall, G., Skern-Mauritzen, R., 2013. The salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) life cycle has only two chalimus stages. PLoS One. 8, e73539

Hamre, L.A., Bui, S., Oppedal, F., Skern-Mauritzen, R., Dalvin, S., 2019. Development of the salmon louse Lepeophtheirus salmonis parasitic stages in temperatures ranging from 3 to 24 C. Aquac. Environ. Interac. 11, 429-443

Hart, B.L., 1990. Behavioral adaptations to pathogens and parasites: Five strategies. Neurosci. Biobehav. Rev. 14, 273-294

Helgesen, K.O., Romstad, H., Aaen, S.M., Horsberg, T.E., 2015. First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse *Lepeophtheirus salmonis* in Norway. Aquaculture Reports. 1, 37-42

Herrero, A., Thompson, K.D., Ashby, A., Rodger, H.D., Dagleish, M.P., 2018. Complex Gill Disease: an Emerging Syndrome in Farmed Atlantic Salmon (*Salmo salar* L.). J. Comp. Pathol. 163, 23-28

Heuch, P.A., Parsons, A., Boxaspen, K., 1995. Diel vertical migration: a possible host-finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids? Can. J. Fish. Aquat. Sci. 52, 681-689

Hevrøy, E., Boxaspen, K., Oppedal, F., Taranger, G., Holm, J., 2003. The effect of artificial light treatment and depth on the infestation of the sea louse *Lepeophtheirus salmonis* on Atlantic salmon (*Salmo salar* L.) culture. Aquaculture. 220, 1-14

Hjeltnes, B., Bang Jensen, B., Bornø, G., Haukaas, A., Walde, C.S., 2019. Fiskehelserapporten 2018. Veterinærinstituttets rapportserie nr. 6a/2019 p. 90-91.

Hodneland, K., Solberg, P., 1995. Infection trials with marine *Eubothrium* in planktonic copepods, with aspects of procercoid development, The 4th International Symposium of Fish Parasitology, Munich, Germany, pp. 3-7.

Hvas, M., Folkedal, O., Imsland, A., Oppedal, F., 2018. Metabolic rates, swimming capabilities, thermal niche and stress response of the lumpfish, *Cyclopterus lumpus*. Biology open. 7, bio036079

Hvas, M., Oppedal, F., 2019. Physiological responses of farmed Atlantic salmon and two cohabitant species of cleaner fish to progressive hypoxia. Aquaculture. 512, 734353

Hvas, M., Folkedal, O., Oppedal, F., 2020. Fish welfare in offshore salmon aquaculture. Rev. Aquac.

ICES, 2020. Working Group on North Atlantic Salmon (WGNAS). ICES Scientific Reports. 2:21. 358 pp.

Imsland, A., Reynolds, P., Eliassen, G., Hangstad, T., Nytrø, A., Foss, A., Vikingstad, E., Elvegård, T., 2015. Assessment of suitable substrates for lumpfish in sea pens. Journal of the European Aquaculture Society. 23, 639-645

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2014. Assessment of growth and sea lice infection levels in Atlantic salmon stocked in small-scale cages with lumpfish. Aquaculture. 433, 137-142

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018a. Assessment of artificial substrates for lumpfish: effect of material thickness and water current speed. Aquacult. Int. 26, 1469-1479

Imsland, A.K.D., Hanssen, A., Nytrø, A.V., Reynolds, P., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018b. It works! Lumpfish can significantly lower sea lice infestation in large-scale salmon farming. Biology Open. 7

Jansen, P.A., Kristoffersen, A.B., Viljugrein, H., Jimenez, D., Aldrin, M., Stien, A., 2012. Sea lice as a density-dependent constraint to salmonid farming. Proc. R. Soc. B. 279, 2330-2338

Johannessen, A., 1977. Early stages of *Lepeophtheirus salmonis* (Copepoda, Caligidae). Sarsia. 63, 169-176

Johansson, D., Ruohonen, K., Kiessling, A., Oppedal, F., Stiansen, J.-E., Kelly, M., Juell, J.-E., 2006. Effect of environmental factors on swimming depth preferences of Atlantic salmon (*Salmo salar* L.) and temporal and spatial variations in oxygen levels in sea cages at a fjord site. Aquaculture. 254, 594-605

Johnsen, I.A., Asplin, L.C., Sandvik, A.D., Serra-Llinares, R.M., 2016. Salmon lice dispersion in a northern Norwegian fjord system and the impact of vertical movements. Aquac. Environ. Interac. 8, 99-116

Jones, M.W., Sommerville, C., Bron, J., 1990. The histopathology associated with the juvenile stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. J. Fish Dis. 13, 303-310

Jónsdóttir, H., Bron, J.E., Wootten, R., Turnbull, J.F., 1992. The histopathology associated with the pre-adult and adult stages of *Lepeophtheirus salmonis* on the Atlantic salmon, *Salmo salar* L. J. Fish Dis. 15, 521-527

Jónsdóttir, K.E., Hvas, M., Alfredsen, J.A., Føre, M., Alver, M.O., Bjelland, H.V., Oppedal, F., 2019. Fish welfare based classification method of ocean current speeds at aquaculture sites. Aquac. Environ. Interac. 11, 249-261

Jónsdóttir, K.E., Volent, Z., Alfredsen, J.A., 2020. Dynamics of dissolved oxygen inside salmon sea cages with lice shielding skirts at two hydrographically different sites. Aquac. Environ. Interac. 12, 559-570

Jónsdóttir, K.E., Klebert, P., Volent, Z., Alfredsen, J.A., 2021a. Characteristic current flow through a stocked conical sea-cage with permeable lice shielding skirt. Ocean Eng. 223, 108639

Jónsdóttir, K.E., Volent, Z., Alfredsen, J.A., 2021b. Current flow and dissolved oxygen in a full-scale stocked fish-cage with and without lice shielding skirts. Applied Ocean Research. 108, 102509

Juell, J.-E., Fernö, A., Furevik, D., Huse, I., 1994. Influence of hunger level and food availability on the spatial distribution of Atlantic salmon, *Salmo salar* L., in sea cages. Aquacult. Res. 25, 439-451

Juell, J.-E., Fosseidengen, J.E., 2004. Use of artificial light to control swimming depth and fish density of Atlantic salmon (*Salmo salar*) in production cages. Aquaculture. 233, 269-282

Kabata, Z., 1982. Copepoda (Crustacea) Parasitic on Fishes: Problems and Perspectives. In: Advances in Parasitology. Lumsden, W.H.R., Muller, R., Baker, J.R. (Eds.), Academic Press, pp. 1-71.

Klebert, P., Su, B., 2020. Turbulence and flow field alterations inside a fish sea cage and its wake. Applied Ocean Research. 98, 102113

Korsøen, Ø.J., Dempster, T., Fjelldal, P.G., Oppedal, F., Kristiansen, T.S., 2009. Long-term culture of Atlantic salmon (*Salmo salar* L.) in submerged cages during winter affects behaviour, growth and condition. Aquaculture. 296, 373-381

Kristoffersen, A.B., Qviller, L., Helgesen, K.O., Vollset, K.W., Viljugrein, H., Jansen, P.A., 2018. Quantitative risk assessment of salmon louse-induced mortality of seaward-migrating post-smolt Atlantic salmon. Epidemics. 23, 19-33

Krkošek, M., 2010. Host density thresholds and disease control for fisheries and aquaculture. Aquac. Environ. Interac. 1, 21-32

Krkošek, M., Connors, B.M., Morton, A., Lewis, M.A., Dill, L.M., Hilborn, R., 2011. Effects of parasites from salmon farms on productivity of wild salmon. Proceedings of the National Academy of Sciences. 108, 14700-14704

Krkošek, M., Revie, C.W., Gargan, P.G., Skilbrei, O.T., Finstad, B., Todd, C.D., 2013. Impact of parasites on salmon recruitment in the Northeast Atlantic Ocean. Proceedings of the Royal Society B: Biological Sciences. 280, 20122359

LaBar, G.W., McCleave, J.D., Fried, S.M., 1978. Seaward migration of hatchery-reared Atlantic salmon (*Salmo salar*) smolts in the Penobscot River estuary, Maine: open-water movements. ICES J. Mar. Sci. 38, 257-269

Ljungfeldt, L.E.R., Quintela, M., Besnier, F., Nilsen, F., Glover, K.A., 2017. A pedigree-based experiment reveals variation in salinity and thermal tolerance in the salmon louse, Lepeophtheirus salmonis. Evolutionary Applications. 10, 1007-1019

Lovdata, 2012. Regulations on combating salmon lice in aquaculture facilities (in Norwegian: Forskrift om bekjempelse av lakselus i akvakulturanlegg) https://lovdata.no/dokument/SF/forskrift/2012-12-05-1140 (accessed September 2020).

Marcos-López, M., Rodger, H., 2020. Amoebic gill disease and host response in Atlantic salmon (*Salmo salar* L.): a review. Parasite Immunol. 42, e12766

McCallum, H., Harvell, D., Dobson, A., 2003. Rates of spread of marine pathogens. Ecol. Lett. 6, 1062-1067

McCallum, H.I., Kuris, A., Harvell, C.D., Lafferty, K.D., Smith, G.W., Porter, J., 2004. Does terrestrial epidemiology apply to marine systems? Trends Ecol. Evol. 19, 585-591

Mo, T., Poppe, T., 2018. Risiko ved bruk av rensefisk i fiskeoppdrett, Norsk Veterinærtidsskrift nr. 2 2018, pp. 90-92.

Moore, G., 2021. New lice treatment method close to commercial launch, *FishFarming expert*. Available at: https://www.fishfarmingexpert.com/article/new-lice-treatment-method-close-to-commercial-launch/ (accessed at 03.03.2021).

Moore, J., 2002. Parasites and the Behavior of Animals. Oxford University Press, Incorporated, Cary, UNITED STATES.

Myksvoll, M.S., Sandvik, A.D., Albretsen, J., Asplin, L., Johnsen, I.A., Karlsen, Ø., Kristensen, N.M., Melsom, A., Skardhamar, J., Ådlandsvik, B., 2018. Evaluation of a national operational salmon lice monitoring system—From physics to fish. PLoS One. 13, e0201338

Nilsen, A., Viljugrein, H., Røsæg, M.V., Colquhoun, D., 2014. Rensefiskhelse–kartlegging av dødelighet og dødelighetsårsaker. Veterinærinstituttets rapportserie. 12, 74

Nilsen, A., Nielsen, K.V., Biering, E., Bergheim, A., 2017. Effective protection against sea lice during the production of Atlantic salmon in floating enclosures. Aquaculture. 466, 41-50

Nilsson, G.E., Östlund-Nilsson, S., 2008. Does size matter for hypoxia tolerance in fish? Biological Reviews. 83, 173-189

Nilsson, J., Folkedal, O., 2019. Sampling of Atlantic salmon *Salmo salar* from tanks and sea cages is size-biased. Aquaculture. 502, 272-279

Nilsson, J., Moltumyr, L., Madaro, A., Kristiansen, T.S., Gåsnes, S.K., Mejdell, C.M., Gismervik, K., Stien, L.H., 2019. Sudden exposure to warm water causes instant behavioural responses indicative of nociception or pain in Atlantic salmon. Veterinary and Animal Science. 8, 100076

Norwegian Directorate of Fisheries, 2020a. Use of cleaner fish 1998-2019. Available at: https://www.fiskeridir.no/English/Aquaculture/Statistics/Cleanerfish-Lumpfish-and-Wrasse (Accessed 09 October 2020).

Norwegian Directorate of Fisheries, 2020b. Sale 1994-2019. Available at: https://www.fiskeridir.no/English/Aquaculture/Statistics/Atlantic-salmon-and-rainbow-trout (Accessed 28 September 2020).

Nowak, B.F., 2012. *Neoparamoeba perurans*. In: Fish parasites: pathobiology and protection. Woo, P.T.K., Buchmann, K. (Eds.), CAB International, Wallingford, p 1–18.

Oldham, T., Rodger, H., Nowak, B.F., 2016. Incidence and distribution of amoebic gill disease (AGD) — An epidemiological review. Aquaculture. 457, 35-42

Oldham, T., Dempster, T., Fosse, J.O., Oppedal, F., 2017. Oxygen gradients affect behaviour of caged Atlantic salmon *Salmo salar*. Aquac. Environ. Interac. 9, 145-153

Oldham, T., Oppedal, F., Dempster, T., 2018. Cage size affects dissolved oxygen distribution in salmon aquaculture. Aquac. Environ. Interac. 10, 149-156

Oppedal, F., Dempster, T., Stien, L.H., 2011. Environmental drivers of Atlantic salmon behaviour in sea-cages: A review. Aquaculture. 311, 1-18

Oppedal, F., Samsing, F., Dempster, T., Wright, D.W., Bui, S., Stien, L.H., 2017. Sea lice infestation levels decrease with deeper 'snorkel' barriers in Atlantic salmon sea-cages. Pest Manag. Sci. 73, 1935-1943

Oppedal, F., Bui, S., Stien, L.H., Overton, K., Dempster, T., 2019. Snorkel technology to reduce sea lice infestations: efficacy depends on salinity at the farm site, but snorkels have minimal effects on salmon production and welfare. Aquac. Environ. Interac. 11, 445-457

Overton, K., Dempster, T., Oppedal, F., Kristiansen, T.S., Gismervik, K., Stien, L.H., 2018a. Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review. Rev. Aquac. 11, 1398-1417

Overton, K., Samsing, F., Oppedal, F., Dalvin, S., Stien, L.H., Dempster, T., 2018b. The use and effects of hydrogen peroxide on salmon lice and post-smolt Atlantic salmon. Aquaculture. 486, 246-252

Overton, K., Barrett, L.T., Oppedal, F., Kristiansen, T.S., Dempster, T., 2020. Sea lice removal by cleaner fish in salmon aquaculture: a review of the evidence base. Aquac. Environ. Interac. 12, 31-44

Plantalech Manella, N., Thorstad, E.B., Davidsen, J.G., Økland, F., Sivertsgård, R., McKinley, R.S., Finstad, B., 2009. Vertical movements of Atlantic salmon post-smolts relative to measures of salinity and water temperature during the first phase of the marine migration. Fish. Manage. Ecol. 16, 147-154

Poppe, T.T., Dalum, A.S., Røislien, E., Nordgreen, J., Helgesen, K.O., 2018. Termisk behandling av laks. Norsk veterinærtidsskrift, 148-156

Powell, A., Treasurer, J., Pooley, C., Keay, A., Lloyd, R., Imsland, A., Garcia de Leaniz, C., 2017. Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. Rev. Aquac. 10, 683-702

Powell, M.D., Reynolds, P., Kristensen, T., 2015. Freshwater treatment of amoebic gill disease and sea-lice in seawater salmon production: Considerations of water chemistry and fish welfare in Norway. Aquaculture. 448, 18

Remen, M., Oppedal, F., Torgersen, T., Imsland, A.K., Olsen, R.E., 2012. Effects of cyclic environmental hypoxia on physiology and feed intake of post-smolt Atlantic salmon: Initial responses and acclimation. Aquaculture. 326-329, 148-155

Remen, M., Sievers, M., Torgersen, T., Oppedal, F., 2016. The oxygen threshold for maximal feed intake of Atlantic salmon post-smolts is highly temperature-dependent. Aquaculture. 464, 582-592

Revie, C.W., Gettinby, G., Treasurer, J.W., Wallace, C., 2005. Evaluating the effect of clustering when monitoring the abundance of sea lice populations on farmed Atlantic salmon. J. Fish Biol. 66, 773-783

Revie, C.W., Hollinger, E., Gettinby, G., Lees, F., Heuch, P.A., 2007. Clustering of parasites within cages on Scottish and Norwegian salmon farms: Alternative sampling strategies illustrated using simulation. Prev. Vet. Med. 81, 135-147

Rikardsen, A.H., Diserud, O.H., Elliott, J.M., Dempson, J.B., Sturlaugsson, J., Jensen, A.J., 2007. The marine temperature and depth preferences of Arctic charr (*Salvelinus alpinus*) and sea trout (*Salmo trutt*a), as recorded by data storage tags. Fish. Oceanogr. 16, 436-447

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

Rozas-Serri, M., 2019. Gill diseases in marine salmon aquaculture with an emphasis on amoebic gill disease. Atlantic. 32, 34-35

Ruud, K., 2019. Infection dynamics of marine *Eubothrium* sp. (Cestoda) in farmed Atlantic salmon. Master thesis. University of Bergen.

Saksvik, M., Nilsen, F., Nylund, A., Berland, B., 2001a. Effect of marine *Eubothrium* sp. (Cestoda: Pseudophyllidea) on the growth of Atlantic salmon, *Salmo salar* L. J. Fish Dis. 24, 111-119

Saksvik, M., Nylund, A., Nilsen, F., Hodneland, K., 2001b. Experimental infection of Atlantic salmon (*Salmo salar)* with marine *Eubothrium* sp.(Cestoda: Pseudophyllidea): observations on the life cycle, aspects of development and growth of the parasite. Folia Parasitol. 48, 118-126

Samsing, F., Johnsen, I., Stien, L.H., Oppedal, F., Albretsen, J., Asplin, L., Dempster, T., 2016a. Predicting the effectiveness of depth-based technologies to prevent salmon lice infection using a dispersal model. Prev. Vet. Med. 129, 48-57

Samsing, F., Oppedal, F., Dalvin, S., Johnsen, I., Vågseth, T., Dempster, T., 2016b. Salmon lice (*Lepeophtheirus salmonis*) development times, body size, and reproductive outputs follow universal models of temperature dependence. Can. J. Fish. Aquat. Sci. 73, 1841-1851

Samsing, F., Johnsen, I., Dempster, T., Oppedal, F., Treml, E.A., 2017. Network analysis reveals strong seasonality in the dispersal of a marine parasite and identifies areas for coordinated management. Landsc. Ecol. 32, 1953-1967

Samuelsen, O.B., Parsons, A.E., Agnalt, A.L., Tjensvoll, T., Lunestad, B.T., Hannisdal, R., 2020. Mortality in the rockpool shrimp *Palaemon elegans* following long-term exposure to low doses of the anti-parasitic drug teflubenzuron. Aquac. Environ. Interac. 12, 23-29

Sandvik, A.D., Johnsen, I.A., Myksvoll, M.S., Sævik, P.N., Skogen, M.D., 2020. Prediction of the salmon lice infestation pressure in a Norwegian fjord. ICES J. Mar. Sci. 77, 746-756

Sievers, M., Korsøen, Ø., Dempster, T., Fjelldal, P., Kristiansen, T., Folkedal, O., Oppedal, F., 2018. Growth and welfare of submerged Atlantic salmon under continuous lighting. Aquac. Environ. Interac. 10, 501-510

Sievers, M., Oppedal, F., Ditria, E., Wright, D.W., 2019. The effectiveness of hyposaline treatments against host-attached salmon lice. Sci. Rep. 9, 6976

Skiftesvik, A.B., Bjelland, R.M., Durif, C.M.F., Johansen, I.S., Browman, H.I., 2013. Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). Aquaculture. 402 403, 113

Skiftesvik, A.B., Durif, C.M.F., Bjelland, R.M., Browman, H.I., 2015. Distribution and habitat preferences of five species of wrasse (Family Labridae) in a Norwegian fjord. ICES J. Mar. Sci. 72, 890-899

Solstorm, D., Oldham, T., Solstorm, F., Klebert, P., Stien, L.H., Vågseth, T., Oppedal, F., 2018. Dissolved oxygen variability in a commercial sea-cage exposes farmed Atlantic salmon to growth limiting conditions. Aquaculture. 486, 122-129

Stehfest, K.M., Carter, C.G., McAllister, J.D., Ross, J.D., Semmens, J.M., 2017. Response of Atlantic salmon *Salmo salar* to temperature and dissolved oxygen extremes established using animal-borne environmental sensors. Sci. Rep. 7, 4545

Stentiford, G.D., Bateman, I.J., Hinchliffe, S.J., Bass, D., Hartnell, R., Santos, E.M., Devlin, M.J., Feist, S.W., Taylor, N.G.H., Verner-Jeffreys, D.W., van Aerle, R., Peeler, E.J., Higman, W.A., Smith, L., Baines, R., Behringer, D.C., Katsiadaki, I., Froehlich, H.E., Tyler, C.R., 2020. Sustainable aquaculture through the One Health lens. Nature Food. 1, 468-474

Stien, L.H., Nilsson, J., Hevrøy, E.M., Oppedal, F., Kristiansen, T.S., Lien, A.M., Folkedal, O., 2012. Skirt around a salmon sea cage to reduce infestation of salmon lice resulted in low oxygen levels. Aquacult. Eng. 51, 21-25

Stien, L.H., Dempster, T., Bui, S., Glaropoulos, A., Fosseidengen, J.E., Wright, D.W., Oppedal, F., 2016. 'Snorkel' sea lice barrier technology reduces sea lice loads on harvest-sized Atlantic salmon with minimal welfare impacts. Aquaculture. 458, 29-37

Stien, L.H., Lind, M.B., Oppedal, F., Wright, D.W., Seternes, T., 2018. Skirts on salmon production cages reduced salmon lice infestations without affecting fish welfare. Aquaculture. 490, 281-287

Stien, L.H., Størkersen, K.V., Gåsnes, S.K., 2020. Analysis of mortality data from survey on cleaner fish. Rapport fra havforskningen 2020-6

Strøm, J.F., Thorstad, E.B., Hedger, R.D., Rikardsen, A.H., 2018. Revealing the full ocean migration of individual Atlantic salmon. Animal Biotelemetry. 6, 2

Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O.T., Nilsen, F., Horsberg, T.E., Jackson, D., 2013. Salmon lice - impact on wild salmonids and salmon aquaculture. J. Fish Dis. 36, 171

Treasurer, J.W., Mordue, A.J., Pike, A.W., 2002. A review of potential pathogens of sea lice and the application of cleaner fish in biological control. Pest Manag. Sci. 58, 546-558

Tully, O., 1992. Predicting infestation parameters and impacts of caligid copepods in wild and cultured fish populations. Invertebr. Reprod. Dev. 22, 91-102

Tully, O., Nolan, D.T., 2002. A review of the population biology and host–parasite interactions of the sea louse *Lepeophtheirus salmonis* (Copepoda: Caligidae). Parasitology. 124, 165-182

Vaughan, D.B., Grutter, A.S., Costello, M.J., Hutson, K.S., 2017. Cleaner fishes and shrimp diversity and a re-evaluation of cleaning symbioses. Fish Fish. 18, 698-716

Vindas, M.A., Johansen, I.B., Folkedal, O., Höglund, E., Gorissen, M., Flik, G., Kristiansen, T.S., Øverli, Ø., 2016. Brain serotonergic activation in growth-stunted farmed salmon: adaption versus pathology. Royal Society Open Science. 3, 160030

Vollset, K.W., Dohoo, I., Karlsen, Ø., Halttunen, E., Kvamme, B.O., Finstad, B., Wennevik, V., Diserud, O.H., Bateman, A., Friedland, K.D., Mahlum, S., Jørgensen, C., Qviller, L., Krkošek, M., Åtland, Å., Barlaup, B.T., 2017. Disentangling the role of sea lice on the marine survival of Atlantic salmon. ICES J. Mar. Sci. 75, 50-60

Wright, D.W., Glaropoulos, A., Solstorm, D., Stien, L.H., Oppedal, F., 2015. Atlantic salmon *Salmo salar* instantaneously follow vertical light movements in sea cages. Aquac. Environ. Interac. 7, 61-65

Wright, D.W., Nowak, B., Oppedal, F., Bridle, A., Dempster, T., 2017a. Free-living *Neoparamoeba perurans* depth distribution is mostly uniform in salmon cages, but reshaped by stratification and potentially extreme fish crowding. Aquac. Environ. Interac. 9, 269-279

Wright, D.W., Stien, L.H., Dempster, T., Vågseth, T., Nola, V., Fosseidengen, J.E., Oppedal, F., 2017b. 'Snorkel' lice barrier technology reduced two co-occurring parasites, the salmon louse (*Lepeophtheirus salmonis*) and the amoebic gill disease causing agent (*Neoparamoeba perurans*), in commercial salmon sea-cages. Prev. Vet. Med. 140, 97-105

Wright, D.W., Geitung, L., Karlsbakk, E., Stien, L.H., Dempster, T., Oldham, T., Nola, V., Oppedal, F., 2018. Surface environment modification in Atlantic salmon sea-cages: effects on amoebic gill disease, salmon lice, growth and welfare. Aquac. Environ. Interac. 10, 255-265

Young, N.D., Crosbie, P.B.B., Adams, M.B., Nowak, B.F., Morrison, R.N., 2007. *Neoparamoeba perurans* n. sp., an agent of amoebic gill disease of Atlantic salmon (*Salmo salar*). Int. J. Parasitol. 37, 1469-1481

Yuen, J.W., Dempster, T., Oppedal, F., Hvas, M., 2019. Physiological performance of ballan wrasse (*Labrus bergylta*) at different temperatures and its implication for cleaner fish usage in salmon aquaculture. Biol. Control. 135, 117-123

Øverlid, M.S., 2017. Characterization of planktonic sea lice distribution and association to fish farm installations. Master Thesis, Norwegian University of Science and Technology.

Individual papers

I

Paper I

Geitung, L., Oppedal, F., Stien, L.H., Dempster, T., Karlsbakk, E., Nola, V., Wright, D.W. (2019)

Snorkel sea cage technology decreases salmon louse infestation by 75% in a full-cycle commercial test.

International Journal for Parasitology 49, 843–846.

https://doi.org/10.1016/j.ijpara.2019.06.003

International Journal for Parasitology 49 (2019) 843–846

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Succinctus

Snorkel sea-cage technology decreases salmon louse infestation by 75% in a full-cycle commercial test

Lena Geitung [a,b,*], Frode Oppedal [c], Lars Helge Stien [c], Tim Dempster [d], Egil Karlsbakk [b], Velimir Nola [c], Daniel W. Wright [c,1]

[a] Bremnes Seashore AS, Øklandsvegen 90, 5430 Bremnes, Norway
[b] Department of Biology, University of Bergen, 5006 Bergen, Norway
[c] Institute of Marine Research, Matre Research Station, 5984 Matredal, Norway
[d] Sustainable Aquaculture Laboratory-Temperate and Tropical (SALTT), School of BioSciences, University of Melbourne, Victoria 3010, Australia

ARTICLE INFO

Article history:
Received 22 February 2019
Received in revised form 28 May 2019
Accepted 3 June 2019
Available online 13 September 2019

Keywords:
Aquaculture
Control
Lepeophtheirus salmonis
Parasite
Salmo salar
Snorkel sea-cage

ABSTRACT

Methods to prevent parasite infestations in farmed fish are becoming widespread, yet tests of their effectiveness often lack commercial relevance and statistical power, which may lead to technology misuse. Here, we examined salmon louse infestation on Atlantic salmon in triplicate commercial snorkel louse barrier and standard cages over a 12 month production cycle. Barrier cages reduced newly settling lice on Atlantic salmon by 75%, with variability in parasite reduction over time depending upon environmental variables. The commercial, triplicate, long-term study design serves as a template to validate performance and detect weaknesses in anti-parasite techniques in fish mariculture.

Intensive animal farming systems are susceptible to parasite outbreaks. However, understanding host-parasite interactions creates opportunities to prevent parasite encounters and infestations in these systems (Bui et al., 2019). In fish mariculture, novel sea-cage designs or host behaviour manipulations that mismatch host and parasite environments have been developed (Wright et al., 2017; Stien et al., 2018) in attempts to overcome the normal free-flow of rapidly spreading marine parasites onto fish stocked in open enclosures (McCallum et al., 2003). These preventive methods appear fruitful against the salmon louse, *Lepeophtheirus salmonis*, the primary parasite causing issues for the world's largest finfish mariculture industry, sea-cage Atlantic salmon, *Salmo salar*, farming. In 2015, Norway produced NOK 49 billion of farmed salmon, but spent > NOK 5 billion to control the parasite (Brooker et al., 2018). Adding a layer of complexity, wild salmonids dying at unacceptable rates from farm-magnified salmon louse populations (Kristoffersen et al., 2018) have triggered the Norwegian government to enforce production volume limits and treatments when salmon louse infestation levels are too high in salmon farms (Lovdata, 2012, 2017).

For management of salmon louse infestation, prophylactic depth-based technologies are emerging (Bui et al., 2019). These include barrier cages (a skirt or snorkel tarpaulin wrapped around the upper depths), submerged cages (repeatedly submerged or submerged with an air dome), semi-enclosed cages (with deep water pumped in), and deep lighting and feeding (motivating salmon to swim deeper). They work by uncoupling salmon from surface-dwelling salmon louse larvae but provide surface air access required for salmon swim bladder reinflation, buoyancy control and optimal welfare. Several trials and case studies report prophylactic depth-based technologies reduce salmon louse infestation levels, however their short-term, research-scale, or sub-optimally replicated nature increases uncertainty surrounding the results (Table 1). Short-term studies will not capture how seasonal variations in louse larvae development and dispersal (Samsing et al., 2016, 2017) and environmental factors that influence host or parasite depths (Heuch et al., 1995; Stien et al., 2016) affect depth-based technologies over full production cycles. In addition, research-scale studies could suffer from scale-dependent differences such as fish numbers and cage volumes that mean their results are not directly transferable to the salmon farming industry

* Corresponding author at: Department of Biology, University of Bergen, Thormøhlens Gate 53A, 5006 Bergen, Norway.
E-mail address: lena.geitung@uib.no (L. Geitung).
[1] Present address: Department of Primary Industries, Narrandera Fisheries Centre, PO Box 182, Narrandera, New South Wales, Australia.

https://doi.org/10.1016/j.ijpara.2019.06.003
0020-7519/

Table 1
The scale, replication and seasonal coverage of studies assessing salmon louse infestation in preventive depth-based cage designs versus standard cages.

Depth-based preventive cage	Study	Commercial scale	≥3 replicates	Seasons covered			
				Autumn	Winter	Spring	Summer
Snorkel	Stien et al. (2016)		x	x			
	Oppedal et al. (2017)		[a]		x		
	Wright et al. (2017)	x				x	x
	Wright et al. (2018)		x	x			
	This study	**x**	**x**	**x**	**x**	**x**	**x**
Skirt	Stien et al. (2018)	x	x				x
	Grøntvedt et al. (2018)	x	x	[d]	[d]	[d]	[d]
Floating enclosed	Nilsen et al. (2017)	x	x[b]	x[c]	x[c]	x[c]	x[c]
Deep light	Hevrøy et al. (2003)				x	x	x
Deep feed and light	Frenzl et al. (2014)	x				x	x
Submerged	Korsøen et al. (2009)		x		x		
	Sievers et al. (2018)		x			x	
	Glaropoulos et al. (2019)		x		x		

The current study is indicated in bold.
[a] Regression design.
[b] Different sites used, with different louse infestation pressures.
[c] Cages were stocked over inconsistent periods using different fish cohorts with variable louse infestation dynamics.
[d] Seasons were not known, but farm sites were tracked for 2–5 months.

(Wright et al., 2017). To have sufficient statistical power, experiments should also use at least three replicate cages to account for expected random environmental variation (Ling and Cotter, 2003), with all cages at the same farm site so they experience similar louse infestation pressures. In a best practice experiment, we compared salmon louse infestation between three commercial-scale snorkel and three standard cages at a single site over 12 months (Fig. 1). Environmental conditions were monitored to assess the influence of periodic brackish water and high surface temperatures, respectively, expected to push lice and fish in standard cages deeper, on snorkel technology effectiveness.

The study was conducted at a commercial salmon sea-cage farm at Låva, Jelsafjorden, Finnøy commune, Norway (59.1° N, 5.6° E). Data were collected through most of a production cycle from sea transfer to harvest, from June 2016 to August 2017. Atlantic salmon (*S. salar*, autumn transferred smolts, Salmobreed strain in four cages and Mowi strain in two cages; the strains were split evenly between cage types) were stocked in triplicate standard and snorkel cages (Fig. 1). The snorkels of 10 m depth were deployed before fish arrival. Two snorkel and two standard cages were stocked between 11–14 June, while one snorkel and one standard cage were stocked on 22 September. At transfer, the number of fish per cage ranged between 147,149–159,775 with an average weight of 82–155 g. Co-stocked cleaner fish were in equal numbers between cage types.

At fortnightly sampling events, we randomly netted and lethally dosed (Benzoak vet., Benzocaine, 200 mg/ml, VESO Vikan, Namsos, Norway) 20 fish per cage, and counted their sessile salmon lice stages (copepodid, chalimus I, chalimus II) while submerged in seawater-filled trays. Sessile lice stages were used to represent new lice encounters when determining the effect of snorkel technology on louse infestation because: (i) they were expected to develop within the fortnightly sampling interval and (ii) were less likely to be influenced by de-lousing measures and cleaner fish compared with mobile stages. We monitored water salinity and temperature between 0–20 m depth daily by profiling a Conductivity, Temperature and Depth (CTD) recorder (SD208, SAIV-AS, Bergen, Norway) at the feed barge. When louse infestations at the farm exceeded the maximum allowed limit of 0.5 adult female lice per fish or 0.2 adult females during weeks 16–21 (Lovdata, 2012), cages over the limit were deloused with hydrogen peroxide or thermolicer treatments. Delousing events that occurred before a sampling event could have reduced the sessile lice numbers recorded to some extent, but as the standard cages were deloused more often, our results on louse reduction in snorkel compared with standard cages are conservative.

Data analyses were performed using R software v.3.1.0 (Copyright 2009, The R Foundation for Statistical Computing, Vienna, Austria). We compared square-root-transformed newly attached lice (copepodid to chalimus II) counts between cage types using linear mixed-effect models, setting cage type as a fixed factor and sampling time as a random effect. Square-root transformed newly attached lice numbers were also compared between cage types at individual times via a Welch's *t*-test. Correlations between

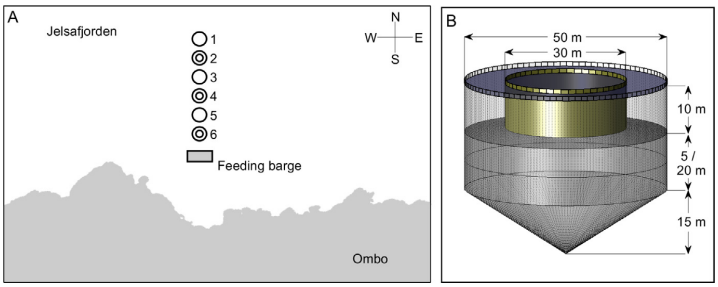


Fig. 1. Schematic of commercial farm used in the study; (A) Låva fish farm, Norway and (B) commercial snorkel sea-cage. The fish farm had six circular cages on a line from the feeding barge and perpendicular to the shoreline. The rectangle represents the feeding barge, the circles represent standard cages and the double circles represent snorkel cages. All cages were 50 m in diameter and 30–50 m deep, while three cages were also fitted with a 30 m diameter and 10 m deep snorkel.

the significance of cage type effects based on P values from t-tests at individual times and the corresponding magnitude of salinity (depth of 28 ppt contour) or temperature stratification (depth of 16 °C contour) in the preceding fortnight were assessed using Pearson's product-moment correlation tests. Error distributions were checked for variance and normality and the significance level was set at $P < 0.05$.

Research data for this article are available in Mendeley Data, DOI: https://doi.org/10.17632/3jn84ngx9t.1.

Overall, throughout the study period newly attached lice were on average 75% lower in snorkel relative to standard cages (mean of 0.17 ± 0.03 versus 0.71 ± 0.07: $\chi^2 = 104{,}18$, $P < 0.001$). When compared at individual times, counts of new infestations were significantly lower in snorkel than in standard cages, 15 of 28 times when 50–100% less lice were observed (Fig. 2). The significance of snorkel effects on newly attached lice was negatively correlated with the intensity of surface brackish water ($t = -2.52$, $P = 0.018$) and surface warm water events ($t = -3.38$, $P = 0.002$) (Fig. 2). Louse bath treatments were reduced by a factor of almost 2 in the three snorkel cages (treated zero, two and two times) in relation to the three standard cages (treated zero, four and three times).

In this study we demonstrate the effectiveness of spatially separating Atlantic salmon from infective salmon louse larvae using depth-based technologies in commercial-scale sea-cages. Over 12 months, approximating a full seawater phase production cycle, installing a 10 m deep snorkel in sea-cages reduced louse infestation by a factor of 4 and louse bath treatments by a factor of almost 2, relative to standard cages (Fig. 2). The reductions are consistent with previous snorkel cage studies at commercial- and research-scales (Stien et al., 2016; Oppedal et al., 2017; Wright et al., 2017). The salmon louse develops through both free-swimming and host-attached stages, and initial host infection involves the infective free-swimming copepodid stage. Infective copepodids vertically migrate into surface waters using positive phototaxis and possibly geotaxis (Bron et al., 1993), using average swimming speeds of 1.55 mm s^{-1} (Heuch et al., 1995). Sea-caged Atlantic salmon typically spend extensive periods in surface waters due to a combination of abiotic and biotic factors and sea-cage structures (Oppedal et al., 2011) which typically expose them to infective lice, and likely explains the success of depth-based prophylactic strategies.

However, depth-based technology effects were weakest when surface brackish water (salinities <28 ppt) and warm surface

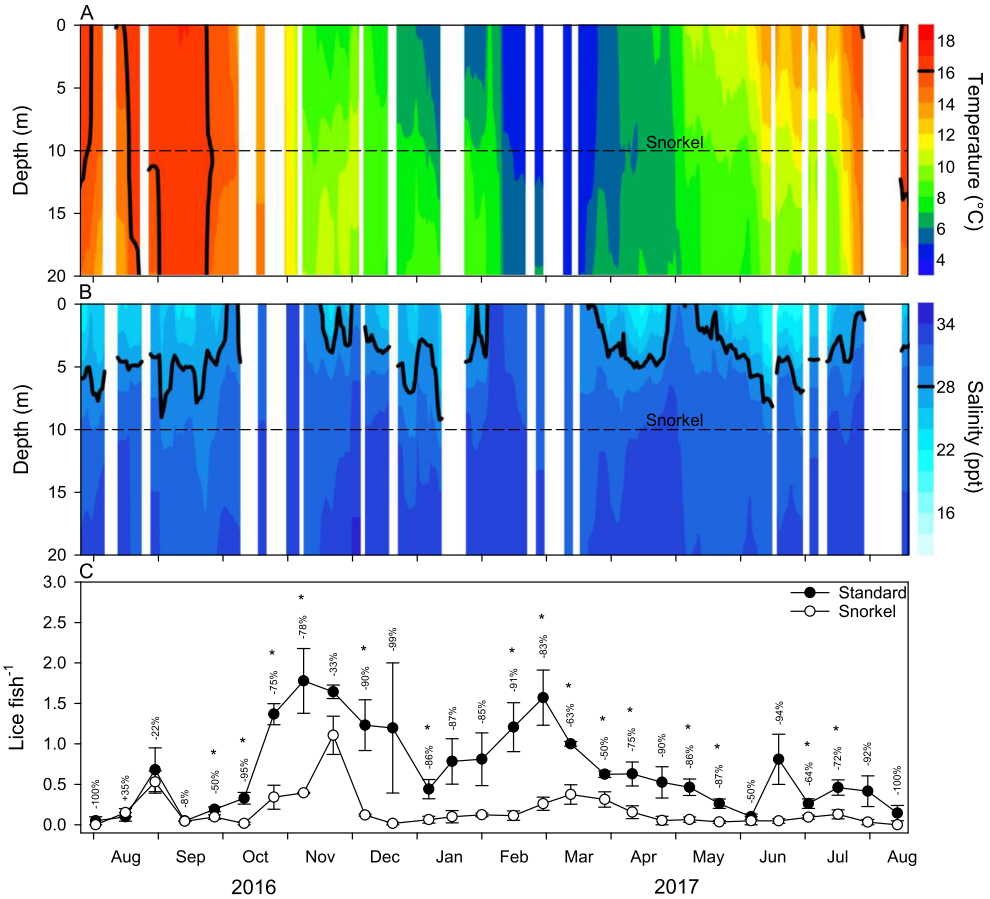


Fig. 2. Daily depth profiles between 0–20 m of (A) temperature (with a black line tracing 16 °C levels) and (B) salinity (with a black line tracing 28 ppt levels) from a reference location at the feed barge at Låva fish farm, Norway. The dashed black line indicates snorkel depth (10 m). Also shown is the mean number (±S.E.) of newly attached lice fish^{-1} (copepodite, chalimus I and chalimus II) per cage type (snorkel and standard cage) for each sampling point (C). The percentage differences between cage types are displayed above each sampling time and significance is indicated with an asterisk when $P < 0.05$.

waters (temperatures >16 °C) occurred. Others have also reported that snorkel sea-cages can make little to no difference in louse infestation in the presence of a strong vertical salinity gradient (Oppedal et al., unpublished data) and at times when fish in standard cages swim deeper and thus both control and snorkel cage fish avoid lice equally (Stien et al., 2016). Infective copepodids have reduced survival at <29 ppt and tend to sink out of low salinity layers to aggregate at haloclines (Heuch et al., 1995; Crosbie et al., 2019), threatening encounters with snorkel fish when these layers penetrate deep enough. Atlantic salmon prefer depths nearest 16 °C for thermoregulation (Oppedal et al., 2011), and likely swim deeper in standard cages when surface temperatures are above this threshold, avoiding infective copepodid encounters. Weak or no effects on louse infestation from snorkel cages during pycnoclines with warm brackish upper layers over late summer and early autumn indicate that depth-based technologies could be abandoned in these situations.

Our experimental design expands on previous studies investigating depth-based technology effects on salmon louse infestation in its combined scale, replication and duration (Table 1). While long-term controlled manipulative experiments in commercial fish production systems are logistically difficult, they are the ultimate test of effectiveness and feasibility for this type of technology. Other fish parasite control methods preventively applied over entire production cycles and lacking data, such as continuous vision-based laser systems (www.stingray.no) and the use of many species of cleaner fish, warrant investigations similar to ours to conclusively reveal performance and weaknesses. Only then can integrated parasite management strategies involving treatments and preventive measures at individual farm and regional scales (Groner et al., 2016) be effectively and adaptively prescribed.

Acknowledgements

We thank Bremnes Seashore, Norway, for commercial salmon farm operations and access, especially Geir Magne Knudsen, Jarle Langvik and Låva farm staff. Funding was provided by Bremnes Seashore, Norway through time-restricted research licenses and SkatteFUNN 279226, Institute of Marine Research, Norway and University of Bergen, Norway. The work was conducted in accordance with the laws and regulations controlling experiments and procedures on live animals in Norway, following the Norwegian Regulations on Animal Experimentation 1996.

References

Bron, J., Sommerville, C., Rae, G., 1837. Aspects of the behaviour of copepodid larvae of the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837). In: Boxshall, G.A., Defaye, D. (Eds.), Pathogens of Wild and Farmed Fish: Sea Lice. Ellis Horwood, Chichester, pp. 125–142.

Brooker, A.J., Skern-Mauritzen, R., Bron, J.E., 2018. Production, mortality, and infectivity of planktonic larval sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837): current knowledge and implications for epidemiological modelling. ICES J. Mar. Sci. 75, 1214–1234.

Bui, S., Oppedal, F., Sievers, M., Dempster, T., 2019. Behaviour in the toolbox to outsmart parasites and improve fish welfare in aquaculture. Rev. Aquac. 11, 168–186.

Crosbie, T., Wright, D.W., Oppedal, F., Johnsen, I.A., Samsing, F., Dempster, T., 2019. Effects of step salinity gradients on salmon lice larvae behaviour and dispersal. Aquac. Environ. Interac. 11, 181–190.

Frenzl, B., Stien, L.H., Cockerill, D., Oppedal, F., Richards, R.H., Shinn, A.P., Bron, J.E., Migaud, H., 2014. Manipulation of farmed Atlantic salmon swimming behaviour through the adjustment of lighting and feeding regimes as a tool for salmon lice control. Aquaculture 424–425, 183–188.

Glaropoulos, A., Stien, L.H., Folkedal, O., Dempster, T., Oppedal, F., 2019. Welfare, behaviour and feasibility of farming Atlantic salmon in submerged cages with weekly surface access to refill their swim bladders. Aquaculture 502, 332–337.

Groner, M.L., Rogers, L.A., Bateman, A.W., Connors, B.M., Frazer, L.N., Godwin, S.C., Krkošek, M., Lewis, M.A., Peacock, S.J., Rees, E.E., Revie, C.W., Schlägel, U.E., 2016. Lessons from sea louse and salmon epidemiology. Philos. Trans. R. Soc. B. Biol. Sci. 371, 20150203.

Grøntvedt, R.N., Kristoffersen, A.B., Jansen, P.A., 2018. Reduced exposure of farmed salmon to salmon louse (*Lepeophtheirus salmonis* L.) infestation by use of plankton nets: Estimating the shielding effect. Aquaculture 495, 865–872.

Heuch, P.A., Parsons, A., Boxaspen, K., 1995. Diel vertical migration: a possible host-finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids? Can. J. Fish. Aquat. Sci. 52, 681–689.

Hevrøy, E., Boxaspen, K., Oppedal, F., Taranger, G., Holm, J., 2003. The effect of artificial light treatment and depth on the infestation of the sea louse *Lepeophtheirus salmonis* on Atlantic salmon (*Salmo salar* L.) culture. Aquaculture 220, 1–14.

Korsøen, Ø.J., Dempster, T., Fjelldal, P.G., Oppedal, F., Kristiansen, T.S., 2009. Long-term culture of Atlantic salmon (*Salmo salar* L.) in submerged cages during winter affects behaviour, growth and condition. Aquaculture 296, 373–381.

Kristoffersen, A.B., Qviller, L., Helgesen, K.O., Vollset, K.W., Viljugrein, H., Jansen, P. A., 2018. Quantitative risk assessment of salmon louse-induced mortality of seaward-migrating post-smolt Atlantic salmon. Epidemics 23, 19–33.

Ling, E.N., Cotter, D., 2003. Statistical power in comparative aquaculture studies. Aquaculture 224, 159–168.

Lovdata, 2012. Regulations on combating salmon lice in aquaculture facilities (in Norwegian: Forskrift om bekjempelse av lakselus i akvakulturanlegg) https://lovdata.no/dokument/SF/forskrift/2012-12-05-1140 (accessed July 2018).

Lovdata, 2017. Forskrift om produksjonsområder for akvakultur av matfisk i sjø av laks, ørret og regnbueørret (produksjonsområdeforskriften) https://lovdata.no/dokument/SF/forskrift/2017-01-16-61#KAPITTEL_2 (accessed July 2018).

McCallum, H., Harvell, D., Dobson, A., 2003. Rates of spread of marine pathogens. Ecol. Lett. 6, 1062–1067.

Nilsen, A., Nielsen, K.V., Biering, E., Bergheim, A., 2017. Effective protection against sea lice during the production of Atlantic salmon in floating enclosures. Aquaculture 466, 41–50.

Oppedal, F., Dempster, T., Stien, L.H., 2011. Environmental drivers of Atlantic salmon behaviour in sea-cages: a review. Aquaculture 311, 1–18.

Oppedal, F., Samsing, F., Dempster, T., Wright, D.W., Bui, S., Stien, L.H., 2017. Sea lice infestation levels decrease with deeper 'snorkel' barriers in Atlantic salmon sea-cages. Pest Manag. Sci. 73, 1935–1943.

Samsing, F., Oppedal, F., Dalvin, S., Johnsen, I., Vågseth, T., Dempster, T., 2016. Salmon lice (*Lepeophtheirus salmonis*) development times, body size, and reproductive outputs follow universal models of temperature dependence. Can. J. Fish. Aquat. Sci. 73, 1841–1851.

Samsing, F., Johnsen, I., Dempster, T., Oppedal, F., Treml, E.A., 2017. Network analysis reveals strong seasonality in the dispersal of a marine parasite and identifies areas for coordinated management. Landsc. Ecol. 32, 1953–1967.

Sievers, M., Korsøen, Ø., Dempster, T., Fjelldal, P., Kristiansen, T., Folkedal, O., Oppedal, F., 2018. Growth and welfare of submerged Atlantic salmon under continuous lighting. Aquac. Environ. Interac. 10, 501–510.

Stien, L.H., Dempster, T., Bui, S., Glaropoulos, A., Fosseidengen, J.E., Wright, D.W., Oppedal, F., 2016. 'Snorkel' sea lice barrier technology reduces sea lice loads on harvest-sized Atlantic salmon with minimal welfare impacts. Aquaculture 458, 29–37.

Stien, L.H., Lind, M.B., Oppedal, F., Wright, D.W., Seternes, T., 2018. Skirts on salmon production cages reduced salmon lice infestations without affecting fish welfare. Aquaculture 490, 281–287.

Wright, D.W., Stien, L.H., Dempster, T., Vågseth, T., Nola, V., Fosseidengen, J.E., Oppedal, F., 2017. 'Snorkel' lice barrier technology reduced two co- occurring parasites, the salmon louse (*Lepeophtheirus salmonis*) and the amoebic gill disease causing agent (*Neoparamoeba perurans*), in commercial salmon sea-cages. Prev. Vet. Med. 140, 97–105.

Wright, D.W., Geitung, L., Karlsbakk, E., Stien, L.H., Dempster, T., Oldham, T., Nola, V., Oppedal, F., 2018. Surface environment modification in Atlantic salmon sea-cages: effects on amoebic gill disease, salmon lice, growth and welfare. Aquac. Environ. Interac. 10, 255–265.

II

Paper II

Geitung, L., Wright, D. W., Stien, L. H., Oppedal, F., Karlsbakk, E.
(*under review*)

Tapeworm (*Eubothrium* sp.) infestation in sea caged Atlantic salmon decreased by lice barrier snorkels during a commercial-scale study.

Tapeworm (*Eubothrium* sp.) infestation in sea caged Atlantic salmon decreased by lice barrier snorkels during a commercial-scale study

Lena Geitung[a,b,*], Daniel W. Wright[c,1], Lars Helge Stien[c], Frode Oppedal[c], Egil Karlsbakk[b]

[a]*Bremnes Seashore AS, Øklandsvegen 90, 5430 Bremnes*

[b]*Department of Biology, University of Bergen, 5006 Bergen, Norway*

[c]*Institute of Marine Research, Matre Research station, 5984 Matredal, Norway*

[1]*Present address: Department of Primary Industries, Narrandera Fisheries Centre, PO Box 182, Narrandera, New South Wales, Australia*

*Corresponding author. Lena Geitung. Email: lena.geitung@uib.no

Postal address: Department of Biology, University of Bergen, Thormøhlens gate 53A, 5006 Bergen, Norway. Telephone: +47 993 89 844

Abstract

Reports of infestation by marine parasitic tapeworms (*Eubothrium* sp.) and an associated growth reduction in Norwegian farmed salmon are on the rise. With few acceptable treatment options available, due to drug resistance evolution in tapeworms or negative drug impacts on fish, alternative controls against the parasite are in demand. In a 10-month commercial-scale study involving standard sea cages and lice barrier snorkel sea cages of different depths (4, 8, 12 and 16 m), we examined if this depth-based preventive technology primarily used against salmon lice (*Lepeophtheirus salmonis*) also reduced tapeworm infestation. A submerged net roof opening to a central barrier tube (snorkel) was added to standard cages to move salmon deeper but retain surface access; a cage manipulation that avoids contact with mostly surface-dwelling salmon lice larvae and may also separate fish from calanoid copepods, the intermediate hosts of *Eubothrium* sp. Salmon populations in unmodified standard cages had higher tapeworm prevalence (63–93 %) and abundances (4.6–5.7 *Eubothrium* sp. fish^{-1}) than those in snorkel cages (20–36 % and 0.2–0.6 *Eubothrium* sp. fish^{-1}). Based on these observations, tapeworm prevention could be another beneficial parasite management outcome of snorkel cage technology or other depth-based prevention techniques against salmon lice.

Keywords: cestode, mariculture, pest control, salmon aquaculture, Salmo salar

1. Introduction

Intensive animal farming systems are often associated with a wide range of pathogens, primarily due to abnormally high host densities and host confinement (Hart, 1990; Krkošek, 2010). This is the case in the world's largest finfish mariculture industry sea cage farming of Atlantic salmon *Salmo salar* (FAO, 2020). Norway is the top producer of Atlantic salmon, producing over 64 billion NOK of farmed salmon in 2018 (Norwegian Directorate of Fisheries, 2020). However, in the last few years, industry growth and production volume has stalled partly owing to outbreaks and control of pathogens. The main challenge for salmon farmers in Norway is the ectoparasitic salmon louse (*Lepeophtheirus salmonis*), as Norwegian regulations require low lice intensities on farmed fish to reduce impacts of the parasite on wild salmonids (Stien et al., 2020). Nevertheless, several other parasites also affect Norwegian farmed salmon, including marine tapeworms (cestodes). Tapeworms belonging to the genus *Eubothrium* have been detected in farmed salmonids for several decades (Berland and Bristow, 1990; Engelstad et al., 1990; Bristow and Berland, 1991a) and have become increasingly problematic in recent years. Reports of *Eubothrium* sp. tapeworm infestations are rising in central and western parts of Norway (Hjeltnes et al., 2018, 2019; Sommerset et al., 2020), and now extend beyond historical ranges into regions north of the Trondheim fjord (Hjeltnes et al., 2019).

Eubothrium spp. tapeworms are intestinal parasites commonly found in salmonids of the northern hemisphere (Shulman, 1961; Wardle et al., 1975; Kennedy, 1978). There are two *Eubothrium* species associated with salmonids in Norway; *E. salvelini* (commonly found in Arctic charr) and *E. crassum* (common in brown trout and Atlantic salmon), both associated with freshwater rivers and lakes (Vik, 1963; Kennedy, 1978). There is also a marine variant of *E. crassum*, often referred to as *Eubothrium* sp., found in sea running trout and Atlantic salmon returning from sea (Kennedy, 1978; Fahy, 1980; Berland, 1997). Controversy has surrounded the identity of this marine *Eubothrium* sp., whether it is identical with the freshwater *E. crassum* (Scholz et al., 2003), a marine form of *E.*

crassum (Kennedy, 1978) or even a different species (Bristow and Berland, 1989). Here, it will be referred to as *Eubothrium* sp. following past studies (Bristow and Berland, 1989; Bristow and Berland, 1991b, a; Saksvik et al., 2001a; Saksvik et al., 2001b; Sundnes, 2003).

Adult *Eubothrium* sp. are often found attached with their scolex to the anterior pyloric caeca of a fish host, while their strobila stretches out into the intestine as the parasite grows (Berland, 1997). Adults can grow very large (> 1 m length) and in some cases take up substantial space in a host's gut. In a field study, Bristow and Berland (1991b) found a significant weight difference between farmed Atlantic salmon with and without marine *Eubothrium* sp. infestation. This was further confirmed in a controlled laboratory study where fish infested with *Eubothrium* sp. grew slower than non-infested fish, even at low intensities of one tapeworm per fish (Saksvik et al., 2001a). Significant reductions in salmon farm production and profit loss may be attributed to *Eubothrium* sp., through the direct loss of salmon growth. In one instance, they were estimated to reduce harvest fish size by 10% (Bristow and Berland, 1991b). Increased food consumption by fish due to tapeworm presence (Walkey and Meakins, 1970; Giles, 1987), potentially leading to additional feeding may also lower farm profits, but this is unstudied in salmon. Anthelminthic drugs administered in feed (Fenbendazole and Praziquantel) have been used by salmon farmers to treat against existing tapeworm infestations. Due to negative side-effects in salmon (e.g. anorexia, growth loss) Fenbendazole is rarely used (Sevatdal and Hellberg, 2006; Sevatdal, 2014) and treatment with Praziquantel have dominated. However, widespread resistance to Praziquantel by *Eubothrium* sp. throughout western Norway has meant this treatment is rarely used against tapeworm infestations (Sevatdal et al., 2008; Sevatdal, 2014; Hjeltnes et al., 2019). Therefore, new control methods are required to optimally treat or reduce *Eubothrium* sp. infestations.

Bothriocephalidean tapeworms generally have a copepod first-intermediate host in their life cycle, but transport hosts may also be involved (Akhmerov, 1962; Vik, 1963; Mulcahy and Kennedy, 1970; Scholz and Kuchta, 2017). Experimental infestations have demonstrated that only the

copepod first-intermediate host is essential for *Eubothrium* sp. to complete their life cycle (Saksvik et al., 2001b). The final fish host can be infected directly via ingestion of a procercoid infected copepod (Saksvik et al., 2001b). Scolex-formation allows attachment to the caeca and further development into an adult tapeworm in the fish host (Berland, 1997; Saksvik et al., 2001b). It is unknown which copepod(s) are the main intermediate hosts leading to tapeworm infestations in wild or cultured salmon, but four calanoid copepods (*Acartia tonsa*, *Acartia clausi*, *Temora longicornis* and *Pseudocalanus elongatus)* are susceptible to infestation by ingesting *Eubothrium* sp. eggs (Hodneland and Solberg, 1995; Saksvik et al., 2001b). While not fully resolved, there is general consensus that *Eubothrium* sp. infestation intensity increases in summer and autumn months, coinciding with increases in intermediate copepod host abundances in the water column (Gundersen, 1953; Matthews, 1967; Saksvik et al., 2001b; Deschutter et al., 2019). Zooplankton, including copepods susceptible to *Eubothrium* sp. infestation, are often associated with surface waters in summer and autumn as primary production is confined to shallow areas.

Infective free-living salmon lice larvae similarly reside in upper depths of the water column (Bron et al., 1993; Heuch et al., 1995; Hevrøy et al., 2003), and several depth-based parasite prevention technologies combatting this parasite could also reduce tapeworm infestations (Bui et al., 2019). These technologies include barrier cages (skirt or snorkel tarpaulin wrapped around upper depths), submerged cages (repeatedly submerged or submerged with an air dome), semi-enclosed cages (deep water pumped in), and deep lighting and feeding (motivating salmon to swim deeper). They work by either moving salmon deeper or shielding salmon from upper depths while still ensuring air access for salmon swim bladder reinflation so buoyancy control and optimal welfare are maintained (Fahlén, 1971; Dempster et al., 2009). Several studies show that these depth-based prevention techniques reduce salmon lice infestation with negligible impact on salmon welfare (Stien et al., 2016; Nilsen et al., 2017; Stien et al., 2018; Geitung et al., 2019; Glaropoulos et al., 2019) and that increasing the shielding depth strengthens lice reductions (Oppedal et al., 2017). In addition, there is

93 the potential to control more than one pathogen by using this technology (Wright et al., 2017;
94 Wright et al., 2018).

95 Here, we examined the effects of a depth-based prevention technology on tapeworm infestations in
96 sea caged Atlantic salmon. In a 10-month study, we observed *Eubothrium* sp. infestations in
97 Atlantic salmon kept in commercial-scale standard cages and lice barrier snorkel cages of different
98 depths (4, 8, 12 and 16 m) to determine if a) the technology alters tapeworm infestations and b)
99 whether a relationship exists between snorkel depth and tapeworm infestation, as previously
100 described for salmon lice (Oppedal et al., 2017). Infestation dynamics were followed to detect the
101 onset and peaks in tapeworm infestations in different sea cage types. We hypothesized that snorkel
102 cage technology would reduce and delay *Eubothrium* sp. infestation and that these effects would
103 strengthen with increasing snorkel depth.

104 **2. Materials and method**

105 *2.1 Experimental setup*

106 The study was conducted at a commercial fish farm (Prestholmane) in Talgjefjorden, Finnøy
107 commune (59.1° N, 5.8° E). Atlantic salmon (autumn transferred smolts, Salmobreed strain) were
108 stocked at sea in 2 standard sea cages and 4 sea cages fitted with snorkels between 21 November –
109 6 December 2017 (Fig. 1). The four snorkels were of 4, 8, 12 and 16 m depth, with net roofs placed
110 accordingly, and were installed before fish arrival. At transfer, the number of fish per cage ranged
111 between 142,473–161,651 with an average weight of 108−168 g. Throughout the experimental
112 period the farm was managed according to standard rearing and feeding procedures in salmon
113 aquaculture.

114 Daily salinity and temperature measurements were performed at the feed barge with a Conductivity,
115 Temperature and Depth (CTD) recorder (SD208, SAIV-AS, Bergen, Norway). Temperature
116 followed normal seasonal variations for the area (Geitung et al., 2019), ranging from 2°C in March

to 18°C in June and with thermal stratification causing warmer surface waters from mid-May to mid-August (Fig. 2a). Salinity varied slightly throughout the trial, but brackish surface water (< 28 ppt) was generally absent (Fig 2b). Minor salinity stratification coincided with thermal stratification (Fig. 2).

Tapeworm infestations in salmon were examined over the first 10 months of production, ceasing in September 2018, when the first snorkel was removed and exposed these fish to surface waters which potentially influenced their subsequent tapeworm infestations. To ascertain whether any tapeworms were of freshwater origin, 60 salmon were examined in the freshwater phase before stocking (Trovåg hatchery, Vindafjord commune). In the marine phase, sampling events were performed every second month with the first done two months after stocking (Table 1). At each sampling event, 20–30 fish per cage were randomly netted and lethally dosed with Benzoak vet. (Benzocaine, 200 mg/ml, VESO Vikan, Namsos, Norway). The fish were then weighed (g) and measured (cm) (Supplementary table 1), before the gastrointestinal tracts of the fish (i.e. pylorus region and intestine) were dissected out and placed in individually labelled bags. The intestines were stored at -20 °C prior to examination.

2.2 Laboratory analyses

In the laboratory (University of Bergen), the intestines were examined for tapeworm infestation. In order to maximise inferential power from a practical number of examinations, intestines from the most extreme groups (standard cages and 16 m snorkel cage) were examined for worms every second month, while all groups were examined at the final sample (Table 1). Before examination, the pylorus region and the intestine were separated and placed in Petri dishes with physiological saline (1% NaCl). The pylorus region was examined by squeezing it between two Petri dishes and viewing under a stereo microscope, noting the presence of *Eubothrium* sp. as well as the number of individuals per fish based on scolex counts. The intestines were cut open and mucosa scraped off with a scalpel before being squeezed and examined. For smaller fish, the pylorus region and

intestine could be squeezed whole, while for larger fish both the pylorus region and intestine were cut into smaller pieces to sufficiently squeeze regions and observe tapeworms. Small (0.4–1.0 mm long) unstrobilated juveniles were referred to as 'plerocercoids', juveniles (<5 mm long) with a few proglottids as 'plerocerciform' and larger immature small worms as 'juveniles'. All worms from each fish were dissected out, washed and either weighed (g) after removing excess moisture on absorbent paper or measured (length mm). The latter was necessary for specimens too small or fragile to be weighed and weight was then estimated from length using a standard length-weight relationship (Ruud, 2019).

2.3 Data analysis

Data analyses were performed in R software v.3.1.0 (package stats, R Core Team (2019)). The parasitological terms in this study are used as defined by Bush et al. (1997), with prevalence being the proportion of fish that are infected, abundance being the number of individual parasites in a host regardless of whether or not the host is infected and intensity being the number of individual parasites in an infected host. Tapeworm prevalence was compared using one-way Fishers Exact test (FET) (function fisher.test), while tapeworm abundances and total weights were compared using nonparametric Mann-Whitney U-tests (MW) (function wilcox.test). Differences between standard and snorkel cages were compared for the last sampling point which were representative of tapeworm infestations accumulated over the course of the study. Bootstrapping (function boot, Davison and Hinkley (1997)) was used to obtain 95% confidence intervals and the significance level was set at $P < 0.05$.

3. Results

3.1 Snorkel versus standard cages

Tapeworm infestations were low in all examined cages until May and were lower in snorkel compared to standard cages at the end of the study (Fig. 3). At the final sampling time (September

2018), sample prevalence appeared to decrease with increasing snorkel depth (Fig. 3a), but the only significant step was between standard cages (or 0 m depth) and the first snorkel depth (Fig. 3a, FET, $p<0.001$ and $p=0.024$ respectively). Similarly, mean abundance of *Eubothrium* sp. (Fig. 3b, MW, $w=1562$, $p<0.001$ and $w=1158$, $p=0.019$) and mean worm weight fish^{-1} was highest in standard cages (Fig 3c, MW, $w=1577$, $p<0.001$ and $w=1173$, $p=0.014$). Final mean numbers of *Eubothrium* sp. fish^{-1} were 5.7 [2.4–11.6] and 4.6 [0.8–12.3] (standard cage), 0.6 [0.3–1.0] (4 m snorkel), 0.5 [0.1–1.2] (8 m snorkel), 0.3 [0.1–0.6] (12 m snorkel) and 0.2 [0.1–0.4] (16 m snorkel) *Eubothrium* sp. fish^{-1} (details in Supplementary table 2). This equated to salmon in standard cages having 10–20 times more tapeworms than those in snorkel cages at the end of the experiment.

3.2 Infestation dynamics

No tapeworms were found in fish sampled in the freshwater phase. Tapeworm growth is variable and cannot be used to accurately back-calculate the duration of infestation (Saksvik et al., 2001b). Therefore, in the present study, significant increases in tapeworm prevalence and abundances between sampling times were taken as evidence that infestation had been recently acquired. In standard cage 1, in the cage row most distant from shore (Fig. 1), no tapeworm infestations were registered until May, when a 2 mm plerocerciform worm was found. Prevalence then markedly increased to 83% in July and 93% in September (Fig. 4a), while abundances increased to a mean of 2.5 *Eubothrium* sp. fish^{-1} in July and 5.7 *Eubothrium* sp. fish^{-1} in September (Fig. 4b). At the last sampling time in September both plerocerciform juveniles and larger adult worms (max. 75 cm) occurred. In standard cage 2, closest to shore (Fig. 1), the first tapeworm, a 14 mm juvenile, was found in January (70 days post sea transfer). The prevalence thereafter increased gradually, reaching 25% in May and 63% in September (Fig. 4a). Abundances also gradually increased, reaching a mean of 0.5 *Eubothrium* sp. fish^{-1} in May and 4.7 *Eubothrium* sp. fish^{-1} in September (Fig. 4b). In the 16 m snorkel cage, the first evidence for infestation was seen in July (13 %) with a mean abundance of 0.63 *Eubothrium* sp. fish^{-1}, where both plerocercoids and juvenile worms were

found (0.5–14 mm long) (Fig. 4). In September, mean abundance (0.23 *Eubothrium* sp. fish^{-1}) and prevalence (20%) remained similar, but both plerciform and larger subgravid worms (≤37 cm) were present (Fig. 4).

4. Discussion

In this study we demonstrated the potential for depth-based technologies, currently used to prevent salmon lice, to also reduce *Eubothrium* sp. tapeworm infestations in Atlantic salmon kept in commercial-scale sea cages. Over the 10-month trial from winter to autumn, standard cages had 3–5 times as many fish infected with 10–20 times more worms than lice barrier snorkel cages. All snorkel cages, even those with barriers of 4 m depth, decreased tapeworm prevalence and abundance. This suggests that prevention of *Eubothrium* sp. could be an additional benefit when using snorkel sea cages or other depth-based prevention techniques against salmon lice, as these are generally of 10 m depth when used in commercial-scale sea cages (Wright et al., 2017; Stien et al., 2018; Geitung et al., 2019). To address additional aspects of the effects of depth-based prevention technologies further studies using alternate designs (e.g. cage replication, longer duration) are needed.

Tapeworm prevalence appeared to decrease with increasing snorkel depth, however there was no clear relationship between tapeworm infestation and snorkel depth as previously observed for salmon lice (Oppedal et al., 2017). Effectiveness of a depth-based technology in this study suggests that *Eubothrium* sp. transmission events are more likely in surface waters. Transmission of *Eubothrium* sp. involves salmon ingesting infected copepods (Hodneland and Solberg, 1995; Saksvik et al., 2001b). Atlantic salmon are usually fed from the surface in commercial sea cages and typically spend extensive periods in surface waters due to a combination of abiotic and biotic factors (Oppedal et al., 2011). Calanoid copepods in the upper water masses may be voluntary or accidentally ingested by salmon in upper cage depths and forcing fish into deeper water in snorkel cages likely minimises exposure to them.

Eubothrium sp. can affect salmon growth at both high and low intensities, even at one tapeworm fish^{-1} (Saksvik et al., 2001a). One reason for this is the 'crowding effect' causing worms at high intensities in a single host to remain small (Read, 1951; Roberts, 2000), while a single worm in a host can grow to > 1 m in length and 5.9 g in weight and weigh more than hundreds of smaller individuals (Berland and Bristow, 1994; Ruud, 2019). Hence worm biomass may be a better predictor of any effects on salmon growth than worm intensities. If the effects on host growth relates to parasite mass, it is vital that a prevention technique not only reduces the number of worms in each fish but also the proportion of fish infected, as observed in this study. However, contrary to the expectation from crowding (Saksvik et al., 2001b), worm weight per fish was lower for the lighter infestations occurring in snorkel cages compared to the higher intensity infestations occurring in standard cages. One reason is that infestations were delayed in snorkel compared to standard cages and more time may be needed before worm weight per fish in cages with lighter infestations exceed those in cages with higher intensity infestations. In addition, slower worm development may have occurred in snorkel fish exposed to lower temperatures below or within snorkels (filled with water at the snorkel depth) during thermal stratification over summer. As salmon and tapeworms are ectotherms, their growth rates are influenced by external temperatures with cooler water slowing growth (Chubb, 1982; Handeland et al., 2008). While depth-based manipulations like snorkel sea cages can alter the temperatures experienced by salmon and their parasites, previous research has found no difference in salmon growth between snorkel and standard sea cages (Stien et al., 2016; Oppedal et al., 2017; Wright et al., 2018; Oppedal et al., 2019). Our results, from this long-term 10-month study suggest that snorkels of 4–16 m depth lower tapeworm prevalence, abundance and worm weight which should minimise growth losses normally experienced due to worm presence. However, studies of even longer duration, at different locations (e.g. with strong vertical salinity gradients (Oppedal et al., 2019)), with designs adding cage replication and using different depth-based technologies should be conducted to ascertain the consistency of these effects on tapeworm infestations in salmon aquaculture.

Determining tapeworm induced effects on fish in a field study can be difficult. Competition for limited food resources between parasite and host explains the reduced condition often observed in fish hosts with internal tapeworms (Smith, 1973; Hoffmann et al., 1986). However, sufficient food supply for the host is thought to diminish these effects (Rees, 1967). A normal feeding regime in salmon aquaculture may therefore reduce or eliminate growth differences caused by tapeworms compared with situations where fish are not fed to excess (Boyce, 1979; Saksvik et al., 2001a). In addition, as tapeworm growth is highly variable and size cannot be used to estimate the age of an infestation (Saksvik et al., 2001a), it is difficult to control for the time of infestation and potential concurrent infestations in field studies. Nonetheless, the potential for tapeworm presence to reduce growth rates (Boyce, 1979; Bristow and Berland, 1991b; Saksvik et al., 2001a), increase food consumption (Walkey and Meakins, 1970; Giles, 1987), or potentially cause immunodepression in fishes (Boyce and Yamada, 1977; Bristow and Berland, 1991b; Saksvik et al., 2001a) should be of concern to salmon farmers from both economic and fish welfare perspectives. Further controlled laboratory studies of tapeworm impacts on salmon are required to properly gauge the extent of these problems.

Eubothrium sp. infestations observed in this study varied seasonally, with the first evidence of parasite acquisition in winter-spring and increasing prevalence and abundance from late May to September. Elevated infestation pressure during summer and autumn is in line with previous studies (Berland and Bristow, 1991; Ruud, 2019). Several cestodes show seasonal fluctuations in infestation pressure (Chubb, 1982; Kennedy, 1996; Scholz and Moravec, 1996; Hanzelová and Gerdeaux, 2003), often associated with the availability of infectious stages and changes in host behaviour throughout the seasons (Chubb, 1982; Williams and Jones, 1994). Information on the seasonality of *Eubothrium* sp. infestation is scarce but appears to be linked to the presence of possible intermediate hosts. The relevant calanoid copepods in Norwegian fjords peak in abundance from May–September (Gundersen, 1953; Matthews, 1967) covering the period when the highest infestation pressure of *Eubothrium* sp. are observed in farmed salmon. An alternative infestation

route is through smaller fish acting as paratenic hosts (Rosen, 1919; Vik, 1963). However, this is unlikely since few possible paratenic hosts enter salmon sea cages holding large fish. Larger salmon may have a lower chance of infestation, as their coarser gill rakers make it more difficult to filter copepods (adult size range: 1.1–2.5 mm) (Enckell, 1980; Ruud, 2019). Based on these infestation dynamics, depth-based technologies such as snorkel cages should ideally be deployed from May-September (possibly longer) for optimal tapeworm prevention and preferably in the first part of the seawater production cycle while fish are small.

The use of depth-based prevention techniques to reduce or limit salmon lice infestations are increasing in salmon aquaculture (Bui et al., 2019), yet commercial-scale testing and effects on co-occurring parasites are seldom documented (Geitung et al., 2019). Here we show that snorkel lice barrier cages, which reduce salmon lice (Stien et al., 2016; Oppedal et al., 2017; Wright et al., 2017; Geitung et al., 2019; Oppedal et al., 2019), also have the potential to limit *Eubothrium* sp. infestations in commercial-scale salmon sea cages. This adds to previous research on controlling co-occurring parasites in snorkel sea cages where freshwater-filling of snorkels has been tested as a prophylactic control method for Amoebic Gill Disease (AGD) outbreaks (Wright et al., 2017; Wright et al., 2018), which appear to worsen in snorkel sea cages (Wright et al., 2017). This work underlines the importance and potential advantages of considering multiple parasites when developing new parasite control strategies (Groner et al., 2016) and testing these strategies at commercial-scale (Geitung et al., 2019).

Acknowledgements

We wish to thank Bremnes Seashore Norway, for commercial salmon farm operations and access, especially Geir Magne Knudsen, Hanne Grytdal and Prestholmane farm staff. We also want to thank Velimir Nola for his valuable assistance with practical work. Funding was provided by Bremnes Seashore, through time-restricted research licenses and SkatteFUNN 279226, as well as from the Institute of Marine Research and University of Bergen. The work was conducted in

accordance with the laws and regulations controlling experiments and procedures on live animals in Norway, following the Norwegian Regulations on Animal Experimentation 1996.

Reference list

Akhmerov, A.K., 1962. Biology of the cestode *Eubothrium crassum* (Bloch, 1779). Trudy Gel'mintologicheskoi Laboratorii. Akademiya Nauk SSSR. 12, 5-8

Berland, B., Bristow, G.A., 1990. Om bendelmark i laksefisk (On tapeworms in salmonids). Bulletinen. 2, 14-20

Berland, B., Bristow, G.A., 1991. The cestode *Eubothrium* sp. in farmed marine salmon - infection pattern and treatment. Bull. Scand. Soc. Parasitol. 1, 53

Berland, B., Bristow, G.A., 1994. The cestode *Eubothrium* sp. in farmed marine salmon." Abstract book, 8th International Congress of Parasitology, Izmir, Turkey 10th-14th October 1994, page 53.

Berland, B., 1997. Current fish parasite problems-Scandinavia and adjoining area. Acta Parasitologica Turcica. 20, 429-443

Boyce, N.P., Yamada, S.B., 1977. Effects of a parasite, *Eubothrium salvelini* (Cestoda: Pseudophyllidea), on the resistance of juvenile sockeye salmon, *Oncorhynchus nerka*, to zinc. Journal of the Fisheries Board of Canada. 34, 706-709

Boyce, N.P., 1979. Effects of *Eubothrium salvelini* (Cestoda: Pseudophyllidea) on the growth and vitality of sockeye salmon, *Oncorhynchus nerka*. Can. J. Zool. 57, 597-602

Bristow, G.A., Berland, B., 1989. *Eubothrium crassum* (Cestoda: Pseudophyllidea)-one species or two? Electrophoretic evidence for species status of sea-water and fresh-water forms, The 14th symposium of the Scandinavian Society for Parasitology, Helsingør, Denmark, pp. 2-4.

Bristow, G.A., Berland, B., 1991a. A report on some metazoan parasites of wild marine salmon (*Salmo salar* L.) from the west coast of Norway with comments on their interactions with farmed salmon. Aquaculture. 98, 311-318

Bristow, G.A., Berland, B., 1991b. The effect of long term, low level *Eubothrium* sp. (Cestoda: Pseudophyllidea) infection on growth in farmed salmon (*Salmo salar* L.). Aquaculture. 98, 325-330

Bron, J., Sommerville, C., Rae, G., 1993. Aspects of the behaviour of copepodid larvae of the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837). In: Pathogens of wild and farmed fish: Sea lice. Boxshall, G.A., Defaye, D. (Eds.), Ellis Horwood, Chichester, pp. 125-142.

Bui, S., Oppedal, F., Sievers, M., Dempster, T., 2019. Behaviour in the toolbox to outsmart parasites and improve fish welfare in aquaculture. Rev. Aquac. 11, 168-186

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology Meets Ecology on Its Own Terms: Margolis et al. Revisited. The Journal of Parasitology. 83, 575-583

Chubb, J.C., 1982. Seasonal Occurrence of Helminths in Freshwater Fishes Part IV. Adult Cestoda, Nematoda and Acanthocephala. In: Advances in Parasitology. Lumsden, W.H.R., Muller, R., Baker, J.R. (Eds.), Academic Press, pp. 1-292.

Davison, A.C., Hinkley, D.V., 1997. Bootstrap Methods and Their Applications. Cambridge University Press, Cambridge. ISBN 0-521-57391-2.

Dempster, T., Korsøen, Ø., Folkedal, O., Juell, J.-E., Oppedal, F., 2009. Submergence of Atlantic salmon (*Salmo salar* L.) in commercial scale sea-cages: A potential short-term solution to poor surface conditions. Aquaculture. 288, 254-263

Deschutter, Y., De Schamphelaere, K., Everaert, G., Mensens, C., De Troch, M., 2019. Seasonal and spatial fatty acid profiling of the calanoid copepods *Temora longicornis* and *Acartia clausi* linked to environmental stressors in the North Sea. Mar. Environ. Res. 144, 92-101

Enckell, P.H., 1980. Kräftdjur. Signum, Lund, Sweden. p. 685.

Engelstad, M., Berland, B., Bristow, G.A., 1990. Bendelmark i laks. Norsk Fiskeoppdrett. 15, 50

Fahlén, G., 1971. The functional morphology of the gas bladder of the genus Salmo. Cells Tissues Organs. 78, 161-184

Fahy, E., 1980. *Eubothrium crassum* in migratory trout, *Salmo trutta* L., in the sea. J. Fish Biol. 16, 99-104

345 FAO, 2020. Global Aquaculture Production Dataset 1950–2017. Food and Agriculture Organisation
346 of the United Nations. Available at: http://www.fao.org/fishery/statistics/global-aquaculture-
347 production/en (Accessed 28 September 2020).
348 Geitung, L., Oppedal, F., Stien, L.H., Dempster, T., Karlsbakk, E., Nola, V., Wright, D.W., 2019.
349 Snorkel sea-cage technology decreases salmon louse infestation by 75% in a full-cycle
350 commercial test. Int. J. Parasitol. 49, 843-846
351 Giles, N., 1987. Predation risk and reduced foraging activity in fish: experiments with parasitized
352 and non-parasitized three-spined sticklebacks, *Gasterosteus aculeatus* L. J. Fish Biol. 31,
353 37-44
354 Glaropoulos, A., Stien, L.H., Folkedal, O., Dempster, T., Oppedal, F., 2019. Welfare, behaviour and
355 feasibility of farming Atlantic salmon in submerged cages with weekly surface access to
356 refill their swim bladders. Aquaculture. 502, 332-337
357 Groner, M.L., Rogers, L.A., Bateman, A.W., Connors, B.M., Frazer, L.N., Godwin, S.C., Krkošek,
358 M., Lewis, M.A., Peacock, S.J., Rees, E.E., Revie, C.W., Schlägel, U.E., 2016. Lessons
359 from sea louse and salmon epidemiology. Philos. Trans. R. Soc. B. Biol. Sci. 371, 20150203
360 Gundersen, K.R., 1953. Zooplankton investigations in some fjords in Western Norway during 1950-
361 1951. Directorate of Fisheries. Report on Norwegian Fishery and Marine Investigations.
362 Vol. X. No. 6.
363 Handeland, S.O., Imsland, A.K., Stefansson, S.O., 2008. The effect of temperature and fish size on
364 growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic
365 salmon post-smolts. Aquaculture. 283, 36-42
366 Hanzelová, V., Gerdeaux, D., 2003. Seasonal occurrence of the tapeworm *Proteocephalus*
367 *longicollis* and its transmission from copepod intermediate host to fish. Parasitol. Res. 91,
368 130-136
369 Hart, B.L., 1990. Behavioral adaptations to pathogens and parasites: Five strategies. Neurosci.
370 Biobehav. Rev. 14, 273-294

371 Heuch, P.A., Parsons, A., Boxaspen, K., 1995. Diel vertical migration: a possible host-finding
372 mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids? Can. J. Fish. Aquat.
373 Sci. 52, 681-689
374 Hevrøy, E., Boxaspen, K., Oppedal, F., Taranger, G., Holm, J., 2003. The effect of artificial light
375 treatment and depth on the infestation of the sea louse *Lepeophtheirus salmonis* on Atlantic
376 salmon (*Salmo salar* L.) culture. Aquaculture. 220, 1-14
377 Hjeltnes, B., Bang Jensen, B., Bornø, G., Haukaas, A., Walde, C.S., 2018. Fiskehelserapporten
378 2017. Veterinærinstituttets rapportserie nr. 1a/2018 p. 78.
379 Hjeltnes, B., Bang Jensen, B., Bornø, G., Haukaas, A., Walde, C.S., 2019. Fiskehelserapporten
380 2018. Veterinærinstituttets rapportserie nr. 6a/2019 p. 90-91.
381 Hodneland, K., Solberg, P., 1995. Infection trials with marine *Eubothrium* in planktonic copepods,
382 with aspects of procercoid development, The 4th International Symposium of Fish
383 Parasitology, Munich, Germany, pp. 3-7.
384 Hoffmann, R., Kennedy, C.R., Meder, J., 1986. Effects of *Eubothrium salvelini* Schrank, 1790 on
385 arctic charr, *Salvelinus alpinus* (L.), in an alpine lake. J. Fish Dis. 9, 153-157
386 Kennedy, C.R., 1978. The biology, specificity and habitat of the species of *Eubothrium* (Cestoda:
387 Pseudophyllidea), with reference to their use as biological tags: a review. J. Fish Biol. 12,
388 393-410
389 Kennedy, C.R., 1996. Establishment, survival and site selection of the cestode *Eubothrium crassum*
390 in brown trout, *Salmo trutta*. Parasitology. 112, 347-355
391 Krkošek, M., 2010. Host density thresholds and disease control for fisheries and aquaculture.
392 Aquac. Environ. Interac. 1, 21-32
393 Matthews, J.B.L., 1967. On the calanoid gopepods of Raunefjorden, western Norway. Sarsia. 29,
394 159-164
395 Mulcahy, M.F., Kennedy, C.R., 1970. *Eubothrium crassum* (Bloch) (Cestoda: Pseudophyllidea) in
396 salmon parr. Ir. Nat. J. 16, 312-313

Nilsen, A., Nielsen, K.V., Biering, E., Bergheim, A., 2017. Effective protection against sea lice during the production of Atlantic salmon in floating enclosures. Aquaculture. 466, 41-50

Norwegian Directorate of Fisheries, 2020. Sale 1994-2019. Available at: https://www.fiskeridir.no/English/Aquaculture/Statistics/Atlantic-salmon-and-rainbow-trout (Accessed 28 September 2020).

Oppedal, F., Dempster, T., Stien, L.H., 2011. Environmental drivers of Atlantic salmon behaviour in sea-cages: A review. Aquaculture. 311, 1-18

Oppedal, F., Samsing, F., Dempster, T., Wright, D.W., Bui, S., Stien, L.H., 2017. Sea lice infestation levels decrease with deeper 'snorkel' barriers in Atlantic salmon sea-cages. Pest Manag. Sci. 73, 1935-1943

Oppedal, F., Bui, S., Stien, L.H., Overton, K., Dempster, T., 2019. Snorkel technology to reduce sea lice infestations: efficacy depends on salinity at the farm site, but snorkels have minimal effects on salmon production and welfare. Aquac. Environ. Interac. 11, 445-457

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org.

Read, C.P., 1951. The "Crowding Effect" in Tapeworm Infections. The Journal of Parasitology. 37, 174-178

Rees, G., 1967. Pathogenesis of adult cestodes. Helminthological Abstracts 36(1): 1-23.

Roberts, L.S., 2000. The Crowding Effect Revisited. The Journal of Parasitology. 86, 209-211

Rosen, F., 1919. Recherches sur le développement des Cestodes. 1. Le cycle évolutif des Bothriocéphales." Bull. Soc. Naeuchateloise Sci. Nat. 1917-1918 43: 241-300.

Ruud, K., 2019. Infection dynamics of marine *Eubothrium* sp. (Cestoda) in farmed Atlantic salmon. Master thesis. University of Bergen.

Saksvik, M., Nilsen, F., Nylund, A., Berland, B., 2001a. Effect of marine *Eubothrium* sp. (Cestoda: Pseudophyllidea) on the growth of Atlantic salmon, *Salmo salar* L. J. Fish Dis. 24, 111-119

Saksvik, M., Nylund, A., Nilsen, F., Hodneland, K., 2001b. Experimental infection of Atlantic salmon (*Salmo salar)* with marine *Eubothrium* sp.(Cestoda: Pseudophyllidea): observations on the life cycle, aspects of development and growth of the parasite. Folia Parasitol. 48, 118-126

Scholz, T., Moravec, F., 1996. Seasonal occurrence and maturation of *Bathybothrium rectangulum* (Cestoda: Amphicotylidae) in barbel *Barbus barbus* (Pisces) of the Jihlava River, Czech Republic. Parasite. 3, 39-44

Scholz, T., Kuchta, R., Shinn, A.P., Šnábel, V., Hanzelová, V., 2003. Host specificity and geographical distribution of *Eubothrium* in European salmonid fish. J. Helminthol. 77, 255-262

Scholz, T., Kuchta, R., 2017. A digest of fish tapeworms. Vie Milieu. 67, 43-58

Sevatdal, S., Hellberg, H., 2006. Bendelorm (*Eubothrium* sp.) hos laks (*Salmo salar*): Utprøving av nye behandlingsmidler og utvikling av et *in vitro* bioassay for måling av resistens overfor praziquantel. Sluttrapport FHF-project 551024.

Sevatdal, S., Kleppa, A.L., Danielsen, T., 2008. Resistens hos bendelorm (*Eubothrium* sp.). Sluttrapport VESO. FHF-project 552024.

Sevatdal, S., 2014. Validering av bioassay med praziquantel og bendelmark (*Eubothrium* sp.). Sluttrapport VESO, FHF-project 900988.

Shulman, S., 1961. Zoogeography of parasites of USSR freshwater fishes. Parasitology of fishes, 180-229

Smith, H.D., 1973. Observations on the Cestode *Eubothrium salvelini* in Juvenile Sockeye Salmon (*Oncorhynchus nerka*) at Babine Lake, British Columbia. Journal of the Fisheries Research Board of Canada. 30, 947-964

Sommerset, I., Walde, C.S., Bang Jensen, B., Bornø, G., Haukaas, A., Brun, E., 2020. Fiskehelserapporten 2019. Veterinærinstituttets rapportserie nr. 5a/2020. p. 102-103.

447 Stien, L.H., Dempster, T., Bui, S., Glaropoulos, A., Fosseidengen, J.E., Wright, D.W., Oppedal, F.,
448 2016. 'Snorkel' sea lice barrier technology reduces sea lice loads on harvest-sized Atlantic
449 salmon with minimal welfare impacts. Aquaculture. 458, 29-37
450 Stien, L.H., Lind, M.B., Oppedal, F., Wright, D.W., Seternes, T., 2018. Skirts on salmon production
451 cages reduced salmon lice infestations without affecting fish welfare. Aquaculture. 490,
452 281-287
453 Stien, L.H., Tørud, B., Gismervik, K., Lien, M.E., Medaas, C., Osmundsen, T., Kristiansen, T.S.,
454 Størkersen, K.V., 2020. Governing the welfare of Norwegian farmed salmon: Three conflict
455 cases. Mar. Policy. 117, 103969
456 Sundnes, G.A., 2003. Cestodes in Atlantic salmon (*Salmo salar* L.) at a W Norwegian hatchery:
457 Infection dynamics, aspects of development and pathology. Master thesis. University of
458 Bergen.
459 Vik, R., 1963. Studies of the helminth fauna of Norway. IV. Occurrence and distribution of
460 *Eubothrium crassum* (Bloch, 1779) and *E. salvelin*i (Schrank, 1790) (Cestoda) in Norway,
461 with notes on their life cycles. Nytt Magasin for Zoologi. 11, 47-73
462 Walkey, M., Meakins, R.H., 1970. An attempt to balance the energy budget of a host-parasite
463 system. J. Fish Biol. 2, 361-372
464 Wardle, R.A., McLeod, J.A., Radinovsky, S., 1975. Advances in the Zoology of tapeworms, 1950-
465 1970. U of Minnesota Press.
466 Williams, H.H., Jones, A., 1994. Parasitic worms of fish. Taylor & Francis London.
467 Wright, D.W., Stien, L.H., Dempster, T., Vågseth, T., Nola, V., Fosseidengen, J.E., Oppedal, F.,
468 2017. 'Snorkel' lice barrier technology reduced two co- occurring parasites, the salmon
469 louse (*Lepeophtheirus salmonis*) and the amoebic gill disease causing agent (*Neoparamoeba*
470 *perurans*), in commercial salmon sea-cages. Prev. Vet. Med. 140, 97-105
471 Wright, D.W., Geitung, L., Karlsbakk, E., Stien, L.H., Dempster, T., Oldham, T., Nola, V.,
472 Oppedal, F., 2018. Surface environment modification in Atlantic salmon sea-cages: effects

on amoebic gill disease, salmon lice, growth and welfare. Aquac. Environ. Interac. 10, 255-265

Figure legends

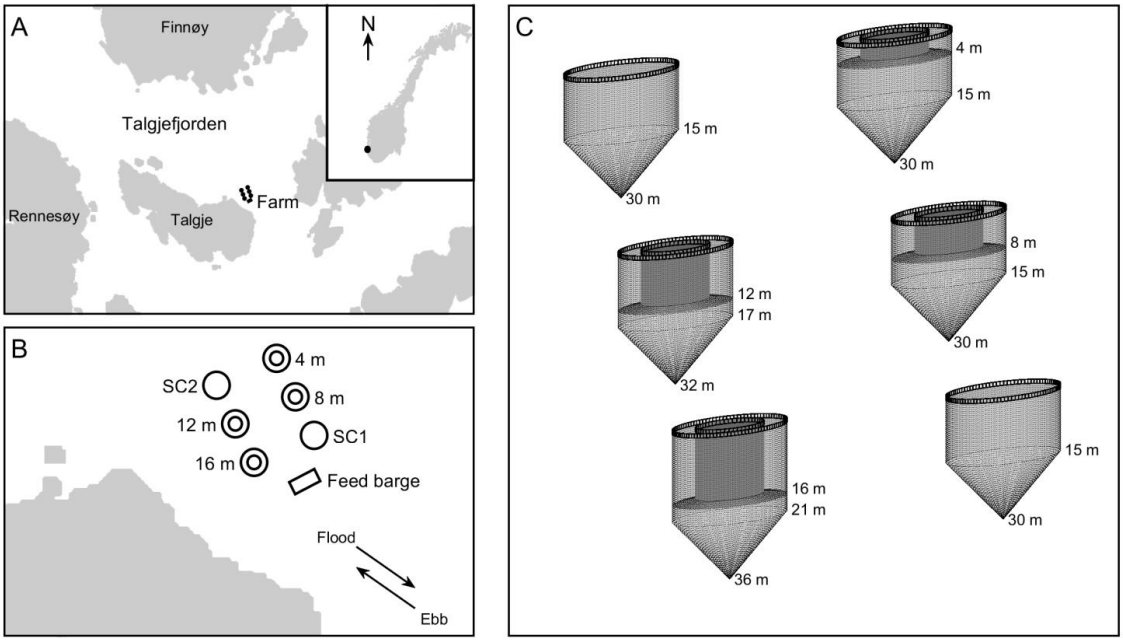
Figure 1. a) Overview of Norway and area surrounding Prestholmane fish farm, b) Prestholmane fish farm with arrows representing main current and c) cage setup. For b) Prestholmane fish farm, the rectangle represents the feeding barge, the circles represent standard cages (SC) and the double circles represent snorkel cages. All cages were 50 m in diameter and 30-36 m deep, while four cages were also fitted with a 30 m in diameter and 4, 8, 12 or 16 m deep snorkel.

Figure 2. Daily a) temperature and b) salinity measurements at depths representing surface (1 m), mid cage (12 m) and bottom cage (25 m) from a reference location at the feed barge.

Figure 3. a) Prevalence of *Eubothrium* sp., b) mean numbers (abundance) and c) mean worm weight (g) of *Eubothrium* sp. $fish^{-1}$ in salmon examined from all cage types in September 2018. Standard cages (SC) are presented at 0 m depth (grey dot = SC1; black dot = SC2) while snorkel cages are presented with a dot at their respective shielding depths (4, 8, 12 and 16 m). The whiskers indicate the respective 95 % confidence interval. Stars mark significance level * = $p < 0.05$, *** = $p < 0.001$.

Figure 4. a) Prevalence of *Eubothrium* sp. and b) mean numbers (abundance) of *Eubothrium* sp. $fish^{-1}$ in salmon examined in two standard cages (SC) and one 16 m snorkel cage every second months from October 2017 until September 2018. Arrows indicate stocking time with the black arrow (21.11.2017) representing stocking time for SC2 and the grey arrow (06.12.2018) stocking time for SC1 and 16 m snorkel. The whiskers indicate the respective 95 % confidence interval.

511 **Geitung et al. Figure 1**



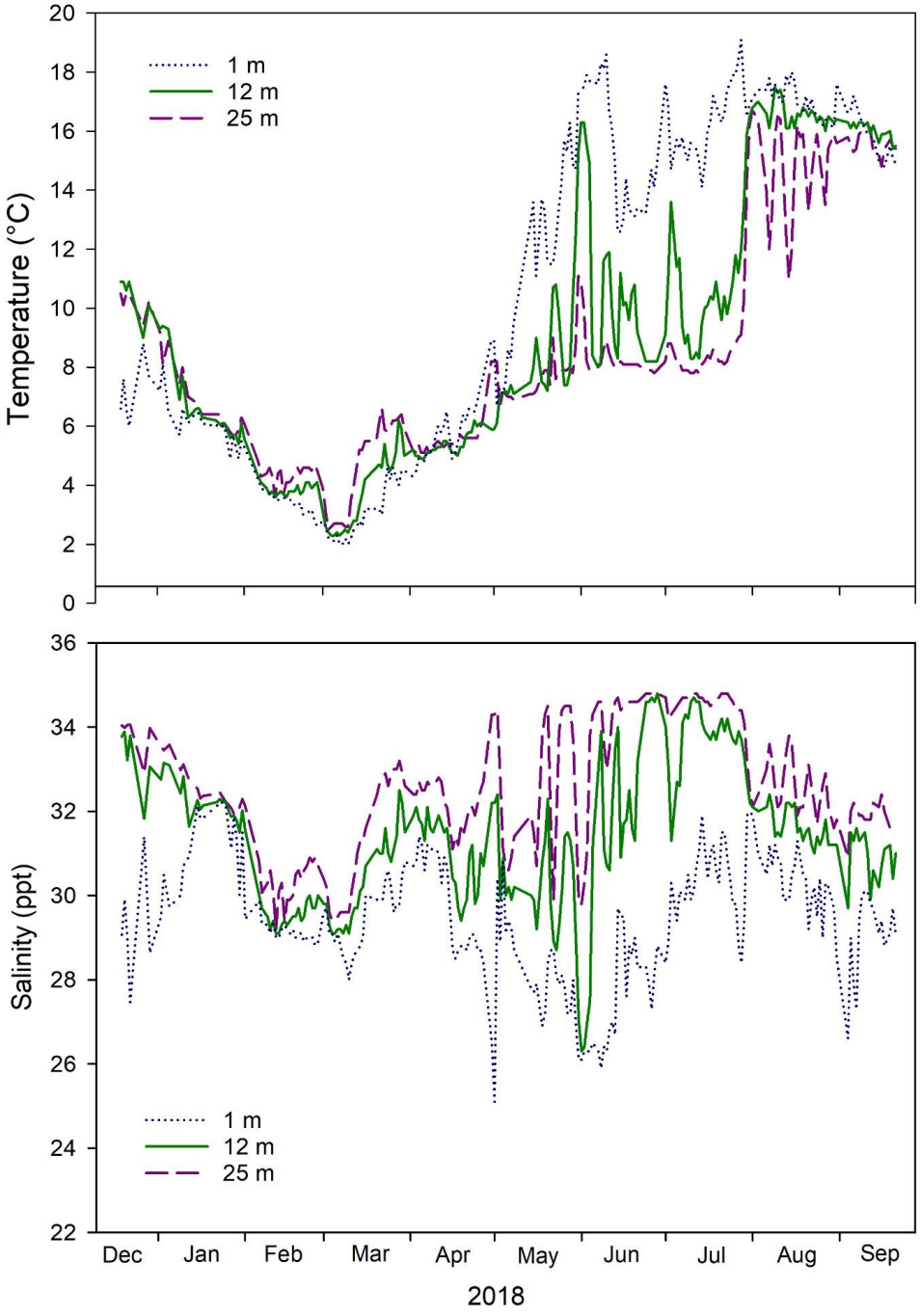
512

513

514

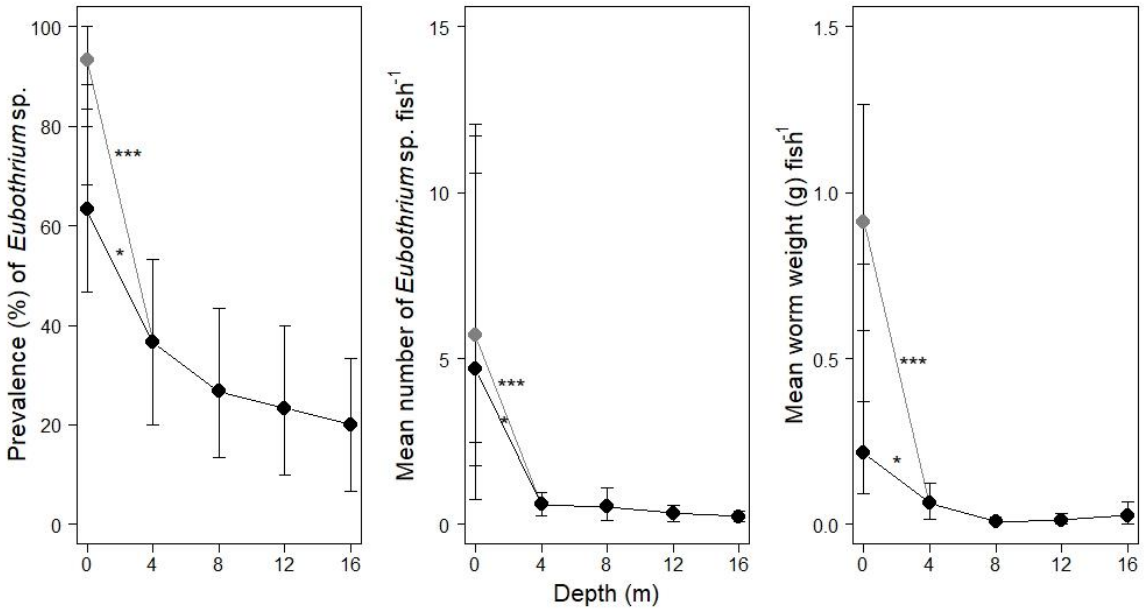
515

516 **Geitung et al. Figure 2**



517

518 **Geitung et al. Figure 3**



519

520

521

522

523

524

525

526

527

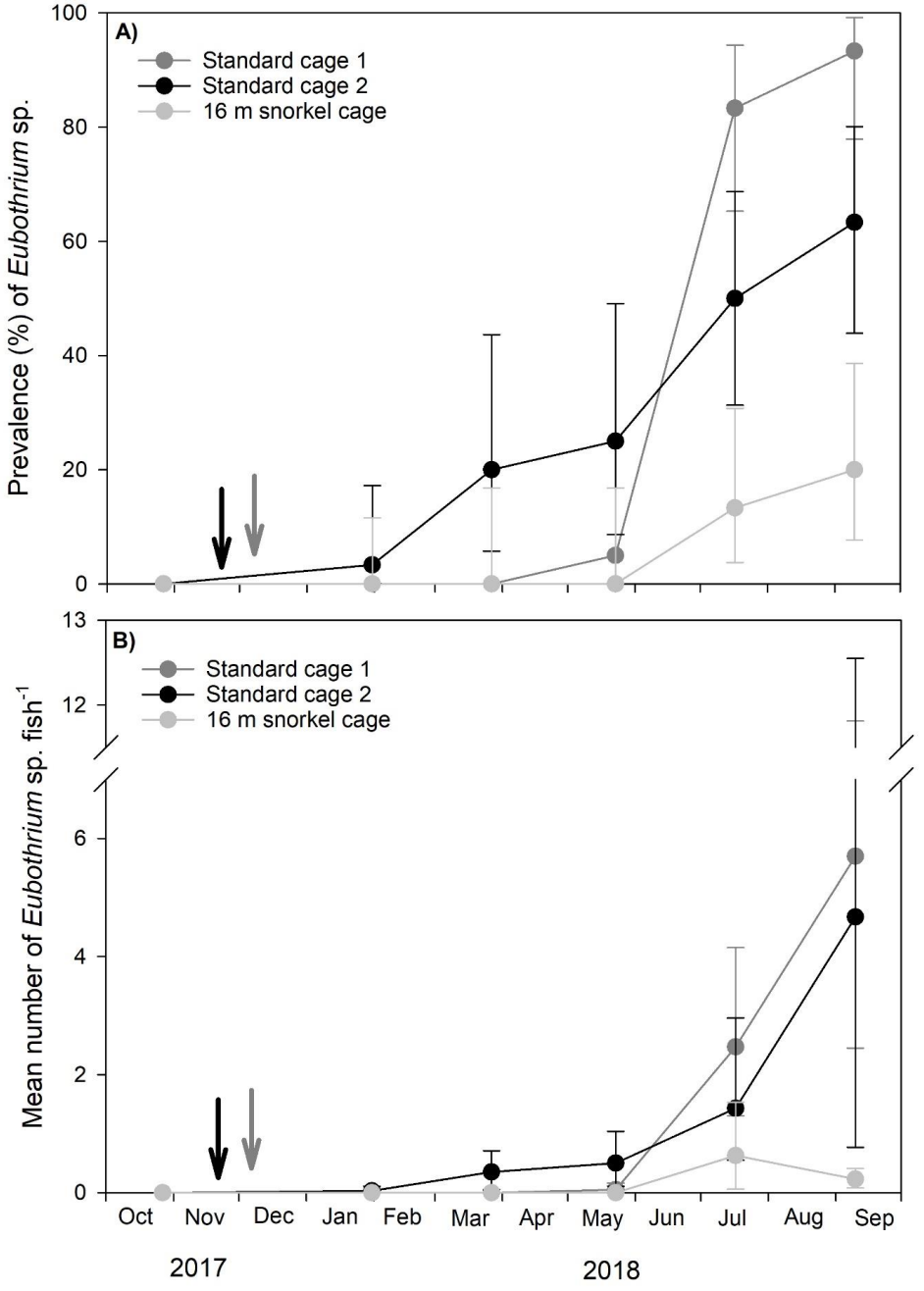

A)
Standard cage 1
Standard cage 2
16 m snorkel cage
Prevalence (%) of Eubothrium sp.
100
80
60
40
20
0
B)
Standard cage 1
Standard cage 2
16 m snorkel cage
Mean number of Eubothrium sp. fish-1
13
12
6
4
2
0
Oct
Nov
Dec
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
2017
2018

Table legends

Table 1: Dates for sampling events, number of fish and sampled cages.

Supplementary table 1: Overview of mean length (cm), weight (g) and condition factor for salmon at all sampling times.

Supplementary table 2: Occurrence of *Eubothrium* sp. in Atlantic salmon for all cages at the last sampling time (September 2018).

548 **Geitung et al. Table 1**

Sampling	Date	Time at sea	N fish sampled from each cage	Cages analysed for *Eubothrium* sp.
0	27 Oct 2017	Freshwater	60*	
1	31 Jan 2018	2 months	30	Standard cage 1, 2 and 16m snorkel cage
2	27 Mar 2018	4 months	20	Standard cage 1, 2 and 16m snorkel cage
3	25 May 2018	6 months	20	Standard cage 1, 2 and 16m snorkel cage
4	17 Jul 2018	8 months	30	Standard cage 1, 2 and 16m snorkel cage
5	19 Sep 2018	10 months	30	All cages

549 **total number of fish sampled*

550

551

552

553

554

555

556

557

558

559

560

561

Sample	Cage		Length (cm)	Weight (g)	Condition factor
Freshwater	All	Mean ± SD	19.5 ± 1.8	105.2 ± 30.2	1.39 ± 0.08
		Minimum	15.6	54.0	1.24
		Maximum	23.7	194.0	1.58
Sample 1	Standard cage 1	Mean ± SD	22.5 ± 3.4	152.3 ± 62.2	1.26 ± 0.13
		Minimum	14.7	35.0	1.08
		Maximum	27.5	260.0	1.72
	Standard cage 2	Mean ± SD	29.4 ± 2.1	313.5 ± 76.4	1.21 ± 0.09
		Minimum	26.0	195.0	1.00
		Maximum	34.5	475.0	1.45
	16 m snorkel	Mean ± SD	25.7 ± 2.9	213.8 ± 71.6	1.22 ± 0.17
		Minimum	19.2	75.0	0.51
		Maximum	31.5	380.0	1.55
Sample 2	Standard cage 1	Mean ± SD	23.0 ± 2.6	150.8 ± 53.7	1.22 ± 0.20
		Minimum	18.0	70.0	0.70
		Maximum	28.8	290.0	1.84
	Standard cage 2	Mean ± SD	34.3 ± 2.1	519.5 ± 95,5	1.27 ± 0.07
		Minimum	29.5	310.0	1.13
		Maximum	36.8	670.0	1.40
	16 m snorkel	Mean ± SD	28.8 ± 4.1	307.2 ± 113.7	1.23 ± 0.13
		Minimum	18.5	80.0	1.00
		Maximum	34.2	490.0	1.60
Sample 3	Standard cage 1	Mean ± SD	35.2 ± 2.4	520.0 ± 119.3	1.17 ± 0.07
		Minimum	31.2	350.0	1.04
		Maximum	40.6	785.0	1.36
	Standard cage 2	Mean ± SD	39.1 ± 2.9	730.8 ± 186.6	1.20 ± 0.09
		Minimum	33.7	465.0	1.01
		Maximum	44.6	1155.0	1.37
	16 m snorkel	Mean ± SD	36.5 ± 3.0	653.0 ± 197.4	1.31 ± 0.14
		Minimum	29.7	330.0	0.88
		Maximum	41.6	1040.0	1.50
Sample 4	Standard cage 1	Mean ± SD	38.6 ± 3.8	596.7 ± 187.1	1.01 ± 0.07
		Minimum	32.0	295.0	0.85
		Maximum	45.5	1025.0	1.16
	Standard cage 2	Mean ± SD	42.4 ± 3.5	789.8 ± 253.4	1.01 ± 0.18
		Minimum	35.5	365.0	0.79
		Maximum	49.5	1385.0	1.62
	16 m snorkel	Mean ± SD	36.3 ± 4.8	475.8 ± 184.5	0.97 ± 0.27
		Minimum	28.0	195.0	0.72
		Maximum	45.2	1005.0	2.28
Sample 5	Standard cage 1	Mean ± SD	46.1 ± 4.3	1154.3 ± 400.3	1.13 ± 0.15
		Minimum	37.0	465.0	0.78
		Maximum	56.5	2235.0	1.37
	Standard cage 2	Mean ± SD	49.6 ± 6.9	1435.8 ± 789.3	1.05 ± 0.25
		Minimum	38.2	435.0	0.66

	Maximum	59.0	2660.0	1.46
4 m snorkel	Mean ± SD	44.2 ± 6.4	923.5 ± 658.4	0.93 ± 0.19
	Minimum	36.6	320.0	0.65
	Maximum	61.0	2930.0	1.30
8 m snorkel	Mean ± SD	40.1 ± 5.3	564.8 ± 286.7	0.80 ± 0.12
	Minimum	29.0	180.0	0.57
	Maximum	52.0	1425.0	1.10
12 m snorkel	Mean ± SD	39.8 ± 4.6	564.0 ± 296.4	0.84 ± 0.15
	Minimum	29.0	215.0	0.44
	Maximum	51.5	1635.0	1.22
16 m snorkel	Mean ± SD	39.4 ± 4.8	552.7 ± 302.7	0.83 ± 0.18
	Minimum	30.5	200.0	0.65
	Maximum	53.6	1775.0	1.56

	Standard cage 1	Standard cage 2	4 m snorkel	8 m snorkel	12 m snorkel	16 m snorkel
Number of fish	30	30	30	30	30	30
Number of fish infected	28	19	11	8	7	6
Prevalence (%) [95% CI]	93.3 [83.3–100.0]	63.3 [46.7–80.0]	36.7 [20.0–56.7]	26.7 [13.3–43.3]	23.3 [10.0–40.0]	20.0 [6.7–36.7]
Abundance, mean [95% CI]	5.7 [2.4–11.5]	4.6 [0.8–12.2]	0.6 [0.3–1.0]	0.5 [0.1–1.2]	0.3 [0.1–0.6]	0.2 [0.1–0.4]
Abundance, range	0–85	0–109	0–4	0–8	0–3	0–2
Intensity, mean [95% CI]	6.1 [3.0–10.8]	7.4 [1.6–15.9]	1.6 [1.2–2.3]	2.0 [1.0–3.8]	1.4 [1.0–2.0]	1.2 [1.0–1.5]
Intensity, range	1–85	1–109	1–4	1–8	1–3	1–2
Worm weight fish^{-1}, mean [95% CI]	0.91 [0.59–1.26]	0.22 [0.10–0.37]	0.07 [0.02–0.13]	0.01 [0.00–0.02]	0.01 [0.00–0.03]	0.03 [0.00–0.07]
Worm weight fish^{-1}, range	0.00–3.27	0.00–1.60	0.00–0.62	0.00–0.20	0.00–0.22	0.00–0.41

577

III

Paper III

Wright, D. W.*, Geitung, L.*, Karlsbakk, E., Stien, L. H., Dempster, T., Oldham, T., Nola, V., Oppedal, F. (2018)

Surface environment modification in Atlantic salmon sea-cages: effects on amoebic gill disease, salmon lice, growth and welfare.

Aquaculture Environment Interactions 10, 255–265.

https://doi.org/10.3354/aei00269

*Joint first author

Vol. 10: 255–265, 2018
https://doi.org/10.3354/aei00269

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published June 14

Surface environment modification in Atlantic salmon sea-cages: effects on amoebic gill disease, salmon lice, growth and welfare

Daniel W. Wright[1,*,], Lena Geitung[2,**], Egil Karlsbakk[2], Lars H. Stien[1], Tim Dempster[3], Tina Oldham[4], Velimir Nola[1], Frode Oppedal[1]**

[1]Institute of Marine Research, Matre Research Station, 5984 Matredal, Norway
[2]Department of Biology, University of Bergen, 5005 Bergen, Norway
[3]Sustainable Aquaculture Laboratory – Temperate and Tropical, School of BioSciences, Parkville, VIC 3010, Australia
[4]Institute of Marine and Antarctic Studies, University of Tasmania, Launceston, TAS 7250, Australia

ABSTRACT: Surface environment modification is a potential parasite control strategy in Atlantic salmon sea-cage farming. For instance, a temporary low salinity surface layer in commercial-scale snorkel sea-cages has coincided with reduced amoebic gill disease (AGD) levels after an outbreak. We tested if a permanent freshwater (FW) surface layer in snorkel sea-cages would lower AGD and salmon lice levels of stock relative to snorkel cages with seawater (SW) only and standard production cages with no snorkels. Triplicate cages of each type with 2000 post-smolts were monitored in autumn to winter for 8 wk and sampled 4 times. Lower proportions of individuals with elevated AGD-related gill scores were registered in SW and FW snorkel cages compared to standard cages; however, these proportions did not differ between SW and FW snorkel cages. Individuals positive for AGD-causing *Paramoeba perurans* were reduced by 65% in FW snorkel relative to standard cages, but values were similar between SW snorkel cages and other types. While total lice burdens were reduced by 38% in SW snorkel compared to standard cages, they were unchanged between FW snorkel and other cage types. Fish welfare and growth were unaffected by cage type. Surface activity was detected in all cages; however, more surface jumps were recorded in standard than snorkel cages. Overall, fish in FW snorkel cages appeared to reside too little in freshwater to consistently reduce AGD levels and salmon lice compared to SW snorkel cages. Further work should test behavioural and environmental manipulations aimed at increasing freshwater or low salinity surface layer use.

KEY WORDS: Aquaculture · Cage environment · *Salmo salar* · *Lepeophtheirus salmonis* · *Paramoeba perurans* · Parasite control

INTRODUCTION

Sea-cage Atlantic salmon *Salmo salar* farming produces more than 2.3×10^6 t yr^{-1} (FAO 2017). This new and constant availability of large numbers of hosts has led to an increased scale of salmon parasite outbreaks in many marine ecosystems (Nowak 2007). Outbreaks of the salmon louse *Lepeophtheirus salmonis*, and of the amoeba *Paramoeba perurans* responsible for amoebic gill disease (AGD) (Young et al. 2007, 2008b, Crosbie et al. 2012) are of particular concern to the industry (Murray et al. 2016). Salmon lice outbreaks are thought to harm wild salmonids (Krkošek et al. 2011, 2013) and, as a result, strict regulations limit salmon lice loads on farmed fish. Many farmers must treat their fish repeatedly against sea lice during a production cycle, leading to increased costs and considerable risk to fish welfare (Overton

*Corresponding author: daniel.william.wright@imr.no
**Joint first authors

Publisher: Inter-Research · www.int-res.com

et al. 2018). Norwegian authorities have also recently introduced the 'traffic light system', which limits allowable production volume in defined production zones according to the percentage of wild salmon in each production zone estimated to die due to salmon lice (Lovdata (2012)): <10% = increased production (green), 10–30% = no change in production (yellow), >30% = reduced production (red). In parallel, the expansion of AGD outbreaks to all major salmon farming regions has caused mass mortality events and a surge in AGD treatments (Shinn et al. 2015, Oldham et al. 2016). Innovating parasite controls to reduce both salmon lice and AGD could safeguard the ecological sustainability and future expansions of the salmon farming industry (Wright et al. 2017).

A range of chemotherapeutants can be used to treat salmon lice (organophosphates, emamectin benzoate, benzoyl ureas, hydrogen peroxide and pyrethroids) (Aaen et al. 2015), whilst AGD is currently treated with freshwater baths and hydrogen peroxide (Rodger 2014). Immersion in freshwater baths for 2 to 4 h removes freshwater-sensitive AGD-causing amoebae *P. perurans* from fish gills (Parsons et al. 2001, Clark et al. 2003, Rodger 2014). Unfortunately, short duration freshwater baths are unlikely to affect host-attached salmon lice which survive days to weeks in freshwater after developing past the first copepodid stage (Stone et al. 2002, Wright et al. 2016). In contrast, hydrogen peroxide use is rising rapidly (NIPH 2015, Murray 2016) due to its well known in-field efficacy against both salmon lice and AGD (Thomassen 1993, Adams et al. 2012). However, potential problematic effects on salmon welfare (Overton et al. 2018) and the evolution of chemical resistance against hydrogen peroxide (Helgesen et al. 2015, Helgesen et al. 2017) call into question the continued heavy reliance on this chemical. These factors are driving the development of chemical-free parasite controls. For salmon lice, these controls aim to prevent new lice from establishing themselves (fallowing, lice barrier skirt or snorkel cages, semi-enclosed cages, selective breeding of lice-resistant salmon) (Bron et al. 1993, Stien et al. 2012, Gharbi et al. 2015, Stien et al. 2016, Nilsen et al. 2017), or treat attached lice without chemicals (cleaner fish, laser, thermodelousing, water jets) (Bjordal 1990, Aaen et al. 2015). The challenge for these substitute controls will be to simultaneously diminish both salmon lice and AGD.

Snorkel sea-cages incorporate a deep net roof opening into a central tarpaulin-lined narrow net-tube (snorkel) to the surface in an otherwise standard cage (Stien et al. 2016). This impedes contact between salmon hosts and free-swimming infective larval stages of salmon lice which are positively phototactic and pressure sensitive, causing them to typically aggregate near the surface (Heuch 1995, Heuch et al. 1995). The snorkel allows salmon to swim up and gulp air at the surface to replenish their open swim bladder for buoyancy regulation (Fahlén 1971, Dempster et al. 2011). Snorkel cages can reduce salmon lice infestations relative to standard cages at research- and commercial scales (Stien et al. 2016, Wright et al. 2017), with their effectiveness increasing with increased depth of the snorkel (Oppedal et al. 2017). AGD may also be treated using this technology by adding a freshwater surface layer inside a tarpaulined lined tube in the snorkel space (Wright et al. 2017) that would remove *P. perurans* from gills if the fish expose themselves sufficiently to freshwater (Parsons et al. 2001, Clark et al. 2003, Roberts & Powell 2003, Wright et al. 2016). Producing a temporary low salinity layer within the snorkels of commercial-scale cages has coincided with marked reductions in AGD levels after an outbreak, suggesting there is the potential for this technology to co-manage salmon lice and AGD (Wright et al. 2017). However, further testing is required to examine how variations of this surface environment modification, such as a permanent freshwater layer, affect AGD levels and to validate findings using standard production and seawater-filled snorkel cages for comparison.

In this study, we tested if snorkel sea-cages with a constant freshwater layer reduced AGD levels relative to standard cages and seawater-filled snorkel cages. Even though it is well established that snorkel cages reduce salmon lice levels (Stien et al. 2016, Oppedal et al. 2017), we also examined cage type effects on salmon lice infestations. Introducing freshwater into snorkel cages holding salmon might affect salmon lice infestations by influencing the behaviour and physiology of the host (McCormick et al. 1998, Oppedal et al. 2011) or parasite, particularly at the freshwater-sensitive copepodid stage (Bricknell et al. 2006, Wright et al. 2016). Additionally, we investigated if growth, mortality and other welfare indicators differed between cage types. Environmental conditions were closely monitored at the farm as well as within each snorkel cage to explain observed patterns.

MATERIALS AND METHODS

Study location and design

Nine steel frame sea-cages (12 × 12 m square, 12 m deep) were used at the Institute of Marine Research farm facility in Austevoll, southwest Norway (60° N).

These consisted of 3 unmodified standard cages and 6 snorkel cages (snorkel dimensions were 3 × 3 m square, 4 m deep), with 3 snorkels filled with seawater pumped (135 l min^{-1} pump, Xylem Water Solutions) from 4 m depth (hereafter 'SW snorkel' cages) and 3 snorkels filled with mains ozone-treated freshwater containing no chlorine or fluoride ('FW snorkel' cages). The 2 treatments (SW and FW snorkels) were interspersed in a block design at the facility. We stocked each cage with 2000 post-smolt Atlantic salmon, naïve to both AGD and salmon lice exposure, in a randomized block order from 26 to 28 October 2016. Fish (AquaGen strain) were produced at the Institute of Marine Research tank facility in Matre as 0+ out of season autumn smolts using standard protocols (e.g. Björnsson et al. 2000). Freshwater-filling of FW snorkels began after transfers were complete. Mean (± SD) fish weight was 76 ± 16 g, which led to stocking densities of 0.09 kg m^{-3} in standard and snorkel cages. Fish were continuously fed small portions throughout daylight hours to excess with a commercial diet (3 mm Spirit Supreme pellets, Skretting) via an automated system that operated screw pellet dispensers which released feed centrally in standard cages and into a pipe where it was transported by pumping seawater or freshwater to the top of snorkels. Because fish were fed to excess, no food conversion ratio (FCR) data was recorded in this trial. Inconsistencies in the management of one replicate FW snorkel cage compared to others led to its removal from all analyses.

Environmental depth profiles

Daily depth profiles of salinity and temperature were recorded by an automatic profiling CTD buoy (APB5, SAIV) programmed to measure between 0 to 12 m starting at 12:00 h daily at a reference location near the centre of the farm facility. We supplemented these measurements with weekly depth profiles between 0 and 12 m of salinity, temperature and dissolved oxygen (DO) using a CTD (SD204, SAIV) at the reference location and within each snorkel cage, to record differences between cage environments. Weekly profiles began the week following stocking and once freshwater layer creation was complete. Profiles involved lowering the CTD at a rate of 1 m min^{-1} to ensure the accuracy of oxygen recordings.

Amoebic gill disease and salmon lice

At fortnightly intervals, on 8–9 November (Time 1), 22–24 November (Time 2), 5–7 December (Time 3) and 20–21 December (Time 4), 20 fish from each cage were sampled. Fish were caught by ceasing feeding at least 24 h prior, lowering a hoop net and hand feeding to motivate surfacing of fish, followed by swift lifting of the hoop net. We subjected sampled fish to a lethal dose of anaesthetic (Finquel), then transferred them to seawater-filled trays for counts of all salmon lice stages (copepodid, chalimus I, chalimus II, preadult I, preadult II male, preadult II female, adult male, adult female and adult female with eggstrings). Counts of mobile stages in buckets holding the sampled fish were also included in the total counts. New lice at each sampling time were considered to be attached copepodid, chalimus I and chalimus II lice stages, which developed in ≤2 wk at mean observed temperatures of 9°C in the trial (Stien et al. 2005). Next, AGD-related gill scoring (0–5, with 0 for no gill pathology and 1–5 for increasing severity of gill pathology, using lesion-covered gill surface area categories) was carried out on each of the 8 gill arches (Taylor et al. 2009). The AGD-related gill score given to an individual fish was based on the maximum score of its arches. At Time 3, when gill scores remained elevated, swabbing of the third right gill arch (a half turn on the front and a half turn on the back) was performed on 10 fish in each cage type. The swab was inserted into 1 ml vials of RNAlater and stored at 4°C for 24 h and thereafter at −18°C until PCR analysis for *P. perurans* detection (Pharmaq, Bergen, Norway). Analysed samples returned a cycle threshold (CT) value indicating *P. perurans* presence when below a cut-off of 30.0, with co-analysed control samples recording CT values above it. We created a *P. perurans* load index, where a CT value of 30.0 or greater had a *P. perurans* load of 0, and lower CT values were transformed by subtracting 30 and reversing the sign of the resulting value (e.g. CT value of 28 = *P. perurans* load index of 2). AGD-related gill scores and *P. perurans* load were positively correlated based on individuals swabbed at Time 3 (Pearson's correlation, $t = 2.8$, $p < 0.05$) providing support that gill scores resulted from AGD-causing *P. perurans*, as reported by others (e.g. Young et al. 2008a, Bridle et al. 2010).

Growth, mortality and other welfare indicators

At Time 4, sampled fish were measured for fork length (cm) and weight (g), condition factor (K) calculated as (weight × $length^{-3}$)/100 (Bolger & Connolly 1989), and scores of individual welfare indicators (emaciation, vertebral deformity, sexual maturation,

smoltification state, fin condition, skin condition, eye status, opercula, mouth jaw wound, upper jaw deformity, lower jaw deformity) contributing to the Semantic Welfare Index Model (SWIM) version 2.0. Lice and gill welfare indicators were not incorporated into overall SWIM scores. Numbers of mortalities in each cage were recorded from checks performed 3 times per week.

Surface activity

Beginning from the first sampling time, jumps and rolls were counted in a 5 min period within each cage on the same day at weekly intervals (Dempster et al. 2008). These numbers were recalculated to jumps per fish per day.

Statistical analyses

Proportions of AGD-related 'light plus' gill scores (≥2, with higher scores indicating increased gill pathology) used as a measure of AGD levels in salmon cages within industrial and research settings (Maynard et al. 2016) were compared. Generalised linear models with binomial error distributions, including treatment (standard, SW snorkel and FW snorkel) and cage (1–8) as factors, compared light plus gill scores at each time (using the glm function in R; Crawley 2012). For each comparison, models incorporating treatment × cage, treatment + cage and treatment only were built and the simplest model was selected if no significant difference was identified between them via ANOVA tests (anova function in R). Arcsine-transformed proportions of fish with gills found to be *P. perurans*-positive in each cage were compared between treatments using *t*-tests (t.test function in R).

We assessed differences in new lice per fish (count data with overdispersion) between treatments at each time using generalised linear models with quasi-Poisson error distributions, which included treatment and cage as factors. As before, a simpler model was chosen from more complex ones if no difference was found from ANOVA tests between models. For an overall assessment of lice infestation levels that fish incurred during the study, we examined total lice numbers (including sessile and mobile stages) on sampled fish and in their bucket for each cage at the final sampling (Time 4). These total counts per cage were compared between treatments via *t*-tests.

At Time 4, when fish had experienced the different cage type treatments the longest, growth (based on weight), condition and square-root-transformed SWIM scores of sampled fish were compared using linear mixed-effect models, with treatment as a fixed effect and cage as a random effect (lme function in R). At Time 4, arcsine-transformed proportions of fish with fin (scores ≥3), skin (scores ≥3), eye (scores ≥2) and cumulative mortalities in each cage were also compared between treatments via *t*-tests, which were also used to compare square-root-transformed jumps per fish per day in each cage, pooled from all weekly assessments, between treatments. Error distributions were checked for variance and normality (plot function in R). Results are presented as means (±SE) and 95% confidence intervals (CIs).

RESULTS

Environment

Salinity remained high (>28.3) and non-stratified at the reference location (reflecting conditions in standard cages) and in the SW snorkel cages (Fig. 1, Table S1 in the Supplement at www.int-res.com/articles/suppl/q010p255_supp.pdf). Thermal stratification with cooler upper layers occurred sporadically in both standard and SW snorkel cages, though was less severe in SW snorkels because snorkel water was constantly replenished with pumped warmer seawater from 4 m depth (Fig. 1, Table S1). In FW snorkel cages, a stable freshwater layer was continuously maintained (salinity ≤1 in top 2 m), of predominantly lower temperature than underlying water (Fig. 1, Table S1). As a result, temperatures between 0 and 1 m depth in FW snorkels were 2.6, 1.4, 0.7 and 1.4°C cooler than SW snorkels and 1.5, 1.2, 0.0 and 0.7°C cooler than in standard cages in the sampling interval periods before Times 1, 2, 3 and 4, respectively (Table S1). DO saturation remained stable between 77 and 85% for standard and SW snorkel cages, but levels were much higher (up to 148%) in the freshwater surface layer of FW snorkel cages, particularly preceding Times 1 and 4, due to the ozone treatment of freshwater (Table S1).

Amoebic gill disease

Soon after stocking at Time 1, AGD-related gill scores remained low and the proportion of fish with light plus scores (≥2) were similar between cage

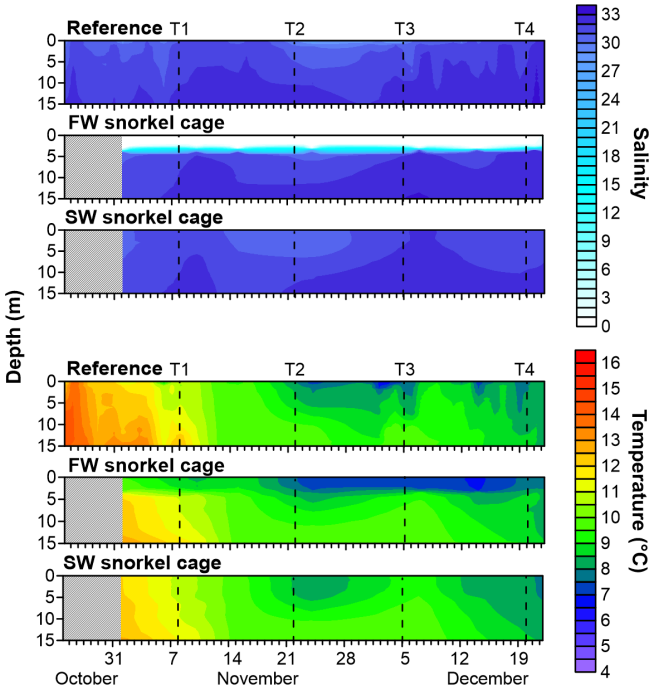


Fig. 1. Depth profiles over time of salinity (top) and temperature (bottom) in a study of the effects of surface environment modification on parasites in farmed Atlantic salmon *Salmo salar* in southwest Norway. Profiles were measured daily by an automatic profiling CTD buoy at a reference location, indicative of standard cage conditions and weekly using a CTD in snorkel cages filled with seawater (SW snorkel) or freshwater (FW snorkel). Measurements at the reference location were taken from 24 October 2016. Measurements in snorkel cages started on 1 November once freshwater layers were established, and the preceding period is shown as grey shading. Values are from a single FW and a single SW snorkel cage, with similar conditions observed in replicate cages. The 4 sampling times (T1 to T4) are shown by dashed vertical lines

types ($z \geq -1.7$, $p \geq 0.1$; Fig. 2, Table S2). Gill scores increased thereafter and became highest in standard cages compared to SW snorkel cages at Times 2 and 3 ($z \geq 3.1$, $p < 0.05$), but not at Time 4 ($z = 1.7$, $p = 0.1$; Fig. 2, Table S2). These scores also remained lower in FW snorkel relative to standard cages at Times 2–4 ($z \leq -2.4$, $p < 0.001$; Fig. 2, Table S2). No differences were observed in gill scores between SW and FW snorkel fish at Times 2–4 ($z = 0.01$ to 1.9, $p > 0.06$; Fig. 2, Table S2). Cage and treatment × cage interactions were present for most comparisons between cage types at Times 2–4 (Fig. 2, Table S2). At Time 3, there was a 65% reduction in the proportion of fish with gills testing positive for *Paramoeba perurans* in FW snorkel (15% of fish) compared to standard cages (43% of fish) ($t = -4.7$, $p < 0.05$), but not between SW snorkel cages (20%) and other types ($t \geq -2.6$, $p \geq 0.1$; Fig. 2).

Salmon lice

New lice (copepodid and chalimus stages) per fish were lower in SW snorkel relative to standard cages at Times 1 (means of 1.6 vs. 2.8) ($t = -4.3$, $p < 0.001$), 2 (means of 1.6 vs. 3.0) ($t = -5.3$, $p < 0.001$) and 4 (means of 3.3 vs. 5.1) ($t = -2.4$, $p < 0.05$), but not at Time 3 (means of 1.6 vs. 2.7) ($t = -1.2$, $p = 0.9$). At Time 3, an interaction between treatment and cage occurred ($t = -2.0$, $p < 0.05$; Fig. 3). FW

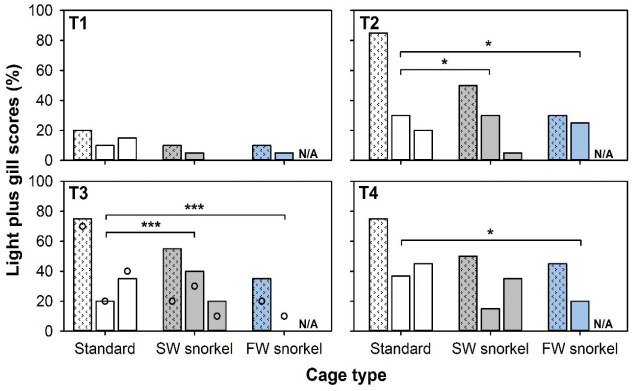


Fig. 2. Proportions of 'light plus amoebic gill disease (AGD)-related gill scores' (scores of ≥2; see 'Materials and methods' for further details) for farmed Atlantic salmon in different cage treatments. Results are shown for each replicate (n = 3 replicates) standard (white bars), SW snorkel (grey bars) and FW snorkel cage (blue bars) at Times 1–4. See Fig. 1 legend for details of cage treatments and sampling times. Stippled bars indicate cages positioned closest to other AGD-affected cages at the farm and expected to be under increased infection pressure. Open circles at Time 3 denote proportions of *Paramoeba perurans*-positive fish from gill swab PCR analysis of 10 fish in each replicate cage. N/A indicates 1 FW snorkel cage discarded from analyses. *p < 0.05, ***p < 0.001

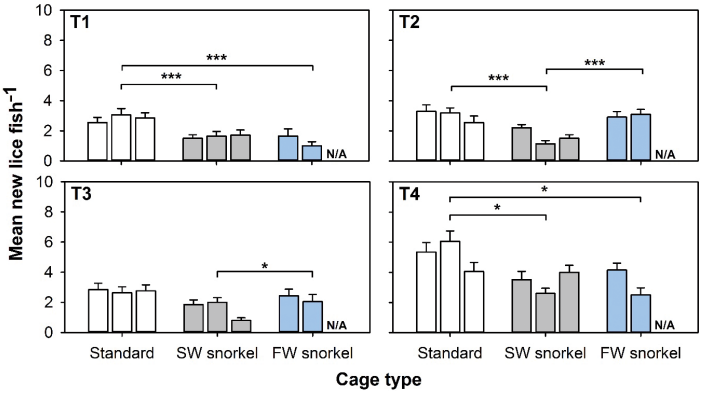


Fig. 3. Mean counts (±SE) of new lice per fish (attached lice or copepodid, chalimus I and chalimus II lice stages) in farmed Atlantic salmon for each replicate standard (white bars), SW snorkel (grey bars) and FW snorkel cage (blue bars) at Times 1–4. See Fig. 1 legend for details of cage treatments and sampling times. N/A indicates 1 FW snorkel cage discarded from analyses. $^{*}p < 0.05$, $^{***}p < 0.001$

snorkel fish also had fewer new lice than those in standard cages at Times 1 (means of 1.3 vs. 2.8 new lice per fish) ($t = -3.9$, $p < 0.001$) and 4 (means of 3.3 vs. 5.1) ($t = -3.3$, $p < 0.05$), although similar counts were observed at Times 2 (means of 3.0 vs. 3.0) ($t = -0.1$, $p > 0.05$) and 3 (means of 2.2 vs. 2.7) ($t = -1.3$, $p < 0.05$) (Fig. 3). Fish had fewer lice in cages with SW snorkels than with FW snorkels at Times 2 ($t = -5.3$, $p < 0.001$) and 3 ($t = -2.1$, $p < 0.05$; Fig. 3). By Time 4, when all lice stages were present in the 3 cage types, total lice per fish differed between standard and SW snorkel cages (means of 15.7 vs. 9.8; i.e. a 38% reduction) ($t = 7.5$, $p < 0.05$), but not between standard and FW snorkel cages (means of 15.7 vs. 12.6) ($t = 0.9$, $p = 0.5$) or SW and FW snorkel cages (means of 9.8 vs. 12.6) ($t = 0.9$, $p = 0.5$; Fig. 4).

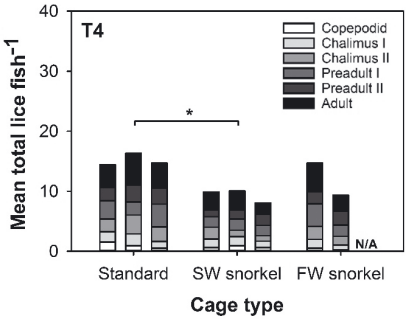


Fig. 4. Mean numbers of copepodids, chalimus I, chalimus II, preadult I, preadult II and adult lice per fish (later stages in increasingly darker shades from white to black) in farmed Atlantic salmon for each replicate standard, SW snorkel and FW snorkel cage at Time 4. See Fig. 1 legend for details of cage treatments and sampling times. N/A represents 1 FW snorkel cage discarded from analyses. $^{*}p < 0.05$

Growth, welfare and mortality

At the last sampling point, there were no differences in the weight ($\chi^2 \leq 2.2$, $p \geq 0.1$) or condition factor ($\chi^2 \leq 2.7$, $p \geq 0.1$) of sampled fish between cage types (Table 1). Adequate and comparable welfare scores of salmon were upheld in all cage types ($\chi^2 \leq 3.5$, $p \geq 0.1$; Table 1). When individual welfare indicators were analysed separately, no differences in observed skin ($t \leq 3.2$, $p \geq 0.1$), fin ($t \geq -0.4$, $p \geq 0.8$) or eye damage ($t \leq 2.5$, $p \geq 0.1$) existed between treatments. Mouth damage was only detected in standard cages (3.4% of stock), and no fish were atypical for other welfare indicators (Table 1). Cumulative mortalities were similar between cage types ($t \geq -0.6$, $p \geq 0.6$).

Table 1. Mean (±SE) values for condition of farmed Atlantic salmon *Salmo salar* in southwest Norway held in standard cages, and in snorkel cages filled with seawater (SW snorkel) or freshwater (FW snorkel). Higher values for condition factor and overall Semantic Welfare Index Model (SWIM) score indicate better condition. Individual welfare indicator scores show proportions of individuals with high scores indicating deviance from the normal condition

Parameter	Standard	SW snorkel	FW snorkel
Mean weight (g)	197.1 ± 13.1	177.3 ± 4.8	179.7 ± 7.6
Mean condition factor	1.15 ± 0.02	1.14 ± 0.02	1.19 ± 0.01
Mean overall SWIM score	0.92 ± 0.01	0.93 ± 0.00	0.93 ± 0.00
Fin damage (scores ≥3)	64.4%	65.0%	62.5%
Skin damage (scores ≥3)	74.6%	63.3%	85.0%
Eye damage (scores ≥2)	84.7%	58.3%	40.0%
Mouth damage (scores ≥2)	3.4%	0.0%	0.0%
Emaciation (scores ≥2)	0.0%	0.0%	0.0%
Smoltification (scores ≥2)	0.0%	0.0%	0.0%
Sexual maturation (scores ≥2)	0.0%	0.0%	0.0%
Vertebral deformity (scores ≥2)	0.0%	0.0%	0.0%
Upper jaw deformity (scores ≥2)	0.0%	0.0%	0.0%
Lower jaw deformity (scores ≥2)	0.0%	0.0%	0.0%

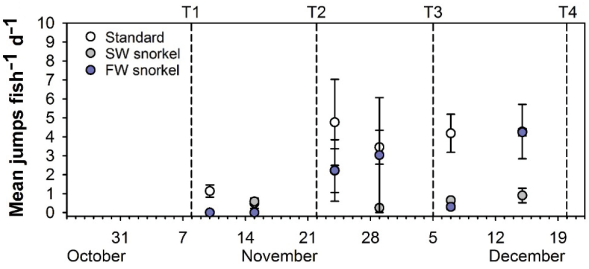


Fig. 5. Mean number (±SE) of jumps per fish per day by farmed Atlantic salmon in standard (white circles), SW snorkel (grey circles) and FW snorkel cages (blue circles) at weekly assessments. Values for each cage type are aggregates from replicate cages (n = 3 replicates). See Fig. 1 legend for details of cage treatments. The 4 sampling times (T1 to T4) are indicated by dashed lines

Surface activity

Surfacing by salmon was observed in all cage types and increased during the study, particularly after Time 2 (Fig. 5). Fish in standard cages performed more jumps per fish per day (mean of 3.0) than SW snorkel (mean of 0.8) and FW snorkel cages (mean of 1.3) ($t \geq 2.8$, $p < 0.05$; Fig. 5). No differences in jump frequency were detected between SW and FW snorkel fish ($t = 0.04$, $p = 0.97$; Fig. 5).

DISCUSSION

SW and FW snorkel cages outperformed standard cages in terms of lowered AGD-related gill scores and reduced numbers of new salmon lice at some time points. All cage types had similar fish welfare and growth outcomes. However, we did not consistently detect reduced AGD and lice levels in FW snorkels compared to SW snorkels as initially predicted, with increases in new lice in FW compared to SW snorkel cages at certain time points. Daytime surfacing behaviour by salmon appeared unaffected between SW and FW snorkel cages. This suggests that while salmon frequently accessed the freshwater surface layer, their exposure durations were likely inadequate to alter AGD or salmon lice levels significantly below those in SW snorkel cages. Our results contrast with the AGD suppression observed in a commercial trial where freshwater was added to a snorkel to combat an AGD outbreak (Wright et al. 2017). There are several possible reasons for this, including differences in snorkel sizes that may affect salmon behaviours, and the multiple ways the freshwater layer water in the FW snorkel differed from the SW snorkel other than salinity, including temperature and oxygen content.

Effects of standard, SW and FW snorkels cages on AGD

AGD-related gill scores, correlated with loads of AGD-causing *Paramoeba perurans* during the study, were often higher in standard cages, but similar between FW and SW snorkel cages. A higher proportion of *P. perurans*-positive fish were also found in standard compared to FW snorkel cages. Harvest-sized fish with high AGD-related gill scores were held in shallow cages within the research farm facility, measuring ~30 m width × 120 m length. As swimming in the same depth and locality as AGD-affected individuals may increase AGD risk (Young et al. 2014), shallow swimming by fish in standard cages could have partially explained their higher AGD-related gill scores than snorkel fish. The lack of difference in AGD-related gill scores between FW and SW snorkel fish suggested that salmon mostly failed to enter freshwater sufficiently to decrease *P. perurans* populations on their gills (2 to 4 h freshwater baths are effective; Parsons et al. 2001, Clark et al. 2003, Rodger 2014).

Effects of standard, SW and FW snorkel cages on salmon lice

The lack of salmon lice reductions in FW snorkel cages indicated that the development of salmon lice on Atlantic salmon was unhindered by regular freshwater exposures during surface jumps (mean of 2.3 jumps per fish per day) and other possible times of residence. Thus, these periods were likely too short to eliminate freshwater-sensitive attached copepodids which takes 1 to 3 h (Wright et al. 2016). High salmon lice infestations of wild sea trout *Salmo trutta* are associated with entry into shallower brackish water or rivers, possibly for self-treatment against lice (Gjelland et al. 2014). Once completing their seaward out-migration, wild post-smolt Atlantic salmon also use less saline environments and this may also be a reaction to new salmon lice recruits (Mitamura et al. 2017). Despite the potential for Atlantic salmon to self-treat against salmon lice by moving from seawater to freshwater or low salinity environments, this did not occur under the conditions created in FW snorkel cages within the current trial.

In some instances, FW snorkel cages increased new salmon lice infestations compared to SW cages. There are several possible reasons for this. Firstly, the freshwater exposures that salmon were subjected to may have removed mucus or induced stress, making them more susceptible to salmon lice infestations as has been documented for other external parasites such as *Neobenedenia girellae* skin flukes (Yamamoto et al. 2011). Reduced sheltering by fish inside snorkels filled with freshwater could also increase salmon lice infestations of FW compared to SW snorkel cages. While harvest-sized salmon have been found to position themselves almost exclusively below 4 m deep SW snorkels in identical cages in autumn (Stien et al. 2016), periodic post-smolt presence inside 4 to 16 m deep SW snorkels, inferred from low oxygen conditions, was detected by Oppedal et al. (2017). Therefore, greater fish residency inside SW snorkels may contribute to lice reduction effects typically seen in this cage type (see Oppedal et al. 2017). More work is needed to reveal differences in depth distribution of Atlantic salmon among standard, SW and FW snorkel cage types.

Effects of standard, SW and FW snorkels cages on fish welfare and growth

Fish welfare indicators and weights were similar between snorkel and standard cages, including where snorkels were filled with freshwater, confirming conclusions reached in previous snorkel cage investigations that use of this technology does not affect fish welfare (Oppedal et al. 2017, Wright et al. 2017). Snout damage, likely due to collisions with net roof and snorkel structures, has been observed in one research scale snorkel cage study (Stien et al. 2016) but we did not observe this negative effect here.

FW in snorkels: contrasting results in commercial and experimental trials

Commercial snorkels (10 m circle diameter × 10 m deep; volume 6448 m^3; Wright et al. 2017) have a volume 179 times greater than our research snorkels. The greater volume within the snorkel may promote greater fish residence time. Greater numbers and densities of fish within larger snorkels may enable them to school in their standard circular swimming pattern (~500 individuals are required to initiate schooling behaviour in 500–2000 m^3 cages; Oppedal et al. 2011) and thus spend longer periods at a given depth. However, the smaller snorkels used in this study may have limited this behaviour and allowed only enough room for surfacing for swim-bladder refilling before returning to swimming in a school formation below the snorkel. Further, while feed entered the snorkel at the surface in both the commercial trial and this experiment, we observed that fish in the commercial trial entered the snorkel to take the feed, while in this trial they mostly waited until the feed had fallen below the snorkel depth. The restricted space in the smaller snorkel may have inhibited formation of the typical feeding aggregation at the surface and limited use by salmon of this layer.

In this trial, the freshwater layer was created by applying mains ozone-treated freshwater, whereas in the commercial trial, where far greater quantities were required, snorkels were filled with freshwater from a local river (Wright et al. 2017). These different methods of application and volumes of added freshwater created quite different outcomes in the surface layer's salinity, temperature and oxygen levels. Due to larger freshwater volumes and greater instability in a larger snorkel, salinity conditions achieved by filling snorkels with freshwater at a commercial scale (salinity of 4–5) were higher than the current study (always <1) (Wright et al. 2017). Salinity gradients also tended to be steeper in this research scale study (stable salinity between 0 and 2 m depth, then constantly increasing salinity between 2 and 4 m) compared to the commercial-scale study (constantly increasing salinity throughout the snorkel) (Young et al. 2014). The higher salinity and its more gradual gradient may have provided a more attractive self-treatment space for Atlantic salmon to enter than an abrupt change to an almost completely fresh layer.

Freshwater filling with cooler temperature water, less preferred by salmon, has been typical in commercial- (Wright et al. 2017) and research-scale snorkels. At the commercial scale, surface water temperatures in individual snorkels filled with freshwater to varying degrees were 0.6 to 1.9°C cooler at the surface than reference conditions at one time (Wright et al. 2017), whereas in this study FW snorkels ranged from 1.5, 1.2, 0 and 0.7°C cooler than reference conditions across 4 sampling points. Similarly lower surface temperatures in FW snorkels between these 2 studies point to these relatively small temperature differences being unimportant in freshwater layer use by salmon. However, a more attractive water temperature within the FW area should be tested to increase fish residence (Oppedal et al. 2011).

Oxygen supersaturation from ozone treatment occurred in the research scale FW snorkels used here,

reaching levels (maximum 148% DO saturation at Time 4) approaching those known to cause stress, gas bubble disease and behavioural and physiological changes in parr and pre-smolt Atlantic salmon (Brauner et al. 2000, Espmark & Baeverfjord 2009, Espmark et al. 2010). In contrast, the low salinity layer in commercial snorkel cages filled from a local river had DO saturations of <100% and was not ozone treated (Wright et al. 2017). Therefore, the oxygen supersaturation and, potentially, residual ozone in the surface freshwater layer in this study may have acted as a deterrent. However, limited information exists on the effects of oxygen supersaturation and residual ozone in post-smolt Atlantic salmon, so we are unable to gauge the extent of this possible effect in this trial.

Mean AGD-related gill scores in snorkel cages (mean gill scores in cages up to 1.9) in this study were lower than in the commercial trial, which experienced a major outbreak (mean gill scores in cages up to 2.8; Wright et al. 2017). Low stocking densities and holding caged fish at declining water temperatures in autumn to winter, rather than increasing temperatures in summer to autumn (elevated water temperature is associated with increased AGD incidence; Oldham et al. 2016), potentially contributed to the lower AGD-related gill scores and limited the detection of gill score differences between cage types. A follow-up investigation, where salmon in SW and FW snorkel cages experience a more severe AGD outbreak, would improve the detectability of AGD differences between these cage types.

CONCLUSIONS

In our autumn to winter study, a permanent freshwater surface layer maintained within snorkel lice barrier sea-cages holding Atlantic salmon did not affect their freshwater-sensitive ectoparasites, *Paramoeba perurans* and salmon lice. Salmon may have had limited contact time with the freshwater layer because of how they vertically positioned within snorkel cages or because they avoided the cool, super-oxygenated freshwater surface layer created to the extent that the parasites were not exposed sufficiently to the freshwater layer to produce an effect. Multiple changes to the freshwater surface layer to attract salmon to it are possible, including temporary night lighting strategies (Juell & Fosseidengen 2004, Wright et al. 2015) and making surface waters warmer, less hyperoxic and more saline (Oppedal et al. 2011). These may intensify freshwater or low salinity layer use by salmon to the point where *P. perurans* and salmon lice are reliably diminished.

Acknowledgements. We thank Jan-Erik Fosseidengen and Tone Vågseth for technical support, along with Gunnar Didriksen, Sjur Åge Skår and others at Austevoll and Matre Research Stations for animal production and farm management. The trial was partly funded by Future Welfare (project 267800) within the Research Council of Norway, R&D projects at Bremnes Seashore and the Norwegian Department of Fisheries and Coastal Affairs. The work was also funded by the Research Council of Norway (project number 256318/E40). The trial was conducted in accordance with the laws and regulations for experiments and procedures on live animals (FOTS ID: 9600/2016).

LITERATURE CITED

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

Adams MB, Crosbie PBB, Nowak BF (2012) Preliminary success using hydrogen peroxide to treat Atlantic salmon, *Salmo salar* L., affected with experimentally induced amoebic gill disease (AGD). J Fish Dis 35:839–848

Bjordal Å (1990) Sea lice infestation of farmed salmon: possible use of cleaner fish as an alternative method for delousing. Can J Fish Aquat Sci 1761:85–89

Björnsson BT, Hemre GI, Bjørnevik M, Hansen T (2000) Photoperiod regulation of plasma growth hormone levels during induced smoltification of underyearling Atlantic salmon. Gen Comp Endocrinol 119:17–25

Bolger T, Connolly PL (1989) The selection of suitable indices for the measurement and analysis of fish condition. J Fish Biol 34:171–182

Brauner CJ, Seidelin M, Madsen SS, Jensen FB (2000) Effects of freshwater hyperoxia and hypercapnia and their influences on subsequent seawater transfer in Atlantic salmon (*Salmo salar*) smolts. Can J Fish Aquat Sci 57:2054–2064

Bricknell IR, Dalesman SJ, O'Shea B, Pert CC, Luntz AJM (2006) Effect of environmental salinity on sea lice *Lepeophtheirus salmonis* settlement success. Dis Aquat Org 71:201–212

Bridle AR, Crosbie PBB, Cadoret K, Nowak BF (2010) Rapid detection and quantification of *Neoparamoeba perurans* in the marine environment. Aquaculture 309:56–61

Bron JE, Sommerville C, Wootten R, Rae GH (1993) Fallowing of marine Atlantic salmon, *Salmo salar* L, farms as a method for the control of sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837). J Fish Dis 16:487–493

Clark G, Powell M, Nowak B (2003) Effects of commercial freshwater bathing on reinfection of Atlantic salmon, *Salmo salar*, with amoebic gill disease. Aquaculture 219: 135–142

Crawley MJ (2012) The R book. John Wiley & Sons, London

Crosbie PBB, Bridle AR, Cadoret K, Nowak BF (2012) In vitro cultured *Neoparamoeba perurans* causes amoebic gill disease in Atlantic salmon and fulfils Koch's postulates. Int J Parasitol 42:511–515

Dempster T, Juell JE, Fosseidengen JE, Fredheim A, Lader P (2008) Behaviour and growth of Atlantic salmon (*Salmo salar* L.) subjected to short-term submergence in com-

mercial scale sea-cages. Aquaculture 276:103–111
Dempster T, Kristiansen TS, Korsøen ØJ, Fosseidengen JE, Oppedal F (2011) Technical note: modifying Atlantic salmon (*Salmo salar*) jumping behavior to facilitate innovation of parasitic sea lice control techniques. J Anim Sci 89:4281–4285
Espmark ÅM, Baeverfjord G (2009) Effects of hyperoxia on behavioural and physiological variables in farmed Atlantic salmon (*Salmo salar*) parr. Aquacult Int 17: 341–353
Espmark ÅM, Hjelde K, Baeverfjord G (2010) Development of gas bubble disease in juvenile Atlantic salmon exposed to water supersaturated with oxygen. Aquaculture 306:198–204
Fahlén G (1971) The functional morphology of the gas bladder of the genus *Salmo*. Acta Anat 78:161–184
FAO (2017) Global aquaculture production. Food and Agriculture Organization of the United Nations. www.fao.org/fishery/statistics/global-aquaculture-production/en. (accessed April 2017)
Gharbi K, Matthews L, Bron J, Roberts R, Tinch A, Stear M (2015) The control of sea lice in Atlantic salmon by selective breeding. J R Soc Interface 12:0574
Gjelland KØ, Serra-Llinares RM, Hedger RD, Arechavala-Lopez P and others (2014) Effects of salmon lice infection on the behaviour of sea trout in the marine phase. Aquacult Environ Interact 5:221–233
Helgesen KO, Romstad H, Aaen SM, Horsberg TE (2015) First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse *Lepeophtheirus salmonis* in Norway. Aquacult Rep 1:37–42
Helgesen KO, Bakke MJ, Kaur K, Horsberg TE (2017) Increased catalase activity—a possible resistance mechanism in hydrogen peroxide resistant salmon lice (*Lepeophtheirus salmonis*). Aquaculture 468:135–140
Heuch PA (1995) Experimental evidence for aggregation of salmon louse copepodids (*Lepeophtheirus salmonis*) in step salinity gradients. J Mar Biol Assoc UK 75: 927–939
Heuch PA, Parsons A, Boxaspen K (1995) Diel vertical migration: a possible host-finding mechanism in salmon louse (*Lepeophtheirus salmonis*) copepodids? Can J Fish Aquat Sci 52:681–689
Juell JE, Fosseidengen JE (2004) Use of artificial light to control swimming depth and fish density of Atlantic salmon (*Salmo salar*) in production cages. Aquaculture 233:269–282
Krkošek M, Connors BM, Morton A, Lewis MA, Dill LM, Hilborn R (2011) Effects of parasites from salmon farms on productivity of wild salmon. Proc Natl Acad Sci USA 108:14700–14704
Krkošek M, Revie CW, Gargan PG, Skilbrei OT, Finstad B, Todd CD (2013) Impact of parasites on salmon recruitment in the Northeast Atlantic Ocean. Proc R Soc B 280: 20122359
Lovdata (2012) Regulations on combating salmon lice in aquaculture facilities (in Norwegian: Forskrift om bekjempelse av lakselus i akvakulturanlegg) http://lovdata.no/dokument/SF/forskrift/2012-12-05-1140 (accessed May 2018)
Maynard BT, Taylor RS, Kube PD, Cook MT, Elliott NG (2016) Salmonid heterosis for resistance to amoebic gill disease (AGD). Aquaculture 451:106–112
McCormick SD, Hansen LP, Quinn TP, Saunders RL (1998) Movement, migration, and smolting of Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 55:77–92
Mitamura H, Thorstad EB, Uglem I, Økland F (2017) In situ measurement of salinity during seaward migration of Atlantic salmon post-smolts using acoustic transmitters with data-storage capabilities and conventional acoustic transmitters. Anim Biotelem 5:5
Murray AG (2016) Increased frequency and changed methods in the treatment of sea lice (*Lepeophtheirus salmonis*) in Scottish salmon farms 2005–2011. Pest Manag Sci 72:322–326
Murray AG, Wardeh M, McIntyre KM (2016) Using the H-index to assess disease priorities for salmon aquaculture. Prev Vet Med 126:199–207
Nilsen A, Nielsen KV, Biering E, Bergheim A (2017) Effective protection against sea lice during the production of Atlantic salmon in floating enclosures. Aquaculture 466: 41–50
NIPH (Norwegian Institute of Public Health) (2015) Consumption of delousing agents remains high. www.fhi.no/hn/legemiddelbruk/fisk/forbruket-av-lakselusmidler-erfort/ (accessed May 2018) (in Norwegian)
Nowak BF (2007) Parasitic diseases in marine cage culture—an example of experimental evolution of parasites? Int J Parasitol 37:581–588
Oldham T, Rodger H, Nowak BF (2016) Incidence and distribution of amoebic gill disease (AGD)—an epidemiological review. Aquaculture 457:35–42
Oppedal F, Dempster T, Stien LH (2011) Environmental drivers of Atlantic salmon behaviour in sea-cages: a review. Aquaculture 311:1–18
Oppedal F, Samsing F, Dempster T, Wright DW, Bui S, Stien LH (2017) Sea lice infestation levels decrease with deeper 'snorkel' barriers in Atlantic salmon sea-cages. Pest Manag Sci 73:1935–1943
Overton K, Samsing F, Oppedal F, Dalvin S, Stien LH, Dempster T (2018) The use and effects of hydrogen peroxide on salmon lice and post-smolt Atlantic salmon. Aquaculture 486:246–252
Parsons H, Nowak B, Fisk D, Powell M (2001) Effectiveness of commercial freshwater bathing as a treatment against amoebic gill disease in Atlantic salmon. Aquaculture 195:205–210
Roberts SD, Powell MD (2003) Reduced total hardness of fresh water enhances the efficacy of bathing as a treatment for amoebic gill disease in Atlantic salmon, *Salmo salar* L. J Fish Dis 26:591–599
Rodger H (2014) Amoebic gill disease (AGD) in farmed salmon (*Salmo salar*) in Europe. Fish Vet J 14:16–26
Shinn AP, Pratoomyot J, Bron JE, Paladini G, Brooker EE, Brooker AJ (2015) Economic costs of protistan and metazoan parasites to global mariculture. Parasitology 142: 196–270
Stien A, Bjørn PA, Heuch PA, Elston DA (2005) Population dynamics of salmon lice *Lepeophtheirus salmonis* on Atlantic salmon and sea trout. Mar Ecol Prog Ser 290: 263–275
Stien LH, Nilsson J, Hevrøy EM, Oppedal F, Kristiansen TS, Lien AM, Folkedal O (2012) Skirt around a salmon sea cage to reduce infestation of salmon lice resulted in low oxygen levels. Aquacult Eng 51:21–25
Stien LH, Dempster T, Bui S, Glaropoulos A, Fosseidengen JE, Wright DW, Oppedal F (2016) 'Snorkel' sea lice barrier technology reduces sea lice loads on harvest-sized Atlantic salmon with minimal welfare impacts. Aquaculture 458:29–37

Stone J, Boyd S, Sommerville C, Rae GH (2002) An evaluation of freshwater bath treatments for the control of sea lice, *Lepeophtheirus salmonis* (Krøyer), infections in Atlantic salmon, *Salmo salar* L. J Fish Dis 25:371–373

Taylor RS, Muller WJ, Cook MT, Kube PD, Elliott NG (2009) Gill observations in Atlantic salmon (*Salmo salar*, L.) during repeated amoebic gill disease (AGD) field exposure and survival challenge. Aquaculture 290:1–8

Thomassen JM (1993) Hydrogen peroxide as a delousing agent for Atlantic salmon. In: Boxshall GA, Defaye D (eds) Pathogens of wild and farmed fish: sea lice. CRC Press, Boca Raton, FL, p 290–295

Wright DW, Glaropoulos A, Solstorm D, Stien LH, Oppedal F (2015) Atlantic salmon *Salmo salar* instantaneously follow vertical light movements in sea cages. Aquacult Environ Interact 7:61–65

Wright DW, Oppedal F, Dempster T (2016) Early-stage sea lice recruits on Atlantic salmon are freshwater sensitive. J Fish Dis 39:1179–1186

Wright DW, Stien LH, Dempster T, Vågseth T, Nola V, Fosseidengen JE, Oppedal F (2017) 'Snorkel' lice barrier technology reduced two co-occurring parasites, the salmon louse (*Lepeophtheirus salmonis*) and the amoebic gill disease causing agent (*Neoparamoeba perurans*), in commercial salmon sea-cages. Prev Vet Med 140: 97–105

Yamamoto S, Shirakashi S, Morimoto S, Ishimaru K, Murata O (2011) Efficacy of oral praziquantel treatment against the skin fluke infection of cultured chub mackerel, *Scomber japonicus*. Aquaculture 319:53–57

Young ND, Crosbie PBB, Adams MB, Nowak BF, Morrison RN (2007) *Neoparamoeba perurans* n. sp., an agent of amoebic gill disease of Atlantic salmon (*Salmo salar*). Int J Parasitol 37:1469–1481

Young ND, Dyková I, Nowak BF, Morrison RN (2008a) Development of a diagnostic PCR to detect *Neoparamoeba perurans*, agent of amoebic gill disease. J Fish Dis 31:285–295

Young ND, Dykova I, Snekvik K, Nowak BF, Morrison RN (2008b) *Neoparamoeba perurans* is a cosmopolitan aetiological agent of amoebic gill disease. Dis Aquat Org 78:217–223

Young ND, Dyková I, Crosbie PBB, Wolf M, Morrison RN, Bridle AR, Nowak BF (2014) Support for the coevolution of *Neoparamoeba* and their endosymbionts, *Perkinsela amoebae*-like organisms. Eur J Protistol 50:509–523

Editorial responsibility: Chris Noble, Tromsø, Norway

Submitted: October 10, 2017; Accepted: March 26, 2018
Proofs received from author(s): May 31, 2018

IV

Paper IV

Bui, S., Geitung, L., Oppedal, F., Barrett, L. T. (2020)

Salmon lice survive the straight shooter: a commercial scale sea cage trial of laser delousing.

Preventive Veterinary Medicine 181, 105063.

https://doi.org/10.1016/j.prevetmed.2020.105063

Preventive Veterinary Medicine 181 (2020) 105063

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Salmon lice survive the straight shooter: A commercial scale sea cage trial of laser delousing

Samantha Bui[a,*], Lena Geitung[b,c], Frode Oppedal[a], Luke T. Barrett[d]

[a] Animal Welfare Research Group, Institute of Marine Research, Matredal 5984, Norway
[b] Bremnes Seashore AS, Øklandsvegen 90, 5430 Bremnes, Norway
[c] Department of Biology, University of Bergen, 5006 Bergen, Norway
[d] Sustainable Aquaculture Laboratory – Temperate and Tropical (SALTT), School of BioSciences, University of Melbourne, Victoria 3010, Australia

ARTICLE INFO

Keywords:
Aquaculture
Snorkel cage
Salmo salar
Lepeophtheirus salmonis
Optical delousing

ABSTRACT

Ectoparasitic salmon louse (*Lepeophtheirus salmonis*) infestations are costly for Atlantic salmon (*Salmo salar*) farmers in Norway. As a result, there is a strong desire for solutions to prevent and control infestations, and new technologies are typically developed and commercialised rapidly, without rigorous validation. Here, we tested the efficacy of a new commercially available control measure—delousing by underwater lasers—using a replicated design at full commercial scale. Laser delousing was used in combination with a preventive method (snorkel cages), with laser nodes deployed in 3 of the 6 sea cages at the site. The trial ran for 54 days, after which time there was no difference in infestation density of mobile salmon louse stages (pre-adult, adult male or adult female) in cages with or without laser nodes installed. By the end of the trial, adult female lice numbers in all cages were close to the legislated trigger for mandatory delousing (0.5 adult female lice per fish). The laser nodes delivered a large number of pulses relative to the number of lice in the cages, indicating that a lack of lethality rather than a lack of target detection was the limiting factor. If all pulses had been effective, they should have removed between 4–38 % of mobile lice each day. There was no effect on salmon welfare indicators such as skin condition or eye status. Our results highlight the importance of rigorous validation of new technologies across a range of conditions before widespread implementation by industry.

1. Introduction

Aquaculture is a relatively young industry compared to terrestrial production systems (Nash, 2011), with systematic research and development only becoming a key focus since the 1970s (Kumar and Engle, 2016). However, commercial production of finfish has become a highly lucrative industrial process analogous to modern agriculture (Asche et al., 2018; Ashche, 2008), with rapid technological advances facilitating substantial productivity growth in recent decades (Asche et al., 2018).

The farming of Atlantic salmon (*Salmo salar*) is a shining example of rapid industry growth, with commercial production initiated only ~50 years ago. Although the production volume of Atlantic salmon is less than 5% of global finfish production, it is the most valuable fish product (FAO, 2018). Norway is the principal producer of salmon, and its success can be partly attributed to technological innovation, productivity advancements, and efficiency at multiple levels of the production process. However, the industry faces significant obstacles in sustainability (Klinger and Naylor, 2012; Olesen et al., 2010), with the most prominent risk factor being the proliferation of the ectoparasitic salmon lice, *Lepeophtheirus salmonis* (Murray et al., 2016). There has been substantial focus on prevention and control of lice in aquaculture, to reduce environmental and welfare impacts of infestations on both wild and farmed salmonids (Costello, 2009; Heuch et al., 2005; Krkosek et al., 2007; McVicar, 2004; Overton et al., 2018; Thorstad et al., 2015; Torrissen et al., 2013).

To manage and reduce the negative impacts of salmon lice, the Norwegian Ministry of Trade, Industry and Fisheries enforces a limit on infestation levels on all Norwegian salmonid farms, whereby companies must ensure less than 0.5 adult female lice on their fish (0.2 during the season of out-migration for wild salmonids) (Norwegian Ministry of Fisheries and Coastal Affairs, 2012). In the last decade, there has been an increase in technological innovation development around the management of lice in farms as traditional chemical or medicinal treatments have become less effective and unsustainable (Aaen et al., 2015; McNair, 2015). Most of these innovations can be considered short-term

* Corresponding author.
E-mail address: samantha.bui@hi.no (S. Bui).

https://doi.org/10.1016/j.prevetmed.2020.105063
Received 4 December 2019; Received in revised form 10 June 2020; Accepted 10 June 2020

(i.e. minor adjustments to current farming practices), and classed as preventive, continuous, or immediate strategies (Brakstad et al., 2019). Preventive approaches aim to minimise exposure of the host to the infective planktonic stages of the lice, for example by shielding a section of the cage (Frank et al., 2015; Stien et al., 2018), changing the cage structure (Stien et al., 2016), or using stimuli to move the school's vertical distribution (Bui et al., 2019). Continuous approaches attempt to control levels of lice on the fish by use of invertivorous cleaner fish (Imsland et al., 2018; Treasurer, 2002) or functional feed (Jensen et al., 2015). Immediate delousing control methods are implemented when preventive or continuous strategies are unsuccessful and lice levels are nearing or exceeded the legislative threshold, requiring rapid reductions in infestation. Delousing treatments can include chemical therapeutants, freshwater bathing, mechanical removal, and thermal treatments (Overton et al., 2018).

Optical or laser delousing is a unique innovation that has overcome multiple technical challenges to produce an instrument that combines machine learning louse detection with targeted louse removal. The method aims to control salmon lice infestation levels using laser pulses (concentrated photons) targeted at machine-identified lice to injure the parasite but not the host. 'Nodes' submerged under buoys within the sea cage contain an automated camera system that scans passing fish, identifies potential lice on the fish, and instigates a pulse of light directed at the suspected lice. The images of lice on salmon collected from nodes are transmitted back to the database and are used to continually train the machine learning system. The pulses apparently do not harm the skin of the salmon, while the system can identify and avoid the fish's eye (Brown, 2016; Frenzl, 2017). Generally, multiple nodes are deployed in each sea cage, and are monitored externally by a technical support team. The system has been commercially available since 2014 and at present, is reportedly in use in around 150 cages in Norway.

Here, we present a case study for a technology that has been adopted by industry, but has not yet been validated scientifically. This study aimed to test the efficacy of laser delousing for the control of salmon lice at a commercial salmon farm, as well as its short-term effects on salmon welfare. The farm had applied an integrated pest management strategy – prevention (snorkel cages; Geitung et al., 2019) in combination with continuous control (lasers). The laser delousing strategy was implemented over 54 days, to a point where the lice level was nearing the threshold that triggers delousing action.

2. Methods

The trial was conducted at a commercial fish farm (Låva) in Jelsafjorden, western Norway (59.1 °N, 5.6 °E). The farm had 6 circular sea cages (Ø = 50.9 m, C = 160 m, 36 m deep) arranged in a single row from the feed barge (from south-west to north-east). All cages had a 16 m deep snorkel (Ø = 28.4 m, C = 90 m) installed before the fish arrived. The cages were stocked with Atlantic salmon (*Salmo salar*, autumn-transferred smolts, SalmoBreed strain) one month before trial start. Throughout the experimental period the farm was managed according to standard rearing and feeding procedures for commercial salmon aquaculture.

The trial ran for 54 days between 6 December 2018 to 28 January 2019. At the start of the experiment, each cage held between 157000–165000 salmon with an average weight of 250–450 g. Every second cage (i.e. the 2nd, 4th and 6th cages heading north-east along the single-row site) was equipped with 2 lasers (Optical Delousing™, Stingray Marine Solutions AS, Norway), as recommended for cages of this size and this number of fish, to undergo the continuous laser delousing treatment. The remaining 3 cages had no additional delousing strategies and therefore acted as controls. The lasers were installed at the location by Stingray's service team in cooperation with the fish farmers on 5 December 2018. As is the standard protocol, daily operation and monitoring of the laser nodes were performed by a technical support team at Stingray's main offices in Oslo, not onsite. Each day had a variable operational time, the period during which the lasers were active; this varied depending on the decision of Stingray's operations team and is directly correlated with the number of pulses emitted. Thus, pulses per day was the best measure of effective operation and was used as such in the analyses. Daily updates on salmon positioning from fish farmers were used to place the laser nodes at the most optimal depth in the cage (the largest proportion of fish visible to the cameras). Nodes could be rotated and moved vertically from the surface to a maximum depth of 25 m. The nodes were cleaned once a month during the experimental period, according to manufacturer recommendations. On day 34 of the experimental period (9 January 2019) one laser was taken out of Cage 6 and sent for repair. Cage 6 continued with a single laser for the remainder of the trial.

Daily salinity and temperature profiles between 0–40 m depth were collected from a reference point (the feed barge) using a Conductivity, Temperature and Depth (CTD) recorder (SD208, SAIV-AS, Bergen, Norway). Thermal stratification with cooler upper layers occurred throughout most of the study period, with temperatures ranging from 9.2 to 4.9 °C in the surface and from 10.8 to 7.4 °C in the deeper waters. The salinity profile was relatively stable throughout, with high salinity (> 28.9 ppt) and only minor stratification.

At fortnightly intervals, 20–50 fish from each cage were sampled (Table 1). Fish were randomly netted and subjected to a lethal dose of anaesthetics (Benzoak vet., benzocaine, 200 mg ml^{-1}, VESO Vikan, Namsos, Norway) before salmon lice were assessed on each fish while submerged in seawater-filled trays. The number of lice were counted and classified according to life stages: copepodid, chalimus I, chalimus II, preadult I, preadult II (male and female) and adult (male, and female with and without egg-strings). Counts of mobile stages that had detached from the host and were found in sedation vessels were also included in the total counts, and divided among the individuals sampled in that vessel. In addition to lice counts, fish welfare was scored according to the Salmon Welfare Index Model 1.0 (SWIM 1.0) (Stien et al., 2013). Ten welfare indicators described the condition of the individual, including assessments of emaciation status, presence of deformities (vertebral, opercula, upper jaw, lower jaw), fin and skin condition, eye and gill status, and presence of mouth/jaw wounds. Individual indicators were scored from 1 (good condition) to a maximum of 3–7, with higher scores representing increasing severity (Stien et al., 2013).

2.1. Data handling and statistical analyses

To test for an effect of the laser treatment on mobile lice abundance, pre-adult, adult male and adult female lice densities were tracked over

Table 1
Dates and corresponding day number since experiment start for sampling events. The number of fish sampled across 3 replicate cages are shown for each sample point.

Experimental Day	Date	N fish sampled in laser-free cages	N fish sampled in laser cages
1	6 Dec 2018	62	61
11	17 Dec 2018	62	61
28	3 Jan 2019	61	59
40	15 Jan 2019	153	158
53	28 Jan 2019	93	93

time in cages with and without lasers deployed. Rates of increase in lice density are not expected to be linear, so lice density data were modelled using generalized additive models (GAMs) fitted using the mgcv package for R (Wood, 2011; R Core Team, 2019). Treatment group (with or without laser) and cage ID were fitted as factors, with trial day as the smoothing term (k = 3). A treatment × cage ID interaction term was also tested and removed from the model if not significant. Model fit was checked using the gam.check function in mgcv. Cage-level mean louse densities at each sampling date were treated as replicates. We avoided using individual fish as replicates due to the non-independence of individuals within the same cage.

To determine the effect of laser treatments on individual welfare indicators, a multivariate ANOVA model was run with all welfare measures included as response variables, and treatment and sample day as predictor variables, with cage ID as a random factor.

3. Results

After the 54 days of laser operation, mean abundance of all mobile lice stages was similar between cages with laser delousing (1.26 ± 0.08 lice fish^{-1}) and those without (1.25 ± 0.20 lice fish^{-1}). This lack of effect remained when focusing on specific lice stages (Table 2); mean abundance of adult females was similar between cages with lasers present (0.17 ± 0.03 females fish^{-1}) and those without lasers (0.15 ± 0.004 females fish^{-1}), and abundance of adult male (laser: 0.32 ± 0.02; no laser: 0.31 ± 0.03 males fish^{-1}) and pre-adult lice stages (laser: 0.29 ± 0.10; no laser: 0.31 ± 0.12 pre-adults fish^{-1}) also did not differ between treatment groups (Supplementary Table 1).

Tracking relative lice abundance over time (GAM analysis) revealed a considerable increase in pre-adult, adult male and adult female lice abundance over the 54-day trial (Sample day smoothing term: Table 2). All cages started with very few lice, then acquired up to 20× more lice during the study period (Fig. 1). This trend was not significantly affected by the presence of lasers, regardless of lice stage (Treatment term: Table 2). A power analysis based on the R^2 of the fitted models indicated that there was adequate power to detect an effect with sampling days within cages treated as replicates (for power = 0.80, required sample size for pre-adult lice: n = 15.1; adult male lice: n = 6.8; adult female lice: n = 11.6).

Operational time of the lasers was 83 % per day on average (range: 22–99 %), i.e. 20 h per day. Rate of laser activity was likely influenced by lice levels in cages but was also controlled externally by the service

Table 2
Results of generalised additive models fitting temporal changes in pre-adult (Model 1), adult male (Model 2) and adult female (Model 3) lice abundance in 6 sea cages (Cage ID term). Three cages contained an operational laser lice removal system, while 3 did not (Treatment term). Sample day was the smoothing term, fitted within treatment groups (k = 3).

Model 1: pre-adult lice				
Term	**df**	**F**	**p**	
Treatment	1	2.1	0.16	
Cage ID	5	1.5	0.24	
Smoothing term	**edf**	**Ref.df**	**F**	**p**
Sample day (with laser)	1.81	1.96	5.8	*0.02*
Sample day (without laser)	1.85	1.98	9.6	*0.002*
Model 2: adult male lice				
Term	**df**	**F**	**p**	
Treatment	1	0.05	0.82	
Cage ID	5	4.1	*0.01*	
Smoothing term	**edf**	**Ref.df**	**F**	**p**
Sample day (with laser)	1.88	1.99	22.7	*< 0.0001*
Sample day (without laser)	1.79	1.96	24.2	*< 0.0001*
Model 3: adult female lice				
Term	**df**	**F**	**p**	
Treatment	1	0.4	0.54	
Cage ID	5	2.8	*0.047*	
Smoothing term	**edf**	**Ref.df**	**F**	**p**
Sample day (with laser)	1.60	1.84	7.9	*0.002*
Sample day (without laser)	1.78	1.95	7.7	*0.002*

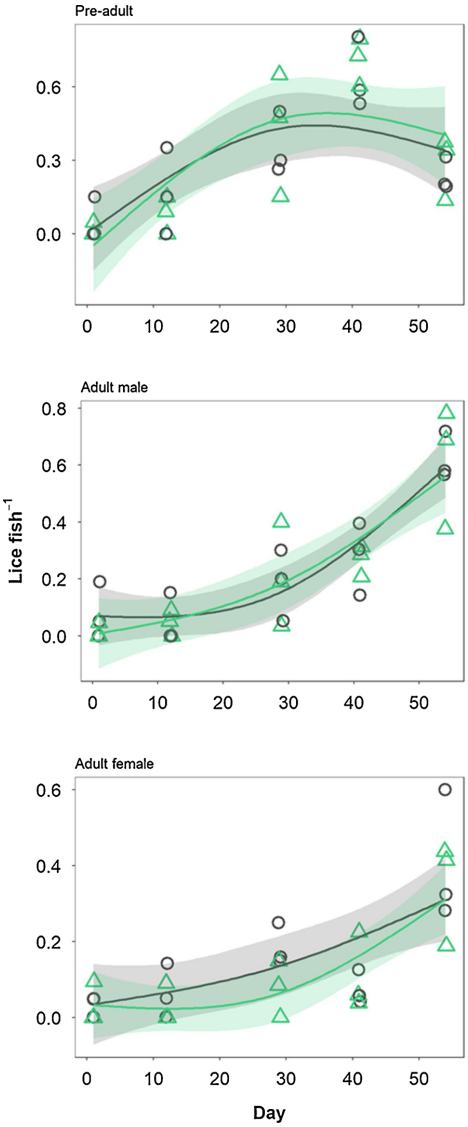


Fig. 1. Mean infestation density of fish in cages with (green triangles) and without (grey circles) lasers present, with the three replicate cages represented. Panels show abundances of three categories of mobile lice stage (top to bottom: pre-adult 1 and 2 with sexes pooled; adult males; adult females). Temporal patterns in infection levels are represented by generalised additive model (GAM) fits, with k = 3. Shaded areas indicate the 95 % confidence interval around the GAM fit (green: lasers; grey: no lasers) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

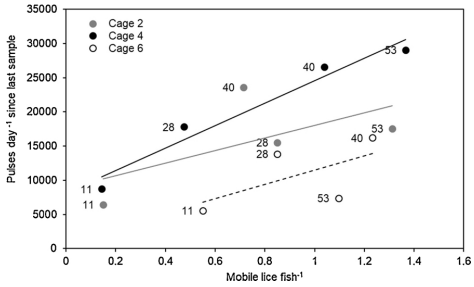


Fig. 2. Correlation between mean pulses per day during the period between sample points (11 – 17 days) in treatment cages with recorded mobile lice per fish, over the experimental period. Regression lines indicate linear correlation between infection intensity and laser delousing function; R^2 values ranged from 0.38 (both Cage 6, black dotted line, and Cage 2, grey solid line) to 0.96 (Cage 4, black solid line). Each data point is labelled with the experimental day of the sample point. Mobile lice includes the pre-adult and adult life-history stages. Note that cage 6 only had one laser node operating from Day 34 onwards, while the remaining two cages continued with 2 operating nodes.

provider and adjusted according to their management strategy. Even so, for each period between sampling days where lice numbers were manually assessed, there was a weak positive relationship between the number of lice per fish recorded on sample days, and the number of pulses per day (since last sample day; i.e. 11–17 days between). More pulses were emitted when higher lice numbers were present on the fish in Cage 2 (R^2 = 0.38) and Cage 6 (R^2 = 0.38), whereas Cage 4 exhibited a stronger correlation (R^2 = 0.96; Fig. 2). After Day 7, 2 cages with 2 lasers installed emitted an average of 17,500–21,664 pulses per day. One cage (Cage 6) only had one laser operational from Day 34 onwards.

To compare the activity of the laser to the number of lice 'available' to detect, the total number of mobile lice in each cage was estimated by multiplying the number of fish by the mean density of lice observed during manual counts. Thus, when considering the possible population of lice available to be targeted in a cage, there were 2–16× more lice available than the number of pulses per day (Table 3), excluding the last sample where Cage 6 (with one operational laser) had 24 times more lice present than pulses shot. This translated to 0.04–0.38 pulses per louse per day (Table 3).

Differences in welfare scores between treatment groups were

Table 3
Estimated number of lice available in a cage to be targeted by the laser delousing technology, and the ratio of laser pulses to available lice. Note that cage 6 only had one laser node operating from Day 34 onwards, while the remaining two cages continued with 2 operating nodes.

Day	Cage	N fish	Mobile lice per fish	Total lice in cage[§]	Pulse per day*	Daily pulse per louse
11	2	156,317	0.15	23,448	6455	0.28
	4	160,378	0.14	22,911	8718	0.38
	6	161,495	0.55	88,822	5562	0.06
28	2	155,996	0.85	132,597	15,523	0.12
	4	160,145	0.47	75,858	17,814	0.23
	6	161,304	0.85	137,108	13,872	0.10
40	2	155,583	0.71	111,131	23,585	0.21
	4	159,725	1.04	165,752	26,586	0.16
	6	160,797	1.23	198,125	16,222	0.08
53	2	154,977	1.31	203,407	17,562	0.09
	4	159,276	1.37	217,677	29,058	0.13
	6	160,237	1.10	175,744	7400	0.04

§ Total number of fish in the cage multiplied by the mean lice density recorded during manual sampling events.

* Mean pulse over the days between sample points.

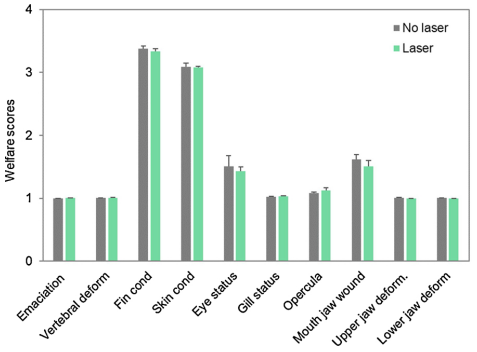


Fig. 3. Mean scores of individual welfare indicators for salmon in cages with laser delousing and cages without, over the 54-day study period.

negligible (Fig. 3), indicating that there was no impact of laser delousing on the salmon welfare indicators monitored (Pillai's trace = 0.33, $F_{(10,12)}$ = 0.59, p = 0.79). There was a slight decrease in welfare scores over time for both treatment groups (Pillai's trace = 0.77, $F_{(10,12)}$ = 4.1, p = 0.01). For skin condition, severe scores (5–7) only occurred on 3 fish out of the 607 assessed across both treatment groups. All 3 were from control cages. For eye status, severe scores (4–5) were observed on 7 out of 607 fish, 5 of which were from cages with lasers. Mortality during the experimental period was on average 1.05 % (± 0.15 % SE) of total fish in control cages, and 0.98 % (± 0.10 % SE) for treatment cages.

4. Discussion

Automated laser delousing had no detectable benefit when deployed for 54 days within snorkel cages at a commercial salmon farm. Legislative thresholds of lice levels on Norwegian farms focus on limiting the adult female stage; abundances of adult female stages increased throughout the trial and were expected to reach the limit within a few weeks of the study's conclusion (Fig. 1).

It is not clear why the laser system was ineffective, but one possibility is that the automated detection system was unable to detect or fire an effective pulse at a sufficient number of lice. Snorkel structures, as used in the present study, can have reduced water exchange and could conceivably concentrate particulate matter or plankton in areas that the nodes occupy. However, conditions in this trial were relatively conducive to laser delousing, with generally low turbidity and algal concentrations at the time of the trial (winter 2018/2019). Salmon behaviour may also be influential; while salmon are not expected to actively avoid nodes, their depth preferences and changes in swimming patterns may frequently draw them away from the nodes' target areas and limited action distance. For example, salmon exhibit strong behavioural responses to feed stimuli and increased activity during feeding periods, particularly during the first feed deliveries of the day (Juell et al., 1994; Oppedal et al., 2011). Environmental conditions such as temperature or light will also drive depth preferences (Oppedal et al., 2007, 2011), with most schools displaying considerable changes in swimming depth (Føre et al., 2017; Johansson et al., 2009). As a result, the node depth must be changed frequently to match that of the school. Further, in a typical commercial cage (~50,000 m^3), only a small proportion of salmon are likely to pass within effective range (~1.5 m) of a node over a given duration. Snorkel structures in the cages confine salmon to a smaller volume of water and could improve encounter rates between salmon and nodes (Geitung et al., 2019; Stien et al., 2016), assuming the school is not beyond the maximum operating depth of the nodes (25 m). However, while all of the above are considerations in deployment of laser delousing nodes, in the present study, the large

number of pulses per day (relative to the estimated number of available lice: Table 3) suggests that infrequent target detection was not the reason for the apparent lack of effect.

A more likely explanation is that most laser pulses did not result in the removal of a louse, either due to an imperfect detection system resulting in the targeting of non-louse objects (we do not have access to data on this), or because the laser pulse is not sufficient to remove a correctly targeted louse. Anecdotal reports from manufacturer testing indicate that each laser pulse is close to 100 % effective, yet results from the present study are consistent with lice staying alive and attached. Specifically, the number of laser pulses relative to the estimated number of mobile lice suggests that if all pulses were lethal, around 4–38 % of the lice population would have been removed each day (Table 3). Instead, we found no significant effect on lice density after 54 days. Occasionally, individual lice were found during manual sampling events with superficial damage that could be attributed to a laser pulse – these were still alive and attached to the host (pers. obs. L. Geitung). It is not known how lice survive the laser pulse (e.g. insufficient power after travelling some distance and attenuating through the water column, or imperfect accuracy resulting in shots to non-lethal areas of the body). Further testing in a range of environmental conditions and cage types may help to identify the potential drivers of poor detection and/or lethality. Adjustments in management tactics of the laser delousing system at a site could also be explored, such as increasing nodes per cage or more frequent changes in depth distribution. Close-range pulses may be more accurate and attenuate less through the water column.

Although our analyses indicated no effect of laser delousing on lice abundance or salmon welfare status at this site, the power to detect such differences must be considered. A post-hoc power analysis indicated that our sample size was adequate for a difference to be detected, however we cannot be certain that the fish sampled were representative of the much larger group in the cage. For the vast majority of studies at commercial scale, the logistical challenge of sampling even 1% of the fish in a sea cage of 150 000 individuals, and repeating that for multiple cages, is impractical. Because estimation of sea lice levels in commercial sites is required for mandatory reporting, there has been a focus on modelling the representativeness of different sampling strategies, and these strategies can similarly be applied to sampling for welfare metrics. With the skewed distribution of abundance of lice infections and correlation with prevalence, current literature emphasises the approach of sampling “few fish from many pens” being more beneficial than “many fish from few pens” (Revie et al., 2007). Even with low sample numbers, prevalence can be indicative of abundance and thus cage-level differences can inform the status of the site (Jeong and Revie, 2020). Similarly, prevalence of poor welfare scores in a sampled group will indicate any rise in negative welfare status. Nevertheless, conclusions from sampling protocols of low sample numbers in such large groups should be interpreted with caution.

Overall welfare score declined over the experimental period for all cages, which is commonly observed in commercial settings (Bui et al., 2018; Folkedal et al., 2016). However, during the period of operation, the laser delousing strategy did not negatively impact salmon welfare in any of the welfare indicators recorded. Concerns have been raised around the potential for injuries to the eyes (which could be mistaken for a louse) and skin, however both of these metrics scored similarly after 50 days of laser operation (Fig. 3). The long-term effect of continuous exposure to light pulses is unknown past 50 days, but any related welfare issues on other commercial sites that have used laser delousing over a full production cycle have not been brought to light. As the exposure rate of individual salmon to laser pulses is unknown, further testing is needed to map impacts on welfare metrics such as cellular skin damage, mucosal layer and eye health, and to ensure that exposure does not cause behavioural distress in salmon. If physical and behavioural welfare indicators are unaffected by laser delousing, then welfare concerns could be allayed for this technology. More powerful lasers may be required to increase lethality; if so, their effects on welfare should be rigorously tested.

In general, there is a disparity between the rapid rate of commercial product development in the aquaculture industry, and scientific validation of those technologies. The Norwegian salmon farming industry benefits from continuous innovation, leading to advances in productivity and efficiency (Asche et al., 2013). There are substantial opportunities for start-up businesses and corporations to develop products that can be available on the market relatively quickly, with anecdotal evidence or personal connections largely driving the acquisition of these new strategies by farming companies (Brakstad et al., 2019). A case study of one relatively small Norwegian salmon farming company estimated production losses from lice and related management expenses totalled approximately 6.89 million NOK per licence (site) in 2016 (Brakstad et al., 2019). This substantial financial and social pressure drives aquaculture companies to seek immediate solutions, and the nature of the Norwegian market is such that rapid commercialisation of technologies is possible. The industry is responsive to emerging innovations and are quick to adopt new strategies to combat salmon lice. This fast-paced acquisition and implementation of technologies can promote progress, but if the strategy is not fully developed or well-tested, investments and resources may be misdirected. Few strategies are robustly validated before implementation.

New technologies, especially those central to production and disease control, should be validated at a commercial scale across a range of conditions. This is particularly important when there are potential welfare implications beyond the scope of Norwegian Food Safety Authority assessments. An example of thorough validation is the snorkel cage for prevention of salmon lice infestations. Its efficacy and potential welfare impacts have been experimentally documented under a range of conditions (Oppedal et al., 2019), with different iterations of the snorkel structure (Oppedal et al., 2017), with a range of fish sizes (Oppedal et al., 2019; Stien et al., 2016), focusing on secondary infections (Wright et al., 2017), and finally at commercial scale over a full production cycle (Geitung et al., 2019). In contrast, there are several examples of lice control technologies that have been widely adopted by the industry without a full understanding of their efficacy or their consequences for animal welfare. These include cleaner fish (small number of studies, mostly poorly replicated and at less than commercial scale: Overton et al., 2019), and thermal and mechanical delousing (Gismervik et al., 2019; Overton et al., 2018). In both cases, the evidence base is now improving, but too late to (a) avoid unacceptable welfare outcomes for billions of vertebrate animals, or (b) guide prudent financial investment by the industry (Brakstad et al., 2019). Proper validation of new techniques before widespread uptake is crucial for the maintenance of high ethical, environmental and financial standards in the industry.

Acknowledgements

We wish to thank Velimir Nola for his valuable assistance with the practical work. We also thank Bremnes Seashore, Norway, for commercial salmon farm operations and access, especially Geir Magne Knudsen, Jarle Langvik and Låva farm staff. Funding was provided by Bremnes Seashore (through time-restricted research licenses and SkatteFUNN 279226), the Norwegian Research Council (267800/E40 and 294730), the Institute of Marine Research, Norway and University of Bergen, Norway. The work was conducted in accordance with the laws and regulations controlling experiments and procedures on live animals in Norway, following the Norwegian Regulations on Animal Experimentation 1996.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prevetmed.2020.105063.

References

Aaen, S.M., Helgesen, K.O., Bakke, M.J., Kaur, K., Horsberg, T.E., 2015. Drug resistance in sea lice: a threat to salmonid aquaculture. Trends Parasitol. 31, 72–81.

Asche, F., Guttormsen, A.G., Nielsen, R., 2013. Future challenges for the maturing Norwegian salmon aquaculture industry: an analysis of total factor productivity change from 1996 to 2008. Aquaculture 396–399, 43–50.

Asche, F., Cojocaru, A.L., Roth, B., 2018. The development of large scale aquaculture production: a comparison of the supply chains for chicken and salmon. Aquaculture 493, 446–455.

Ashche, F., 2008. Farming the sea. Mar. Resour. Econ. 23, 527–547.

Brakstad, O.M., Hagspiel, V., Lavrutich, M.N., Matanovic, D., 2019. Optimal investment decisions in lice-fighting technologies: a case study in Norway. Aquaculture 504, 300–313.

Brown, J.H., 2016. Worth a Shot. Fish Farmer Magazine, Edinburgh.

Bui, S., Stien, L., Nilsson, J., Oppedal, F., 2018. Assessment of Long-term Implementation of Sea Lice Prevention Technologies: Efficiency in Reducing Infestations and Impact on Fish Welfare, Rapport Fra Havforskningen 45-2018. Institute of Marine Research Norway, Bergen.

Bui, S., Oppedal, F., Nilsson, J., Oldham, T., Stien, L.H., 2019. Summary and Status of Deep Lights and Deep Feed Use in Commercial Settings: Welfare, Behaviour and Infestation at Three Case Study Sites, Rapport Fra Havforskningen 2019-4. Institute of Marine Research Norway, Bergen.

Costello, M.J., 2009. The global economic cost of sea lice to the salmonid farming industry. J. Fish Dis. 32, 115–118.

FAO, 2018. The State of World Fisheries and Aquaculture 2018 (SOFIA), State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department, Rome, Italy.

Føre, M., Frank, K., Dempster, T., Alfredsen, J.A., Høy, E., 2017. Biomonitoring using tagged sentinel fish and acoustic telemetry in commercial salmon aquaculture: a feasibility study. Aquac. Eng. 78, 163–172.

Folkedal, O., Pettersen, J.M., Bracke, M.B.M., Stien, L.H., Nilsson, J., Martins, C., Breck, O., Midtlyng, P.J., Kristiansen, T., 2016. On-farm evaluation of the Salmon Welfare Index Model (SWIM 1.0): theoretical and practical considerations. Anim. Welf. 25, 135–149.

Frank, K., Gansel, L.C., Lien, A.M., Birkevold, J., 2015. Effects of a shielding skirt for prevention of sea lice on the flow past stocked salmon fish cages. J. Offshore Mech. Arct. Eng. 137, 011201.

Frenzl, B., 2017. Lasers against parasites: revolutionizing the control of sea louse in Atlantic farmed salmon. J. Ocean Technol. 12, 92–93.

Geitung, L., Oppedal, F., Stien, L.H., Dempster, T., Karlsbakk, E., Nola, V., Wright, D.W., 2019. Snorkel sea-cage technology decreases salmon louse infestation by 75% in a full-cycle commercial test. Int. J. Parasitol. 49, 843–846.

Gismervik, K., Gåsnes, S.K., Gu, J., Stien, L.H., Madaro, A., Nilsson, J., 2019. Thermal injuries in Atlantic salmon in a pilot laboratory trial. Vet. Anim. Sci. 8.

Heuch, P.A., Bjørn, P.A., Finstad, B., Holst, J.C., Asplin, L., Nilsen, F., 2005. A review of the Norwegian 'National Action Plan Against Salmon Lice on salmonids': the effect on wild salmonids. Aquaculture 246, 79–92.

Imsland, A.K.D., Hanssen, A., Nytrø, A.V., Reynolds, P., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018. It works! Lumpfish can significantly lower sea lice infestation in large-scale salmon farming. Biol. Open 7, bio036301.

Jensen, L.B., Provan, F., Larssen, E., Bron, J.E., Obach, A., 2015. Reducing sea lice (*Lepeophtheirus salmonis*) infestation of farmed Atlantic salmon (*Salmo salar* L.) through functional feeds. Aquac. Nutr. 21, 983–993.

Jeong, J., Revie, C.W., 2020. Appropriate sampling strategies to estimate sea lice prevalence on salmon farms with low infestation levels. Aquaculture 518, 734858.

Johansson, D., Ruohonen, K., Juell, J.-E., Oppedal, F., 2009. Swimming depth and thermal history of individual Atlantic salmon (*Salmo salar* L.) in production cages under different ambient temperature conditions. Aquaculture 290, 296–303.

Juell, J.-E., Ferno, A., Furevik, D., Huse, I., 1994. Influence of hunger level and food availability on the spatial distribution of Atlantic salmon, *Salmo salar* L., in sea cages. Aquat. Fish Manage. 25 439/451.

Klinger, D., Naylor, R., 2012. Searching for solutions in aquaculture: charting a sustainable course. Annu. Rev. Environ. Resour. 37, 247–276.

Krkosek, M., Ford, J.S., Morton, A., Lele, S., Myers, R.A., Lewis, M.A., 2007. Declining wild salmon populations in relation to parasites from farm salmon. Science 318, 1772–1775.

Kumar, G., Engle, C.R., 2016. Technological advances that led to growth of shrimp, Salmon, and Tilapia farming. Rev. Fish. Sci. Aquac. 24, 136–152.

McNair, C.M., 2015. Ectoparasites of medical and veterinary importance: drug resistance and the need for alternative control methods. J. Pharm. Pharmacol. 67, 351–363.

McVicar, A.H., 2004. Management actions in relation to the controversy about salmon lice infections in fish farms as a hazard to wild salmonid populations. Aquac. Res. 35, 751–758.

Murray, A.G., Wardeh, M., McIntyre, K.M., 2016. Using the H-index to assess disease priorities for salmon aquaculture. Prev. Vet. Med. 126, 199–207.

Nash, C., 2011. The History of Aquaculture. Wiley-Blackwell, Ames, Iowa.

Norwegian Ministry of Fisheries and Coastal Affairs, 2012. Forskrift om bekjempelse av lakselus i akvakulturanlegg.

Olesen, I., Myhr, A.I., Rosendal, G.K., 2010. Sustainable aquaculture: are we getting there? Ethical perspectives on salmon farming. J. Agric. Environ. Ethics 24, 381–408.

Oppedal, F., Juell, J.-E., Johansson, D., 2007. Thermo- and photoregulatory swimming behaviour of caged Atlantic salmon: implications for photoperiod management and fish welfare. Aquaculture 265, 70–81.

Oppedal, F., Dempster, T., Stien, L.H., 2011. Environmental drivers of Atlantic salmon behaviour in sea-cages: a review. Aquaculture 311, 1–18.

Oppedal, F., Samsing, F., Dempster, T., Wright, D.W., Bui, S., Stien, L.H., 2017. Sea lice infestation levels decrease with deeper' snorkel' barriers in Atlantic salmon sea-cages. Pest Manag. Sci. 73, 1935–1943.

Oppedal, F., Bui, S., Stien, L.H., Overton, K., Dempster, T., 2019. Snorkel technology to reduce sea lice infestations: efficacy depends on salinity at the farm site, but snorkels have minimal effects on salmon production and welfare. Aquac. Environ. Interact. 11, 445–457.

Overton, K., Dempster, T., Oppedal, F., Kristiansen, T.S., Gismervik, K., Stien, L.H., 2019. Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review. Rev. Aquac. 11, 1398–1417.

Overton, K., Barrett, L.T., Oppedal, F., Kristiansen, T.S., Dempster, T., 2019. Sea lice removal by cleaner fish in salmon aquaculture: a systematic review of the evidence base. Aquac. Environ. Interact in press.

R Development Core Team, 2019. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Revie, C.W., Hollinger, E., Gettinby, G., Lees, F., Heuch, P., 2007. Clustering of parasites within cages on Scottish and Norwegian salmon farms: alternative sampling strategies illustrated using simulation. Prev. Vet. Med. 81, 135–147.

Stien, L.H., Bracke, M.B.M., Folkedal, O., Nilsson, J., Oppedal, F., Torgersen, T., Kittilsen, S., Midtlyng, P.J., Vindas, M.A., Øverli, Ø., Kristiansen, T.S., 2013. Salmon Welfare Index Model (SWIM 1.0): a semantic model for overall welfare assessment of caged Atlantic salmon: review of the selected welfare indicators and model presentation. Rev. Aquac. 5, 33–57.

Stien, L.H., Dempster, T., Bui, S., Glaropoulos, A., Fosseidengen, J.E., Wright, D.W., Oppedal, F., 2016. 'Snorkel' sea lice barrier technology reduces sea lice loads on harvest-sized Atlantic salmon with minimal welfare impacts. Aquaculture 458, 29–37.

Stien, L.H., Lind, M.B., Oppedal, F., Wright, D.W., Seternes, T., 2018. Skirts on salmon production cages reduced salmon lice infestations without affecting fish welfare. Aquaculture 490, 281–287.

Thorstad, E.B., Todd, C.D., Uglem, I., Bjørn, P.A., Gargan, P.G., Vollset, K.W., Halttunen, E., Kålås, S., Berg, M., Finstad, B., 2015. Effects of salmon lice *Lepeophtheirus salmonis* on wild sea trout *Salmo trutta*—a literature review. Aquac. Environ. Interact. 7, 91–113.

Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O.T., Nilsen, F., Horsberg, T.E., Jackson, D., 2013. Salmon lice – impact on wild salmonids and salmon aquaculture. J. Fish Dis. 36, 171–194.

Treasurer, J.W., 2002. A review of potential pathogens of sea lice and the application of cleaner fish in biological control. Pest Manag. Sci. 58, 546–558.

Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. R. Stat. Soc. Ser. B 73, 3–36.

Wright, D.W., Stien, L.H., Dempster, T., Vagseth, T., Nola, V., Fosseidengen, J.E., Oppedal, F., 2017. 'Snorkel' lice barrier technology reduced two co- occurring parasites, the salmon louse (*Lepeophtheirus salmonis*) and the amoebic gill disease causing agent (*Neoparamoeba perurans*), in commercial salmon sea-cages. Prev. Vet. Med. 140, 97–105.

V

Paper IV

Geitung, L., Wright, D. W., Oppedal, F., Stien, L. H., Vågseth, T., Madaro, A. (2020)

Cleaner fish growth, welfare and survival in Atlantic salmon sea cages during an autumn-winter production.

Aquaculture 528, 735623.

https://doi.org/10.1016/j.aquaculture.2020.735623

Aquaculture 528 (2020) 735623

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Cleaner fish growth, welfare and survival in Atlantic salmon sea cages during an autumn-winter production

Lena Geitung[a,b,*], Daniel William Wright[c,1], Frode Oppedal[c], Lars Helge Stien[c], Tone Vågseth[c], Angelico Madaro[c]

[a] Department of Biology, University of Bergen, 5005 Bergen, Norway
[b] Bremnes Seashore AS, Øklandsvegen 90, 5340 Bremnes, Norway
[c] Institute of Marine Research, Matre Research station, 5984 Matredal, Norway

ARTICLE INFO

Keywords:
Ballan wrasse
Biological control
Lumpfish
Mariculture
Salmon aquaculture

ABSTRACT

Cleaner fish used as a biological control agent against salmon lice is rapidly increasing in Atlantic salmon aquaculture. However, concerns have been raised about the welfare and mortality of cleaner fish in salmon cage systems, which could in turn affect their performance in controlling salmon lice. In a 4-month autumn-winter study, we monitored growth, welfare, mortality and daytime depth distribution of the most commonly used cleaner fish, farmed ballan wrasse and lumpfish, in six salmon production sea cages where thermo- and haloclines were present. Ballan wrasse did not grow (SGR: small: -0.01% day^{-1}, large: -0.06% day^{-1}), while lumpfish significantly doubled in size (SGR: 0.87% day^{-1}) during the study. High losses (registered mortality + unregistered loss) were observed in both species (57 and 27% of ballan wrasse and lumpfish, respectively). The welfare status of remaining individuals generally improved over the study period, regardless of species. Brief daytime camera observations at hides found ballan wrasse were typically deeper at warmer (median 12.4 °C) more saline (median 31.7 ppt) depths, where salmon were expected to reside during day periods, compared to lumpfish generally occupying colder (median 7.3 °C), brackish (median 18.9 ppt) water in surface layers. Considerable mortalities, minimal feeding (inferred from ceased growth) by ballan wrasse and a possible mismatch in lumpfish and salmon depths (inferred from limited daytime camera observations) suggest that cleaner fish may have low long-term effectiveness against salmon lice in stratified salmon sea cages over autumn-winter. Similar studies across seasons, locations and cage types (e.g. depth-based cage technologies) are vital to understand the extent of these issues in salmon aquaculture more broadly.

1. Introduction

The primary obstacle to production growth for the world's largest finfish mariculture industry, sea-cage Atlantic salmon *Salmo salar* farming (FAO, 2019), is the ectoparasitic salmon louse *Lepeophtheirus salmonis*. Due to potential negative impacts on wild salmonid populations from farm-produced lice (Krkošek et al., 2011; Kristoffersen et al., 2018), the Norwegian government have enforced production volume limits and treatments when infestations exceed 0.5 adult females per fish (0.2 adult females during the out-migration of wild salmon, weeks 16–21) (Lovdata, 2012, 2017). This led the Norwegian industry to spend > 5 billion NOK (or €425 million at present currency exchange rates) in 2015 in attempts to control the parasite, with costs likely to have continued to rise since then (Brooker et al., 2018a). Several delousing methods are currently in use, such as chemical, thermal and mechanical treatments. However, these methods can result in poor welfare and increased mortalities (Overton et al., 2018a, 2018b), in addition to salmon lice developing a resistance to many of the chemical therapeutants (Grøntvedt et al., 2013; Aaen et al., 2015; Helgesen et al., 2015). Lice-eating cleaner fish on the other hand, have become widely accepted as a biological control of salmon lice due to a lack of negative effects on salmon welfare compared to chemical or physical delousing methods (Deady et al., 1995; Treasurer et al., 2002; Skiftesvik et al., 2013; Imsland et al., 2014a).

Wild-caught wrasse species from the Labridae family, primarily ballan (*Labrus bergylta*), corkwing (*Symphodus melops*) and goldsinny wrasse (*Ctenolabrus rupestris*) (Deady et al., 1995; Treasurer et al., 2002), were first used as cleaner fish in salmon aquaculture in the late

* Corresponding author at: Department of Biology, University of Bergen, 5005 Bergen, Norway.
E-mail address: lena.geitung@uib.no (L. Geitung).
[1] Present address: Department of Primary Industries, Narrandera Fisheries Centre, PO Box 182, Narrandera, New South Wales, Australia.

https://doi.org/10.1016/j.aquaculture.2020.735623
Received 20 December 2019; Received in revised form 26 May 2020; Accepted 13 June 2020
Available online 24 June 2020

1980s (Bjordal, 1988, 1991). In recent years, their use in Norway has dramatically increased from 1.7 million cleaner fish in 2008 to over 54 million in 2017 (Norwegian Directorate of Fisheries, 2019). To meet the increasing demand, cleaner fish supply has shifted from being exclusively of wild-caught origin to being increasingly hatchery-produced, which has also improved stock quality and sustainability. Currently there are two species farmed; ballan wrasse (*L. bergylta*) and lumpfish (*Cyclopterus lumpus*) (Brooker et al., 2018b), and in 2017, around 56% (29.7 million lumpfish and 1.0 million ballan wrasse) of all stocked cleaner fish in Norway were hatchery-produced (Norwegian Directorate of Fisheries, 2019).

Although typically cohabiting in salmon sea cages, ballan wrasse and lumpfish differ widely in their biology and life history. Ballan wrasse are a temperate species, inhabiting shallow coastal rocky reefs and kelp beds < 30 m (Dipper et al., 1977; Figueiredo et al., 2005; Villegas-Ríos et al., 2013) in the north-east Atlantic, from Morocco to southern Norway (Quignard and Pras, 1986; Porteiro et al., 1996). Contrastingly, lumpfish is a cold-water semi-pelagic species (Blacker, 1983; Daborn and Gregory, 1983; Ern et al., 2016) dwelling in coastal and offshore habitats, often in association with floating seaweed (Davenport, 1985; Ingólfsson and Kristjánsson, 2002; Kennedy et al., 2016) across the North Atlantic (Stein, 1986). Lumpfish lack a swim bladder but possess an abdominal suction disc formed by a modified pelvic fin (Budney and Hall, 2010) which allows it to adhere onto different surfaces (Imsland et al., 2015). Both species are diurnal (Morel et al., 2013; Villegas-Ríos et al., 2013; Imsland et al., 2015) and neither species are fast swimmers such as Atlantic salmon (Hvas et al., 2018; Yuen et al., 2019). Shelters and hides are therefore offered in sea cages for nocturnal resting, in addition to provide protection from strong currents, rough weather and winter conditions. Due to higher activity with increasing temperature (Yuen et al., 2019) and becoming sedentary at temperatures below 10 °C (Morel et al., 2013), ballan wrasse are often preferred stocked during summer months, while active feeding at low temperatures (Nytrø et al., 2014) as well as a preference and high physiological tolerance to cooler temperatures (Hvas et al., 2018; Mortensen et al., 2020) has led salmon farmers preferring to stock lumpfish during winter months (Brooker et al., 2018b; Eliasen et al., 2018; Imsland et al., 2018d) and in northern Norway (Barrett et al., 2020). While stocking timing may vary, all cleaner fish species can occupy salmon sea cage environments throughout annual cycles, despite possessing different physiological limits and preferences to environmental variables.

Environmental preferences may override typical depth distributions of cleaner fish species when strong vertical gradients in temperature and salinity are present (Oppedal et al., 2011a). Lumpfish have a low thermal range and die from extended periods at 18 °C (Hvas et al., 2018), which likely results in a preference for depths of cooler temperature. Whereas ballan wrasse, which display low activity and become sedentary at temperatures below 10 °C (Morel et al., 2013; Yuen et al., 2019), are expected to prefer depths of warmer temperature. Both species are marine-adapted fish but can tolerate brackish water (Sayer and Reader, 1996; Skiftesvik et al., 2018; Treasurer and Turnbull, 2019). However, ballan wrasse and lumpfish may both prefer depths of high rather than low salinity (Sayer et al., 1993; Powell et al., 2018). It is unknown how each cleaner fish species responds to competing environmental preferences (e.g. temperature and salinity), but this is key to understanding their depth distribution and interactions with salmon in sea cages.

The commercial use of cleaner fish comes with a responsibility to secure their welfare and survival according to animal welfare legislation (Lovdata, 2008). Reports of poor cleaner fish survival in commercial salmon sea cages is cause for concern (Nilsen et al., 2014; Skiftesvik et al., 2014; Mo and Poppe, 2018; Stien et al., 2020). A short 6-week trial involving 5 m deep sea cages recorded high ballan wrasse losses (14.8%) compared to salmon (0.03%) and noted that many losses were not confirmed mortalities at dead fish collection (Skiftesvik et al., 2013). Longer studies in larger salmon sea cages are needed that carefully monitor a) registered mortalities at regular dead fish collections, and b) additional unregistered losses at final whole-of-cage counts of cleaner fish (Overton et al., 2020). Conducting such investigations in a range of environments and sea cage types (Nilsen et al., 2017; Stien et al., 2018; Geitung et al., 2019; Glaropoulos et al., 2019) is required to fully grasp the extent of the issue and potential solutions which would improve the effectiveness of this biological control.

Here, over autumn-winter at a location with thermoclines and haloclines present, we monitored growth, welfare, registered mortality and unregistered loss of the two most common cleaner fish species, farmed ballan wrasse and lumpfish, in salmon production sea-cages over four months. We also explored the effects of Floy and Passive Integrated Transponder (PIT) tags, which are increasingly used in cleaner fish research (Imsland et al., 2014b, 2016a, 2018c), on growth, welfare and mortality by comparing tagged to untagged individuals. We hypothesised ballan wrasse to have more welfare issues and mortalities than lumpfish during the winter period when they feed less and become more inactive. Brief daytime camera observations at hides also monitored daytime depth distribution of cleaner fish throughout the study, with the expectation that lumpfish and ballan wrasse would prefer cooler and warmer depths, respectively, but that both marine-adapted species would avoid low salinity depths.

2. Material and methods

2.1. Experimental setup

The study was conducted in six steel framed sea cages (12 × 12 m square, 12 m deep) at the Institute of Marine Research sea-cage farm facility (Solheim, Masfjorden commune; 60.9° N, 5.46° E) from 17 October 2018 to 20 February 2019 (126 days). The farm is situated in the end of a long fjord system and is rarely affected by strong currents or rough seas. Atlantic salmon (*Salmo salar*, Aquagen strain) were stocked two months before the beginning of the present trial, with 6000–6280 salmon per cage at a mean weight of 240–320 g.

Ballan wrasse were supplied by Mowi ASA in two different year classes termed "small" (n = 900, initial weight ± SD = 33.5 ± 9.0 g) and "large" (n = 180, initial weight ± SD = 96.0 ± 18.4 g). "Small" ballan wrasse were transported directly from the Mowi Øygarden site while "large" ballan wrasse were transported from Institute of Marine Research, Matre (previously delivered from Mowi Øygarden and continued reared at IMR facilities). They were both transported in vehicles with holding tanks ("large": 43.3 kg/m^3 and "small": 60.2 kg/m^3) and were oxygenated and monitored for the duration of these periods. Lumpfish were obtained from Institute of Marine Research (n = 900, initial weight ± SD = 53.0 ± 14.1 g) and vaccinated with AMARINE micro 3–1 (Pharmaq AS, Oslo, Norway). They were transported by boat in holding tanks (15.9 ± 0.2 kg/m^3) with oxygen distributed and monitored throughout the transport. Sedation was not added to the holding tanks during transport. The cleaner fish were regularly monitored and screened for diseases (ex. Amoebic Gill Disease) following normal guidelines in the rearing phase. Ballan wrasse were transported and deployed at the farm on 17 October 2018, while lumpfish were transported and deployed six weeks later on 28 November 2018. Cleaner fish were divided equally between sea cages, with 150 lumpfish, 150 small ballan wrasse and 30 large ballan wrasse in each cage. The cleaner fish were slowly introduced to the sea cages at the surface in close proximity to the hides.

One artificial kelp station (Krantare™, NorseAqua, Norway), with 6 ropes of 10 m depth each, was placed across a corner of each sea cage as substrate and shelter for the cleaner fish. This amounts to 5.5 cleaner fish per metre of artificial kelp and is within the recommended amount of 15–50 cleaner fish per metre of artificial kelp (Lusedata.no, 2017; Rabadan, 2018). Ballan wrasse were offered feeding blocks (Symbio

Blocks, BioMar AS, Norway) at five depths (1, 3, 5, 7 and 9 m) near the shelters, while lumpfish were offered pellet feed (2 mm pellets, Atlantic Gold, Pacific Trading Aqua Ltd., Ireland) dispersed at the surface near the shelters from an automatic feeder (Rognkjeksautomat, NorseAqua, Norway) for four hours every day. All cages were checked for registered mortalities at daily dead fish collections and the number and species were recorded. Dead fish collection was not performed the day after stocking events due to anecdotal evidence from farmers that live cleaner fish would reside at the cage bottom at this time and were likely to be pumped out. Ballan wrasse were not distinguishable between "small" and "large" sizes when recording registered mortalities at dead fish collection. In addition, due to a PIT tag reader malfunction and uncertainty of tag presence when cleaner fish were decomposing, tag type was also not included in the registered mortality data. Daily salinity and temperature depth profiles (0–17 m) were recorded by an automatic profiling CTD (Conductivity, Temperature and Depth) buoy (APB5, SAIV AS, Norway) at a reference location on the outer end of the farm facility.

One day after lumpfish transfer, a hole (30 × 16 cm) at 10–12 m depth was discovered and repaired in the net wall of one of the sea cages. This was suspected to cause the mass escape of ballan wrasse from this cage as only 5 (3.3%) ballan wrasse were left at the end of the trial, leading to abnormally high unregistered losses. Therefore, this cage was removed and only 5 cages were used in analyses involving ballan wrasse. However, this did not appear to affect registered mortalities and unregistered losses of lumpfish, with similar mortalities and losses between the cage with a hole and the other cages, and so all six cages were used in lumpfish analyses.

At the termination of the study, the net bottom was lifted, and cleaner fish were sorted from salmon and netted out for whole-of-cage counts to determine unregistered losses in each sea cage. Artificial kelps were lifted out of the water and closely inspected to retrieve fish that were still attached to (lumpfish) or within it. Finally, cleaner fish of both species were collected and counted after an overdose of anaesthetic (100 mg L^{-1}, Finquel®vet., ScanAqua AS, Årnes, Norway).

2.2. Tagging

Prior to stocking, two-thirds of the cleaner fish (600 lumpfish, 600 small ballan wrasse and 120 large ballan wrasse) were anesthetised (60 mg L^{-1}, Finquel®vet., ScanAqua AS, Norway) and half were tagged intraperitoneally with a Passive Integrated Transponder (PIT) (2 × 12 mm) while the other half were tagged with a Floy tag (1.2 × 55 mm, anchor: 7 mm) in dorsal musculature below the dorsal fin. After tagging, fish were returned to a seawater bucket and monitored for recovery until upright swimming resumed, at which point they were transferred to the sea cages. The remaining cleaner fish (300 lumpfish, 300 small ballan wrasse and 60 large ballan wrasse) were transferred directly to the sea cages.

2.3. Growth and welfare

All tagged cleaner fish at the start of the trial and all remaining cleaner fish at the termination of the trial were individually weighed and measured for length. From this, Fulton's condition factor ($K = 100 \times W\,L^{-3}$, *where W* is the weight of the fish and *L* corresponds to the total length) was calculated to estimate cleaner fish condition. The condition factor of lumpfish is higher than most other teleost, but the species follow an isometric growth pattern so the method of using condition factor is valid (Coull et al., 1989), and has been used as an indicator in several papers describing lumpfish growth (ex. Imsland et al., 2014a, 2018a, 2018b, 2019b). Specific growth rate (SGR) was calculated according to the formula of Houde and Schekter (1981) $SGR = (e^g - 1) \times 100$, where $g = \ln(W_2) - \ln(W_1) / (t_2 - t_1)$ and W_2 and W_1 are weights on days t_2 and t_1, respectively. In addition, cleaner fish were scored according to 7 welfare indicators (fins, skin, eyes, jaw and sucker disc deformity, snout, opercula) based on Operational Welfare Indicators (OWI) from RENSVEL (Noble et al., 2019a, 2019b), Gentry et al. (2020) and Katharine Gentry, pers. comm. (Table 1). At the start of the trial, Floy tagged cleaner fish were scored, while at the termination of the trial a subsample of the remaining tagged and untagged cleaner fish were scored (Table 2).

Table 1
Scores and definitions of welfare indicators.

	Score	Definition
Fins	1	No erosion, splitting or rays exposed
	2	Any minor damage on fins; up to 60% deep fin split, or 1–2 splits, or up to 50% erosion
	3	Split of > 60% depth, or 3 + splits, or > 50% erosion
Skin	1	No damage
	2	Some skin damage (< 0.5 cm^2) or previous wounds (evidence of scars)
	3	Wound present (> 0.5 cm^2)
Eyes	1	No damage
	2	Some minor damage to one or both eyes, but still some vision in both eyes
	3	Blind in one or both eyes, or at least > 50% blind (moderate cataracts) in both eyes
Deformities (Jaw, sucker disc)	0	No damage
	1	Damage or wound present
Snout	0	No damage
	1	Damage or wound present
Opercula	0	No damage
	1	Damage or wound present

2.4. Daytime depth distribution

Daytime depth distribution of cleaner fish was monitored by brief underwater camera observations at hides (Imenco Gemini Aquaculture camera, Imenco AS, Norway) two to four times per week for 12 weeks (42 times during the experimental period). The cameras were situated outside the corner hides, with the ability to be moved up and down by a winch and rotate 360° in the horizontal plane. Depth distribution at hides was classified by performing 1 min observations at each metre from the surface (0 m) down to 16 m, recording the numbers of both cleaner fish species present at each depth. The observations were performed between 10 and 12 am; the period cleaner fish are most active (Blanco Gonzalez and de Boer, 2017; Brooker et al., 2018b; Powell et al., 2018) and believed to be interacting with salmon to remove lice. Although camera observations only gave a snapshot of fish depth behaviour (e.g. compared to continuous monitoring by implanted electronic tags), they were chosen here to a) provide data on large sample sizes, b) minimally disturb fish behaviour, and c) monitor during daylight when interactions between cleaner fish and salmon are expected. Ballan wrasse size was not recorded in the depth observations due to difficulties in determining size from camera observations and therefore ballan wrasse distribution data included both small and large ballan wrasse.

2.5. Data analysis

Data analyses were performed using R software v.3.1.0 (package stats, R Core Team (2019)). Data are presented as mean ± standard error, unless otherwise stated. Data were checked for variance and normality and the significance level was set at $P < .05$. To compare specific growth rate values between tag types (i.e. untagged, Floy tagged, PIT tagged) for each cleaner fish species, a one-way ANOVA was used (function aov). A two-way ANOVA (function aov) was used to compare the effects of sample time and tag types on weight and condition factor. Lumpfish weight data were ln-transformed in order to satisfy the assumptions of parametric analysis. To test for effects of

Table 2
Proportions of welfare scores for small ballan wrasse, large ballan wrasse and lumpfish (fins, skin, eye score ≥ 2; deformities (jaw, sucker disc), snout, opercula score ≥ 1). For each fish type, the start values are from a single sample before stocking, while the end values are mean (± SE) values from samples of individual cages. Higher scores indicate deviance from normal condition (fins, skin, eye score = 1–3; deformities (jaw, sucker disc), snout, opercula score = 0–1). $^{**}p < .01$, $^{***}p < .001$.

Parameter	Small ballan wrasse		Large ballan wrasse		Lumpfish	
	Start (n = 67)	End (n = 87)	Start (n = 50)	End (n = 76)	Start (n = 91)	End (n = 120)
Dorsal fin (scores ≥2)	44.8%	22.1 ± 3.6% **	64.0%	33.2 ± 6.2% **	7.7%	7.1 ± 1.7%
Anal fin (scores ≥2)	N/A	N/A	N/A	N/A	3.3%	4.5 ± 2.9%
Pectoral fin left (scores ≥2)	98.5%	94.5 ± 3.4%	100%	96.1 ± 1.6%	6.6%	5.3 ± 1.9%
Pectoral fin right (scores ≥2)	98.5%	97.8 ± 1.4%	100%	96.1 ± 1.6%	13.2%	5.8 ± 2.1%
Caudal fin (scores ≥2)	71.6%	63.4 ± 6.0%	86.0%	60.7 ± 5.9% **	82.4%	74.3 ± 5.0%
Skin condition (scores ≥2)	14.9%	21.0 ± 3.4%	26.0%	27.5 ± 7.9%	3.3%	4.0 ± 2.5%
Eye condition (scores ≥2)	0.0%	3.9 ± 2.5%	0%	2.9 ± 1.8%	0%	16.4 ± 4.0% ***
Jaw deformity (scores ≥1)	10.4%	7.6 ± 4.3%	22.0%	12.6 ± 4.8%	0%	0.0 ± 0.0%
Sucker disc deformity (scores ≥1)	N/A	N/A	N/A	N/A	0%	0.0 ± 0.0%
Snout damage (scores ≥1)	0.0%	0.0 ± 0.0%	0%	0.0 ± 0.0%	0%	0.0 ± 0.0%
Opercula damage (scores ≥1)	0.0%	1.2 ± 1.2%	0%	0.0 ± 0.0%	0%	0.0 ± 0.0%

mortality, percentage values were arcsine transformed before input to Welsh two-sample *t*-test (function t.test) or one-way ANOVA (function aov) as recommended by Crawley (2007). Fishers Exact Test were used to compare welfare scores between first and last sample points and between different tag types (function fisher.test). Following Nakagawa (2004) we did not use Bonferroni or similar adjustments to correct for multiple comparisons of welfare indicators to be able to observe significant differences, which should be taken into account when observing the results.

3. Results

3.1. Environment

During the experimental period, temperature followed normal seasonal variations (Oppedal et al., 2011a) (Fig. 1a). Throughout the trial there was a distinct thermocline, with warm deep waters and cooler surface waters. The highest temperatures were observed at the beginning of the trial of up to 16 °C in deeper waters, and temperatures cooled to 6–8 °C in deeper waters and 2–4 °C in surface layers at the end of the study. Salinity varied through the trial with long periods of brackish water (< 16 ppt) between 0 and 5 m (Fig. 1b).

3.2. Growth

Ballan wrasse condition decreased, and weight did not change during the trial (Fig. 2) for both sizes and tag types (i.e. untagged, Floy tagged, PIT tagged). In contrast, lumpfish increased in both weight and condition factor, with their mean weight doubling over the trial period, regardless of tagging (Fig. 2). There was no effect from either tag type

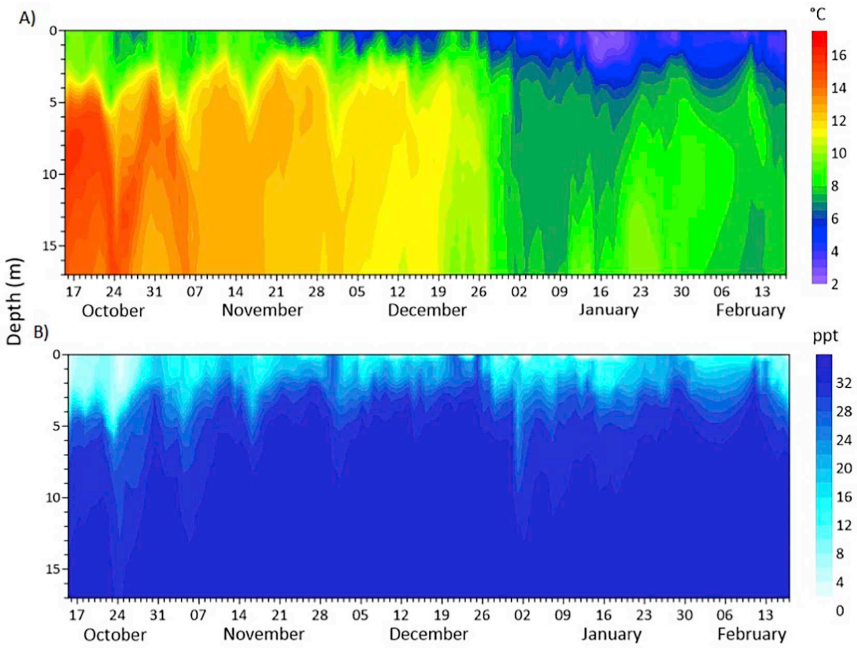


Fig. 1. Daily depth profiles between 0 and 17 m of a) temperature and b) salinity from a reference location at the outer end of the farm at Solheim, Norway.

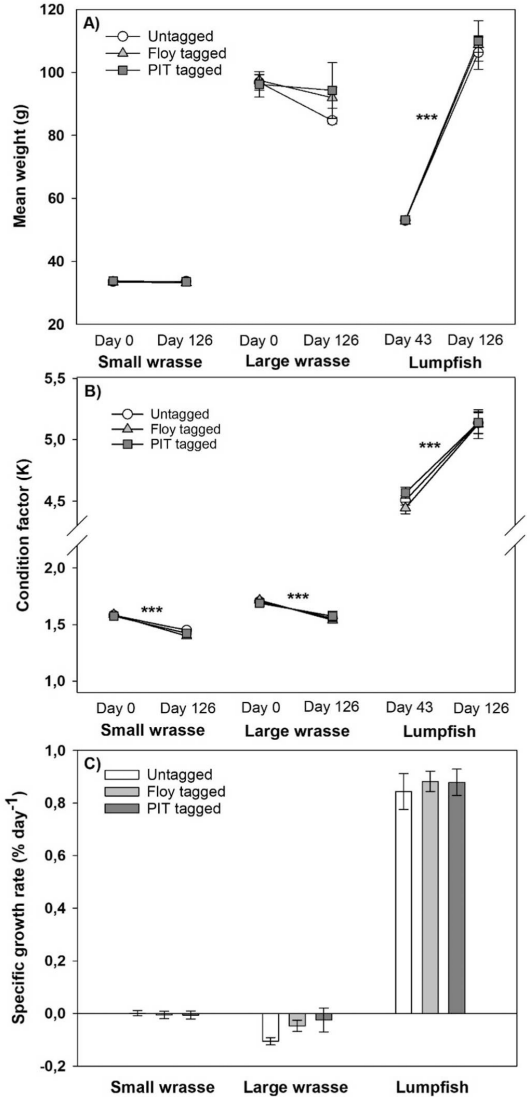


Fig. 2. Overview of a) mean weight (g); b) condition factor (K) and c) specific growth rate (% day^{-1}) from the initial and final sampling points for untagged, floy tagged and PIT tagged lumpfish, small and large ballan wrasse. ***$p < .001$.

compared to untagged individuals in terms of growth or condition factor for both species ($F \leq 2.758$, $p > .08$).

3.3. Mortality and losses

Registered accumulated mortalities of ballan wrasse and lumpfish were similar over the study period, 7.2 ± 1.3% and 9.9 ± 2.3%, respectively, while registered salmon mortality was considerably lower at 0.3 ± 0.1% during the same time interval (Fig. 3). When accounting for deployment interval disparities, there was no difference in registered mortalities between ballan wrasse or lumpfish (0.06 ± 0.01% day^{-1} vs. 0.12 ± 0.03% day^{-1}, $t = -2.1466$, $p = .068$). Ballan wrasse stocking was immediately followed by a rise in mortalities, while lumpfish mortalities were largely absent until a spike in mid-January or week 6 after deployment. Based on the remaining cleaner fish at whole-of-cage counts at the end of the trial, there were substantial additional unregistered losses leading to a cumulative total loss of ballan wrasse and lumpfish of 56.8 ± 1.7% and 27.3 ± 1.7%, respectively (Fig. 3). After correcting for different deployment intervals, ballan wrasse had higher total losses than lumpfish (0.45 ± 0.01% day^{-1} vs. 0.33 ± 0.02% day^{-1}, $t = 4.8113$, $p < .001$). Tagged (floy and PIT respectively) ballan wrasse had similar total cumulative losses to untagged individuals (60.2 ± 3.5% and 58.3 ± 1.8% vs. 52.0 ± 2.7%, $F = 2.402$, $p = .133$), as did tagged lumpfish (25.4 ± 3.0% and 32.0 ± 3.1% vs. 24.3 ± 1.9%, $F = 2.173$, $p = .148$).

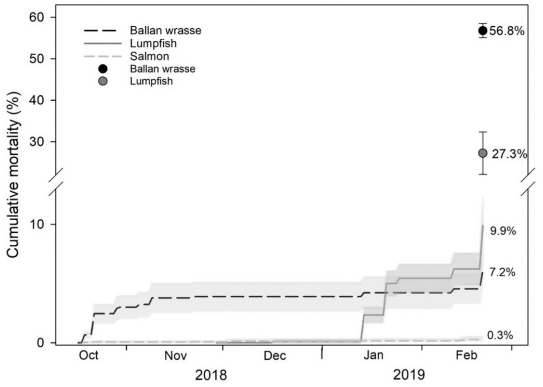


Fig. 3. Overview of mean (± SE) registered mortality (lines) for ballan wrasse, lumpfish and salmon as well as mean (± SE) total losses (dots) for ballan wrasse and lumpfish. Registered mortality is taken from daily mortality registrations while total losses was calculated at the end of the experiment based on how many individuals were left in the cages.

3.4. Welfare

Welfare scores generally improved during the course of the trial for both cleaner fish species (Table 2). Large ballan wrasse had better dorsal and caudal fins at the end of the study (Table 2), with a higher proportion of untagged individuals showing better dorsal fin scores than tagged individuals (17.6 ± 5.0% vs. 41.6 ± 9.4%, $p = .001$, Fishers Exact Test, FET), and a lower proportion of Floy tagged individuals showing an improvement in caudal fin damage than untagged individuals (80.3 ± 5.5% vs. 42.9 ± 12.2, $p = .022$, FET) (Supplementary Table 1). Fin damage was the most common issue for both ballan wrasse and lumpfish, with caudal fin damage most prevalent for lumpfish, while ballan wrasse experienced a high prevalence of both caudal and pelvic fin damage (Table 2). For lumpfish, eye condition decreased during the trial (Table 2), and poor eye condition was seen in a higher proportion of tagged compared to untagged individuals (19.9 ± 5.3% vs. 5.2 ± 3.5%, $p = .0345$, FET) (Supplementary Table 1). These patterns were not evident for ballan wrasse (Table 2).

3.5. Daytime depth distribution

From brief daytime observations at hides, the two cleaner fish species appeared to exhibit different daytime depth distributions and environmental preferences (Fig. 4). Ballan wrasse were observed predominantly below the halocline and thermocline (pycnocline) present at 2–4 m depth (Fig. 4a) while lumpfish were mainly above the

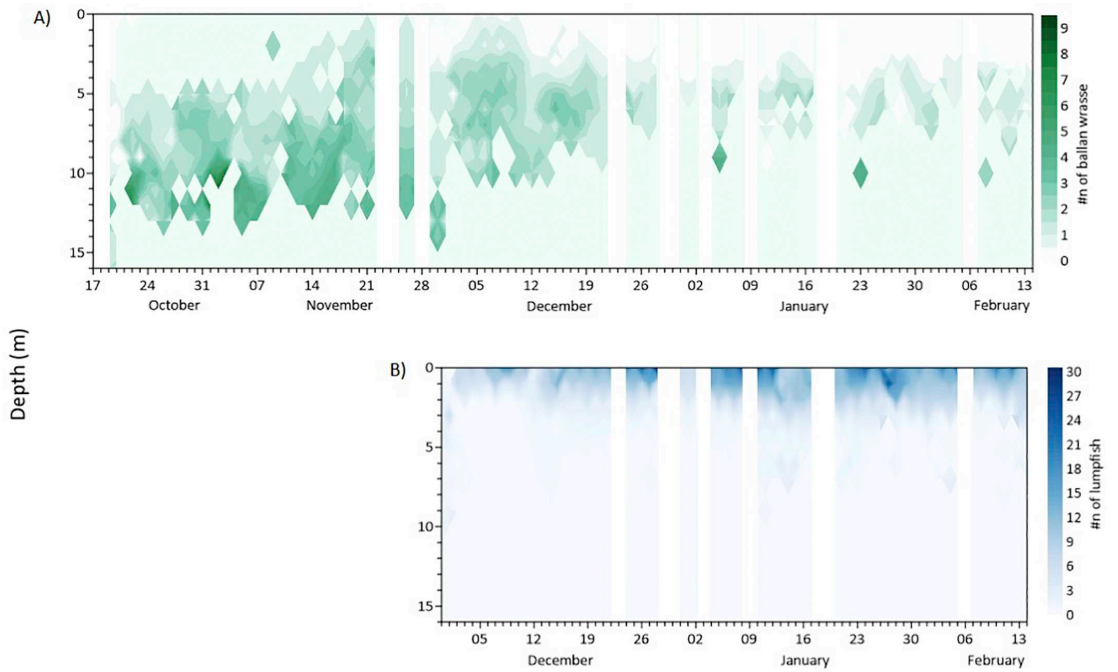


Fig. 4. Depth distribution of a) ballan wrasse and b) lumpfish from brief underwater camera observations at hides every 2–3 days with 1 min observation at every metre from 0 to 16 m depth. Lumpfish was added to the cages four weeks after ballan wrasse.

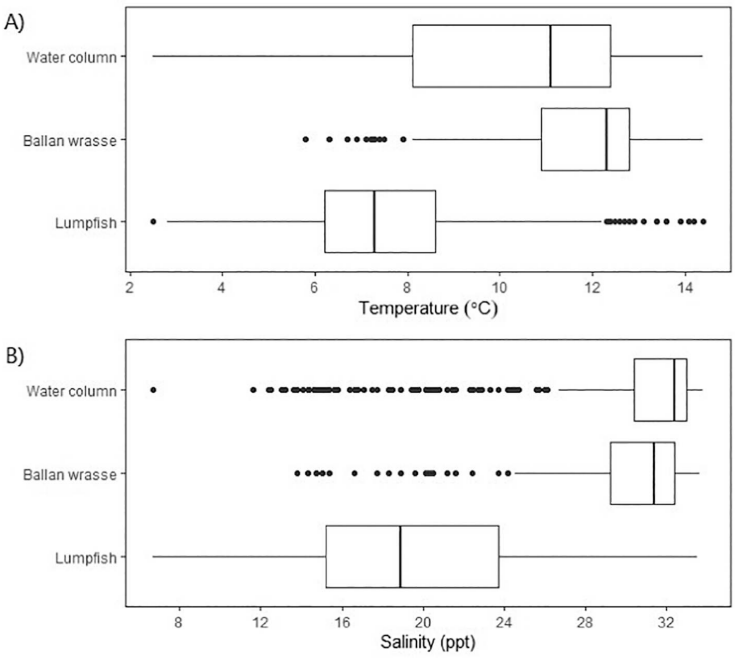


Fig. 5. Boxplots showing range of a) temperature and b) salinity values measured throughout the water column inside the cages as well as temperature and salinity conditions experienced by both ballan wrasse and lumpfish based on their depth from each observation day from the deployment of lumpfish to the end of study (study day 44–126).

pycnocline (Fig. 4b). Compared to the available temperatures (median 11.1 °C) in the water column, ballan wrasse tended to select slightly warmer depths (median 12.4 °C) and lumpfish selected cooler depths (median 7.3 °C) (Fig. 5a). While compared to the available salinities (median 32.4 ppt), ballan wrasse selected depths of higher salinity (median 31.7 ppt) and lumpfish selected depths of considerably lower salinity (median 18.9 ppt) (Fig. 5b). Both cleaner fish species were observed in lower cage sections at the first observation after stocking, before adjusting to shallower depths (Fig. 5).

4. Discussion

Farmed cleaner fish (ballan wrasse and lumpfish) are becoming the dominant species used as biological controls against salmon lice in the Atlantic salmon farming industry (Brooker et al., 2018b). In this autumn-winter study in salmon production cages, we show that temperate ballan wrasse (Yuen et al., 2019) failed to grow, while cold-water specialist lumpfish (Ern et al., 2016; Hvas et al., 2018) doubled in weight, suggesting that ballan wrasse may under-perform as a biological control agent compared to lumpfish over this period. In addition, total cumulative losses were high in both cleaner fish species (27–57%) within our 4-month sea cage trial, suggesting that losses are a key factor in explaining the performance of cleaner fish as biological controls. Finally, brief camera observations suggested that these cleaner fish species vary in their daytime depth distribution and preference for environmental variables in sea cages, which may lead to species-specific differences in salmon-cleaner fish interactions.

Over the course of the study ballan wrasse showed a negative growth rate and had reduced condition, while in lumpfish, weight doubled, and their condition improved. This supports the notion that wrasse species enter a dormant phase and discontinue feeding in cooler winter periods (Sayer and Davenport, 1996; Sayer and Reader, 1996; Morel et al., 2013; Yuen et al., 2019). The increased weight by a growth factor of 0.87% day^{-1} and improved condition of lumpfish, on the other hand, indicated they were actively feeding. The observed growth rate was similar to a previous study in commercial scale salmon sea cages spanning autumn-winter months (0.68% day^{-1}) (Imsland et al., 2018d), but faster growth rates have been recorded in tank trials over a range of temperatures (Nytrø et al., 2014). Our findings suggest that lumpfish but not ballan wrasse will actively feed during autumn-winter periods in salmon sea cages. However, as lumpfish prefer colder temperatures (Mortensen et al., 2020) a repeat of the study during spring-summer could be interesting to observe if ballan wrasse out-perform lumpfish in warmer conditions.

Total losses were high regardless of cleaner fish species, reaching 27% (0.33% day^{-1}) in 12 weeks for lumpfish and 57% (0.45% day^{-1}) in 18 weeks for ballan wrasse. This draws attention to current concerns about the utilization of cleaner fish in salmon aquaculture (Nilsen et al., 2014; Mo and Poppe, 2018). According to industry reports, cleaner fish mortalities in commercial sea cages range from 18 to 48%, with individual farms observing up to 100% mortality or loss (Nilsen et al., 2014). A recent study reported > 65% mortality of ~193,000 cleaner fish in 12 commercial salmon sea cages during most of a production cycle (Bui et al., 2018) and a recent industry survey reported a registered cleaner fish mortality of 42% (Stien et al., 2020). Such high registered mortalities and unregistered losses over short periods as described here, have rarely been observed in more controlled studies using small-scale sea cages and highlight the need for larger scale experiments to gather industry relevant data on both mortalities and losses. Heavy losses of cleaner fish in this study and in other commercial-scale sea cage studies suggest that this could be a major determinant of their long-term effectiveness in controlling salmon lice in salmon sea cages.

Primary causes of cleaner fish mortality or loss are purportedly escape, disease, handling and predation (Nilsen et al., 2014; Skiftesvik et al., 2014). Most losses in this study were unregistered, especially for ballan wrasse, making it difficult to determine an exact cause of death. However, registered mortalities of ballan wrasse spiked in the first two weeks after stocking, suggesting that initial acclimation, handling and dead fish pumping played a role. Acclimatization of farmed ballan wrasse to sea cage conditions before stocking have been suggested to make them more efficient biological control agents (Brooker et al., 2020), however further studies is required to determine if this would improve cleaner fish welfare and survival. Pumping of live fish from the cage bottom (16 m depth here and 20–40 m depth in commercial sea cages) would be most harmful to physoclistous ballan wrasse, as their closed swim bladder can over-inflate causing barotrauma from rapid depth changes towards the surface (Helfman et al., 2009). In contrast, lumpfish lack a swim bladder (Davenport and Kjørsvik, 1986). Lumpfish registered mortalities were low after stocking, but increased in mid-January when temperatures in surface waters occupied by this species (0–4 m) decreased to < 4 °C for several days. Imsland et al. (2018d) also reported high registered mortalities of lumpfish at temperatures < 4 °C, which may represent the lower thermal niche of the species. However, no lumpfish mortalities have been registered in smaller scale tank studies at temperatures ≤4 °C (Nytrø et al., 2014; Hvas et al., 2018). Low temperatures may also have explained ballan wrasse mortality, although this species tended to reside in depths with warmer waters during the winter period. Loss of wrasse during winter has often been observed in commercial sea cages (Bjelland et al., 1996; Sayer and Reader, 1996; Treasurer et al., 2002). There were no reports of disease outbreaks during the study, however disease cannot be ruled out as a factor contributing to the large numbers of unregistered losses. Another reason could have been that dead ballan wrasse may get stuck and decompose on the net side and are therefore not taken up by the dead fish pumping system. In addition, ballan wrasse are often associated with net sides and corners (Tully et al., 1996; Leclercq et al., 2018), so predation of resting or dead cleaner fish from outside piscivorous predators (Dempster et al., 2009; Uglem et al., 2014; Stien et al., 2020) could also explain the unregistered losses. While lumpfish mortalities were similar between all cages, almost 100% ballan wrasse loss in one cage was attributed to mass escape through a hole (the cage was discounted from ballan wrasse mortality analysis). As ballan wrasse escaped so efficiently in this one cage, one may argue that the 50–60% loss in the other cages was most likely due to other causes, however, smaller less detectable holes could be another potential source of the high unregistered losses in other cages. We therefore suggest that handling, cold water, predation, escapees and possibly disease contribute to cleaner fish losses in salmon sea cages over autumn-winter.

Of the welfare indicators assessed, fin damage (degree of splitting and erosion) was the most common issue for both cleaner fish species, which is in accordance with other studies (Treasurer and Feledi, 2014; Gentry et al., 2020). However, damage here was not only acquired in sea cages, as fin splitting and erosion was prevalent before trial commencement. During the trial some welfare indicators (fin and jaw damage) for both cleaner fish species improved, either due to healing or mortalities of individuals experiencing poor welfare, thereby "improving" the welfare condition of remaining fish. The only indicator that deteriorated was lumpfish eye condition which reached a moderate level of cataract prevalence and severity. Only severe cataracts are expected to reduce feed intake (Savino et al., 1993), which were not observed over the 12-week study. However, cataract prevalence and severity has been shown to increase with time (Jansson et al., 2017; Imsland et al., 2018c, 2019a). Therefore, this may become problematic over extended periods and impact their ability to prey on lice and source feed for growth and survival.

Ballan wrasse and lumpfish displayed different daytime depth distributions based on brief camera observations at hides. During day periods ballan wrasse were rarely observed above the thermocline or halocline, seemingly preferring the highest temperatures and salinities available deeper in the cage. This coincides with vertical behaviours previously observed (Leclercq et al., 2018), higher activity and coping

at warmer temperatures up to 25 °C (Yuen et al., 2019), and avoidance of low salinity habitats (Sayer et al., 1993; Tully et al., 1996). In contrast, lumpfish stayed at the surface during all the daytime observations, seemingly preferring cold, brackish water. This could be explained by lumpfish being a cold water species (Ern et al., 2016) that fail to cope with temperatures > 15 °C (Hvas et al., 2018), and which tolerate periods in both fresh- and brackish water despite being marine-adapted (Skiftesvik et al., 2018; Treasurer and Turnbull, 2019). The surface daytime depth use by lumpfish in this study was at odds with the expected deeper daytime swimming depths of salmon, due to surface avoidance in daylight and a temperature preference of ~16 °C (Oppedal et al., 2011a). This suggests that stratified sea-cage conditions over autumn winter may result in lumpfish having limited salmon interactions in day periods, when they are thought to be most active (Brooker et al., 2018b; Powell et al., 2018).

While ballan wrasse and lumpfish stocked together were studied here, single species stocking or combined species stocking where more than two cleaner fish species are used can occur and could alter how fish behave. For instance, lumpfish is the only species stocked in Northern Norway and when using wild-caught wrasse several species are often stocked together (i.e. goldsinny, corkwing, cuckoo and ballan wrasse) (Barrett et al., 2020). Lumpfish have been shown to be aggressive towards each other in tank rearing phases (Noble et al., 2019a) and towards goldsinny wrasse in small (1.5 m^3) tanks (Imsland et al., 2016b). However, in larger cage-based studies no apparent intra- or interspecific aggression has been observed (Imsland et al., 2014b, 2016a; Skiftesvik et al., 2018) and the cleaner fish species displayed similar depth preferences regardless of which species they were stocked together with (Skiftesvik et al., 2018). Thus, the authors suspect that depth distributions may vary little between the stocking of one or more cleaner fish species, but further study is required to test this hypothesis.

Neither of the two tag types (Floy - 1.2 × 55 mm, anchor: 7 mm and PIT - 2 × 12 mm) used during this study had a major influence on growth or mortality of ballan wrasse or lumpfish. Several previous studies have used these tag types on lumpfish (Imsland et al., 2014b, 2016a, 2018c), but did not assess tagging effects compared to untagged fish. Using larger acoustic tags (6.8 × 20.0 mm), on 115 g ballan wrasse and 281 g lumpfish, Leclercq et al. (2018) observed high tag signal loss due to reasons that included mortality, and tagging effects compared to untagged fish was not assessed. While not necessarily the case in all instances, large tags and the tagging process can lead to potential negative effects, such as altered behaviour, decreased swimming performance, reduced feeding and growth, and increased mortality (Cooke et al., 2011; Thorstad et al., 2013; Jepsen et al., 2015; Wright et al., 2018), and it is therefore important to be aware of these effects when choosing to use tags. Our study suggests smaller Floy and PIT tags have minor effects on growth, welfare and mortality, but there is still the possibility that these tags could cause deviations from normal behaviour.

High losses of the most commonly stocked farmed ballan wrasse and lumpfish in salmon sea cages, observed here, could be a) severely reducing the effectiveness of this biological agent as a lice control method and b) markedly increasing the expense needed to replace cleaner fish stocks. The potential for substantial cleaner fish mortalities in the salmon industry also raises an ethical dilemma about the widespread use of cleaner fish (Hvas and Oppedal, 2019; Stien et al., 2019; Yuen et al., 2019). Farmed ballan wrasse appeared prone to escape from sea cages and if escape is a major source of unregistered mortalities or losses in salmon sea cages, hybridization with wild populations could be significantly weakening the genetic composition and local population structure (Faust et al., 2018). Autumn-winter conditions and associated low water temperatures halted growth and reduce condition in ballan wrasse, and so this species may be unlikely to substantially reduce lice during such periods. In contrast, cold water specialist lumpfish appear to feed and grow well over autumn-winter periods, but a stratified environment could cause them to occupy cooler surface waters during the day when salmon are predicted to swim in warmer, deeper waters (Oppedal et al., 2011a, 2011b, 2019). These environments may also drive a lack of interaction between salmon and lumpfish. It is hoped that this study expedites broader research into the status and optimised husbandry of cleaner fish in the full range of situations the animals are used in salmon farming, including different locations, seasons and sea cage types (e.g. lice barrier skirt or snorkel cages, submerged cages, enclosed cages) (Korsøen et al., 2012; Nilsen et al., 2017; Stien et al., 2018; Geitung et al., 2019; Glaropoulos et al., 2019).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We wish to thank the staff at the Matre Research station and Solheim for the excellent care of research animals and management of experimental facilities. We also thank Mowi and in particular Olav Breck and Espen Grøtan for providing us with ballan wrasse for the experiment, and Velimir Nola for transporting fish and helping with the experiment. The experiment was funded by IMR's Surveillance of Fish Welfare project (project number: 14930). The work was conducted in accordance with the laws and regulations controlling experiments and procedures on live animals in Norway, following the Norwegian Regulations on Animal Experimentation 1996 (Application 16943).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2020.735623.

References

Aaen, S.M., Helgesen, K.O., Bakke, M.J., Kaur, K., Horsberg, T.E., 2015. Drug resistance in sea lice: a threat to salmonid aquaculture. Trends Parasitol. 31, 72–81.

Barrett, L.T., Overton, K., Stien, L.H., Oppedal, F., Dempster, T., 2020. Effect of cleaner fish on sea lice in Norwegian salmon aquaculture: a national scale data analysis. Int. J. Parasitol. https://doi.org/10.1016/j.ijpara.2019.12.005.

Bjelland, R., Løkke, J., Simensen, L., Kvenseth, P., 1996. Successful survival of wrasse through winter in submersible netcages in a fjord in western Norway. In: Sayer, M.D.J., Treasurer, J.W., Costello, M.J. (Eds.), Wrasse: Biology and Use in Aquaculture. Fishing News Books, Oxford, UK, pp. 265–271.

Bjordal, Å., 1988. Cleaning symbiosis between wrasses (Labridae) and lice infested salmon (*Salmo salar*) in mariculture. Int. Con. Explor. Sea, C.M. 1988, F:17 1–8.

Bjordal, Å., 1991. Wrasse as cleaner-fish for farmed salmon. Prog. Underwater Sci. 16, 17–28.

Blacker, R.W., 1983. Pelagic records of the lumpsucker, *Cyclopterus lumpus* L. J. Fish Biol. 23, 405–417.

Blanco Gonzalez, E., de Boer, F., 2017. The development of the Norwegian wrasse fishery and the use of wrasses as cleaner fish in the salmon aquaculture industry. Fish. Sci. 83, 661–670.

Brooker, A.J., Skern-Mauritzen, R., Bron, J.E., 2018a. Production, mortality, and infectivity of planktonic larval sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837): current knowledge and implications for epidemiological modelling. ICES J. Mar. Sci. 75, 1214–1234.

Brooker, A.J., Papadopoulou, A., Gutierrez, C., Rey, S., Davie, A., Migaud, H., 2018b. Sustainable production and use of cleaner fish for the biological control of sea lice: recent advances and current challenges. Vet. Rec. 183, 383.

Brooker, A.J., Davie, A., Leclercq, E., Zerafa, B., Migaud, H., 2020. Pre-deployment acclimatisation of farmed ballan wrasse (*Labrus bergylta*) to sea-cage conditions promotes behaviour analogous to wild conspecifics when used as cleaner fish in Atlantic salmon (*Salmo salar*) farms. Aquaculture. 520, 734771.

Budney, L.A., Hall, B.K., 2010. Comparative morphology and osteology of pelvic fin-derived midline suckers in lumpfishes, snailfishes and gobies. J. Appl. Ichthyol. 26, 167–175.

Bui, S., Stien, L.H., Nilsson, J., Oppedal, F., 2018. Assessment of long-term implementation of sea lice prevention technologies: efficiency in reducing infestations and impact on fish welfare. Rapport fra havforskningen 45–2018.

Cooke, S.J., Woodley, C.M., Brad Eppard, M., Brown, R.S., Nielsen, J.L., 2011. Advancing the surgical implantation of electronic tags in fish: a gap analysis and research agenda based on a review of trends in intracoelomic tagging effects studies. Rev. Fish Biol.

Fish. 21, 127–151.
Coull, K.A., Jermyn, A.S., Newton, A.W., Henderson, G.I., Hall, W.B., 1989. Length/weight relationships for 88 species of fish encountered in the North East Atlantic. In: Scottish Fisheries Research Report (43):80 p. Environment and Fisheries Department, The Scottish Office Agriculture.
Crawley, M.J., 2007. The R Book. M.J. Crawley Proportion data, West Sussex, Wiley.
Daborn, G.R., Gregory, R.S., 1983. Occurrence, distribution, and feeding habits of juvenile lumpfish, *Cyclopterus lumpus* L. in the Bay of Fundy. Can. J. Zool. 61, 797–801.
Davenport, J., 1985. Synopsis of biological data on the lumpsucker, *Cyclopterus lumpus* (Linnaeus, 1758). FAO Fish. Synop. 147, 1–31.
Davenport, J., Kjørsvik, E., 1986. Buoyancy in the Lumpsucker *Cyclopterus Lumpus*. J. Mar. Biol. Assoc. UK 66, 159–174.
Deady, S., Varian, S.J.A., Fives, J.M., 1995. The use of cleaner-fish to control sea lice on two Irish salmon (*Salmo salar*) farms with particular reference to wrasse behaviour in salmon cages. Aquaculture. 131, 73–90.
Dempster, T., Uglem, I., Sanchez-Jerez, P., Fernandez-Jover, D., Bayle-Sempere, J., Nilsen, R., Bjørn, P.A., 2009. Coastal salmon farms attract large and persistent aggregations of wild fish: an ecosystem effect. Mar. Ecol. Prog. Ser. 385, 1–14.
Dipper, F.A., Bridges, C.R., Menz, A., 1977. Age, growth and feeding in the ballan wrasse *Labrus bergylta* Ascanius 1767. J. Fish Biol. 11, 105–120.
Eliasen, K., Danielsen, E., Johannesen, Á., Joensen, L.L., Patursson, E.J., 2018. The cleaning efficacy of lumpfish (*Cyclopterus lumpus* L.) in Faroese salmon (*Salmo salar* L.) farming pens in relation to lumpfish size and seasonality. Aquaculture. 488, 61–65.
Ern, R., Norin, T., Gamperl, A.K., Esbaugh, A.J., 2016. Oxygen dependence of upper thermal limits in fishes. J. Exp. Biol. 219, 3376–3383.
FAO, 2019. Global Aquaculture Production Dataset 1950–2017. Food and Agriculture Organisation of the United Nations Available at: http://www.fao.org/fishery/statistics/global-aquaculture-production/en, Accessed date: 7 August 2019.
Faust, E., Halvorsen, K.T., Andersen, P., Knutsen, H., André, C., 2018. Cleaner fish escape salmon farms and hybridize with local wrasse populations. R. Soc. Open Sci. 5, 171752.
Figueiredo, M., Morato, T., Barreiros, J.P., Afonso, P., Santos, R.S., 2005. Feeding ecology of the white seabream, *Diplodus sargus*, and the ballan wrasse, *Labrus bergylta*, in the Azores. Fish. Res. 75, 107–119.
Geitung, L., Oppedal, F., Stien, L.H., Dempster, T., Karlsbakk, E., Nola, V., Wright, D.W., 2019. Snorkel sea-cage technology decreases salmon louse infestation by 75% in a full-cycle commercial test. Int. J. Parasitol. 49, 843–846.
Gentry, K., Bui, S., Oppedal, F., Dempster, T., 2020. Sea lice prevention strategies affect cleaner fish delousing efficacy in commercial Atlantic salmon sea cages. Aquac. Environ. Interac. 12, 67–80.
Glaropoulos, A., Stien, L.H., Folkedal, O., Dempster, T., Oppedal, F., 2019. Welfare, behaviour and feasibility of farming Atlantic salmon in submerged cages with weekly surface access to refill their swim bladders. Aquaculture. 502, 332–337.
Grøntvedt, R., Jansen, P., Horsberg, T., Helgesen, K., Tarpai, A., 2013. The surveillance programme for resistance to chemotherapeutants in *L. salmonis* in Norway. In: Surveillance Programmes for Terrestrial and Aquatic Animals in Norway. Annual Report.
Helfman, G., Collette, B.B., Facey, D.E., Bowen, B.W., 2009. The Diversity of Fishes: Biology, Evolution, and Ecology. John Wiley & Sons.
Helgesen, K.O., Romstad, H., Aaen, S.M., Horsberg, T.E., 2015. First report of reduced sensitivity towards hydrogen peroxide found in the salmon louse *Lepeophtheirus salmonis* in Norway. Aquacult. Rep. 1, 37–42.
Houde, E.D., Schekter, R.C., 1981. Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. Rapp. P.-V. Reun. Cons. Int. Explor. Mer. 178, 441–453.
Hvas, M., Oppedal, F., 2019. Physiological responses of farmed Atlantic salmon and two cohabitant species of cleaner fish to progressive hypoxia. Aquaculture. 512, 734353.
Hvas, M., Folkedal, O., Imsland, A., Oppedal, F., 2018. Metabolic rates, swimming capabilities, thermal niche and stress response of the lumpfish, *Cyclopterus lumpus*. Biology open 7 bio036079.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018b. Assessment of artificial substrates for lumpfish: effect of material thickness and water current speed. Aquac. Int. 26, 1469–1479.
Imsland, A., Reynolds, P., Eliassen, G., Hangstad, T., Foss, A., Vikingstad, E., Elvegård, T., 2014a. The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). Aquaculture. 18, 424–425.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2014b. Notes on the behaviour of lumpfish in sea pens with and without Atlantic salmon present. J. Ethol. 32, 117–122.
Imsland, A., Reynolds, P., Eliassen, G., Hangstad, T., Nytrø, A., Foss, A., Vikingstad, E., Elvegård, T., 2015. Assessment of suitable substrates for lumpfish in sea pens. J. Eur. Aquacult. Soc. 23, 639–645.
Imsland, A.K., Reynolds, P., Nytrø, A.V., Eliassen, G., Hangstad, T.A., Jónsdóttir, Ó.D.B., Emaus, P.-A., Elvegård, T.A., Lemmens, S.C.A., Rydland, R., Jonassen, T.M., 2016a. Effects of lumpfish size on foraging behaviour and co-existence with sea lice infected Atlantic salmon in sea cages. Aquaculture. 465, 19–27.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Jónsdóttir, Ó.D.B., Elvegård, T.A., Lemmens, S.C.A., Rydland, R., Nytrø, A.V., 2016b. Investigation of behavioural interactions between lumpfish (*Cyclopterus lumpus*) and goldsinny wrasse (*Ctenolabrus rupestris*) under controlled conditions. Aquac. Int. 24, 1509–1521.
Imsland, A.K., Reynolds, P., Hangstad, T.A., Jónsdóttir, Ó.D., Noble, T., Wilson, M., Mackie, J.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018a. Feeding behaviour and growth of lumpfish (*Cyclopterus lumpus* L.) fed with feed blocks. Aquac. Res. 49, 2006–2012.
Imsland, A.K., Reynolds, P., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018c. Effects of three commercial diets on growth, cataract development and histopathology of lumpfish (*Cyclopterus lumpus* L.). Aquac. Res. 49, 3131–3141.
Imsland, A.K.D., Hanssen, A., Nytrø, A.V., Reynolds, P., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018d. It works! Lumpfish can significantly lower sea lice infestation in large-scale salmon farming. Biol. Open. 7.
Imsland, A.K.D., Reynolds, P., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Hanssen, A., Mikalsen, B., 2019a. Effects of different feeding frequencies on growth, cataract development and histopathology of lumpfish (*Cyclopterus lumpus* L.). Aquaculture. 501, 161–168.
Imsland, A.K.D., Frogg, N., Stefansson, S.O., Reynolds, P., 2019b. Improving sea lice grazing of lumpfish (*Cyclopterus lumpus* L.) by feeding live feeds prior to transfer to Atlantic salmon (*Salmo salar* L.) net-pens. Aquaculture 511, 734224.
Ingólfsson, A., Kristjánsson, B.K., 2002. Diet of Juvenile Lumpsucker *Cyclopterus lumpus* (Cyclopteridae) in Floating Seaweed: Effects of Ontogeny and Prey Availability. Copeia. 2002, 472–476.
Jansson, E., Quintela, M., Dahle, G., Albretsen, J., Knutsen, H., André, C., Strand, Å., Mortensen, S., Taggart, J.B., Karlsbakk, E., Kvamme, B.O., Glover, K.A., 2017. Genetic analysis of goldsinny wrasse reveals evolutionary insights into population connectivity and potential evidence of inadvertent translocation via aquaculture. ICES J. Mar. Sci. 74.
Jepsen, N., Thorstad, E.B., Havn, T., Lucas, M.C., 2015. The use of external electronic tags on fish: an evaluation of tag retention and tagging effects. Ani. Biotelemetry. 3, 49.
Kennedy, J., Jónsson, S.Þ., Ólafsson, H.G., Kasper, J.M., 2016. Observations of vertical movements and depth distribution of migrating female lumpfish (*Cyclopterus lumpus*) in Iceland from data storage tags and trawl surveys. ICES J. Mar. Sci. 73, 1160–1169.
Korsøen, Ø.J., Fosseidengen, J.E., Kristiansen, T.S., Oppedal, F., Bui, S., Dempster, T., 2012. Atlantic salmon (*Salmo salar* L.) in a submerged sea-cage adapt rapidly to re-fill their swim bladders in an underwater air filled dome. Aquac. Eng. 51, 1–6.
Kristoffersen, A.B., Qviller, L., Helgesen, K.O., Vollset, K.W., Viljugrein, H., Jansen, P.A., 2018. Quantitative risk assessment of salmon louse-induced mortality of seaward-migrating post-smolt Atlantic salmon. Epidemics. 23, 19–33.
Krkošek, M., Connors, B.M., Morton, A., Lewis, M.A., Dill, L.M., Hilborn, R., 2011. Effects of parasites from salmon farms on productivity of wild salmon. Proc. Natl. Acad. Sci. 108, 14700–14704.
Leclercq, E., Zerafa, B., Brooker, A.J., Davie, A., Migaud, H., 2018. Application of passive-acoustic telemetry to explore the behaviour of ballan wrasse (*Labrus bergylta*) and lumpfish (*Cyclopterus lumpus*) in commercial Scottish salmon sea-pens. Aquaculture. 495, 1–12.
Lovdata, 2008. Forskrift om drift av akvakulturanlegg (akvakulturdriftsforskriften). https://lovdata.no/dokument/SF/forskrift/2008-06-17-822#KAPITTEL_4, Accessed date: August 2019.
Lovdata, 2012. Regulations on combating salmon lice in aquaculture facilities (in Norwegian: Forskrift om bekjempelse av lakselus i akvakulturanlegg). https://lovdata.no/dokument/SF/forskrift/2012-12-05-1140, Accessed date: July 2019.
Lovdata, 2017. Forskrift om produksjonsområder for akvakultur av matfisk i sjø av laks, ørret og regnbueørret (produksjonsområdeforskriften). https://lovdata.no/dokument/SF/forskrift/2017-01-16-61#KAPITTEL_2, Accessed date: July 2019.
Lusedata.no, 2017. Veileder for bruk og hold av rensefisk. http://lusedata.no/for-naeringen/veiledere-leppefisk/, Accessed date: September 2019.
Mo, T., Poppe, T., 2018. Risiko ved bruk av rensefisk i fiskeoppdrett. Norsk Veterinærtidsskrift nr. 2 (2018), 90–92.
Morel, G., Shrives, J., Bossy, S., Meyer, C., 2013. Residency and behavioural rhythmicity of ballan wrasse (*Labrus bergylta*) and rays (*Raja* spp.) captured in Portelet Bay, Jersey: implications for Marine Protected Area design. J. Mar. Biol. Assoc. UK 93, 1407–1414.
Mortensen, A., Johansen, R.B., Hansen, Ø.J., Puvanendran, V., 2020. Temperature preference of juvenile lumpfish (*Cyclopterus lumpus*) originating from the southern and northern parts of Norway. J. Therm. Biol. 89, 102562.
Nakagawa, S., 2004. A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav. Ecol. 15, 1044–1045.
Nilsen, A., Viljugrein, H., Røsæg, M.V., Colquhoun, D., 2014. Rensefiskhelse–kartlegging av dødelighet og dødelighetsårsaker. Veterinærinstituttets rapportserie. 12, 74.
Nilsen, A., Nielsen, K.V., Biering, E., Bergheim, A., 2017. Effective protection against sea lice during the production of Atlantic salmon in floating enclosures. Aquaculture. 466, 41–50.
Noble, C., Iversen, M.H., Lein, I., Kolarevic, J., Johansen, L.H., Berge, G.M., Burgerhout, E., Puvanendran, V., Mortensen, A., Stene, A., Espmark, Å.M., 2019a. RENSVEL OWI FACT SHEET SERIES: An introduction to Operational and Laboratory-based Welfare Indicators for lumpfish (*Cyclopterus lumpus* L.). FHF project 901136.
Noble, C., Iversen, M.H., Lein, I., Kolarevic, J., Johansen, L.H., Burgerhout, E., Puvanendran, V., Kousoulaki, K., Aas, G.H., Stene, A., Espmark, Å.M., 2019b. RENSVEL OWI FACT SHEET SERIES: An introduction to Operational and Laboratory based Welfare Indicators for ballan wrasse (*Labrus bergylta*). FHF project 901136.
Norwegian Directorate of Fisheries, 2019. Use of Cleaner Fish 1998–2017. Available at: https://www.fiskeridir.no/English/Aquaculture/Statistics/Cleanerfish-Lumpfish-and-Wrasse, Accessed date: 9 April 2019.
Nytrø, A., Vikingstad, E., Foss, A., Hangstad, T., Reynolds, P., Eliassen, G., Elvegård, T., Falk-Petersen, I.-B., Imsland, A., 2014. The effect of temperature and fish size on growth of juvenile lumpfish (*Cyclopterus lumpus* L.). Aquaculture 434, 296.
Oppedal, F., Dempster, T., Stien, L.H., 2011a. Environmental drivers of Atlantic salmon behaviour in sea-cages: A review. Aquaculture. 311, 1–18.
Oppedal, F., Vågseth, T., Dempster, T., Juell, J., Johansson, D., 2011b. Stocking density affects seasonal variation in observed swimming density of caged Atlantic salmon (*Salmo salar* L.) measured by echo sounders. Aquaculture. 315, 361–368.

Oppedal, F., Bui, S., Stien, L.H., Overton, K., Dempster, T., 2019. Snorkel technology to reduce sea lice infestations: efficacy depends on salinity at the farm site, but snorkels have minimal effects on salmon production and welfare. Aquac. Environ. Interac. 11, 445–457.

Overton, K., Dempster, T., Oppedal, F., Kristiansen, T.S., Gismervik, K., Stien, L.H., 2018a. *Salmon* lice treatments and salmon mortality in Norwegian aquaculture: a review. Rev. Aquac. 11, 1398–1417.

Overton, K., Samsing, F., Oppedal, F., Dalvin, S., Stien, L.H., Dempster, T., 2018b. The use and effects of hydrogen peroxide on salmon lice and post-smolt Atlantic salmon. Aquaculture. 486, 246–252.

Overton, K., Barrett, L.T., Oppedal, F., Kristiansen, T.S., Dempster, T., 2020. Sea lice removal by cleaner fish in salmon aquaculture: a review of the evidence base. Aquac. Environ. Interac. 12, 31–44.

Porteiro, F.M., Barreiros, J.P., Santos, R.S., 1996. Wrasses (Teleostei: Labridae) of the Azores. ARQUIPÉLAGO. Ciências Biológicas e Marinhas. Life Marine Sci. 14, 23–40.

Powell, A., Pooley, C., Scolamacchia, M., Garcia de Leaniz, C., 2018. Review of lumpfish biology. In: Cleaner Fish Biology and Aquaculture Applications. 5M Publishing Ltd, Sheffield, pp. 98–121.

Quignard, J.P., Pras, A., 1986. Labridae. In: Whitehead, P.J.P., Beuchot, M.L., Hureau, J.C., Nielsen, J., Tortonese, E. (Eds.), Fishes of the North-Eastern Atlantic and the Mediterranean. UNESCO, Paris, pp. 919–942.

R Core Team, 2019. R: A language and environment for statistical computing. In: R Foundation for Statistical Computing. Austria. URL, Vienna. https://www.R-project.org.

Rabadan, C.G., 2018. Improving the use of wrasse (Labridae) stocked in commercial sea cages with Atlantic salmon (*Salmo salar*) for sea lice control: Key features for a successful deployment. In: Treasurer, J. (Ed.), Cleaner Fish Biology and Aquaculture Applications. 5m Publishing, Sheffield, pp. 54–68.

Savino, J.F., Henry, M.G., Kincaid, H.L., 1993. Factors affecting feeding behavior and survival of juvenile lake trout in the Great Lakes. Trans. Am. Fish. Soc. 122, 366–377.

Sayer, M.D.J., Davenport, J., 1996. Hypometabolism in torpid goldsinny wrasse subjected to rapid reductions in seawater temperature. J. Fish Biol. 49, 64–75.

Sayer, M.D.J., Reader, J.P., 1996. Exposure of goldsinny, rock cook and corkwing wrasse to low temperature and low salinity: survival, blood physiology and seasonal variation. J. Fish Biol. 49, 41–63.

Sayer, M.D.J., Gibson, R.N., Atkinson, R.J.A., 1993. Distribution and density of populations of goldsinny wrasse (*Ctenolabrus rupestris*) on the west coast of Scotland. J. Fish Biol. 43, 157–167.

Skiftesvik, A.B., Bjelland, R.M., Durif, C.M.F., Johansen, I.S., Browman, H.I., 2013. Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). Aquaculture 402–403, 113–118.

Skiftesvik, A.B., Blom, G., Agnalt, A.-L., Durif, C.M.F., Browman, H.I., Bjelland, R.M., Harkestad, L.S., Farestveit, E., Paulsen, O.I., Fauske, M., Havelin, T., Johnsen, K., Mortensen, S., 2014. Wrasse (Labridae) as cleaner fish in salmonid aquaculture – The Hardangerfjord as a case study. Mar. Biol. Res. 10, 289–300.

Skiftesvik, A.B., Bjelland, R.M., Durif, C., Moltumyr, L., Hjellum, R.B., Halvorsen, K.A.T., 2018. Program rensefisk: Adferd og artssamspill i laksemerder. Rapport fra havforskningen 33–2018.

Stein, D.L., 1986. Cyclopteridae. In: Whitehead, P.J.P., Beuchot, M.L., Hureau, J.C., Nielsen, J., Tortonese, E. (Eds.), Fishes of the North-Eastern Atlantic and the Mediterranean. UNESCO, Paris, pp. 1269–1274.

Stien, L.H., Lind, M.B., Oppedal, F., Wright, D.W., Seternes, T., 2018. Skirts on salmon production cages reduced salmon lice infestations without affecting fish welfare. Aquaculture. 490, 281–287.

Stien, L.H., Kristiansen, T., Folkedal, O., Mortensen, S., Skiftesvik, A.B., Waagbø, R., Sæle, Ø., Nilsson, J., 2019. Velferd hos laks og rensefisk i merder i sjøen. In: Grefsrud, E.S., Svåsand, T., Glover, K., Husa, V., Hansen, P.K., Samuelsen, O., Sandlund, N., Stien, L.H. (Eds.), Risikorapport norsk fiskeoppdrett 2019, Institute of Marine Research.

Stien, L.H., Størkersen, K.V., Gåsnes, S.K., 2020. Analysis of mortality data from survey on cleaner fish. Rapport fra havforskningen 6.

Thorstad, E.B., Rikardsen, A.H., Alp, A., Økland, F., 2013. The use of electronic tags in fish research–an overview of fish telemetry methods. Turk. J. Fish. Aquat. Sci. 13, 881–896.

Treasurer, J., Feledi, T., 2014. The physical condition and welfare of five species of wild-caught wrasse stocked under aquaculture conditions and when stocked in Atlantic Salmon, *Salmo salar*, production cages. J. World Aquacult. Soc. 45, 213–219.

Treasurer, J., Turnbull, T., 2019. Tolerance of lumpfish, *Cyclopterus lumpus*, to freshwater bath treatment for amoebic gill disease, *Neoparamoeba perurans*, infection and efficacy of different treatment regimens. J. World Aquacult. Soc. 50, 42–53.

Treasurer, J.W., Mordue, A.J., Pike, A.W., 2002. A review of potential pathogens of sea lice and the application of cleaner fish in biological control. Pest Manag. Sci. 58, 546–558.

Tully, O., Daly, P., Lysaght, S., Deady, S., Varian, S.J.A., 1996. Use of cleaner-wrasse (*Centrolabrus exoletus* (L.) and *Ctenolabrus rupestris* (L.)) to control infestations of *Caligus elongatus* Nordmann on farmed Atlantic salmon. Aquaculture. 142, 11–24.

Uglem, I., Karlsen, Ø., Sanchez-Jerez, P., Sæther, B.S., 2014. Impacts of wild fishes attracted to open-cage salmonid farms in Norway. Aquac. Environ. Interac. 6, 91–103.

Villegas-Ríos, D., Alós, J., March, D., Palmer, M., Mucientes, G., Saborido-Rey, F., 2013. Home range and diel behavior of the ballan wrasse, *Labrus bergylta*, determined by acoustic telemetry. J. Sea Res. 80, 61–71.

Wright, D.W., Stien, L.H., Dempster, T., Oppedal, F., 2018. Differential effects of internal tagging depending on depth treatment in Atlantic salmon: a cautionary tale for aquatic animal tag use. Curr. Zoo. 65, 665–673.

Yuen, J.W., Dempster, T., Oppedal, F., Hvas, M., 2019. Physiological performance of ballan wrasse (*Labrus bergylta*) at different temperatures and its implication for cleaner fish usage in salmon aquaculture. Biol. Control 135, 117–123.

Graphic design: Communication Division, UiB / Print: Skipnes Kommunikasjon AS

uib.no

ISBN: 9788230845530 (print)
9788230856628 (PDF)