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
0044-8486/

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

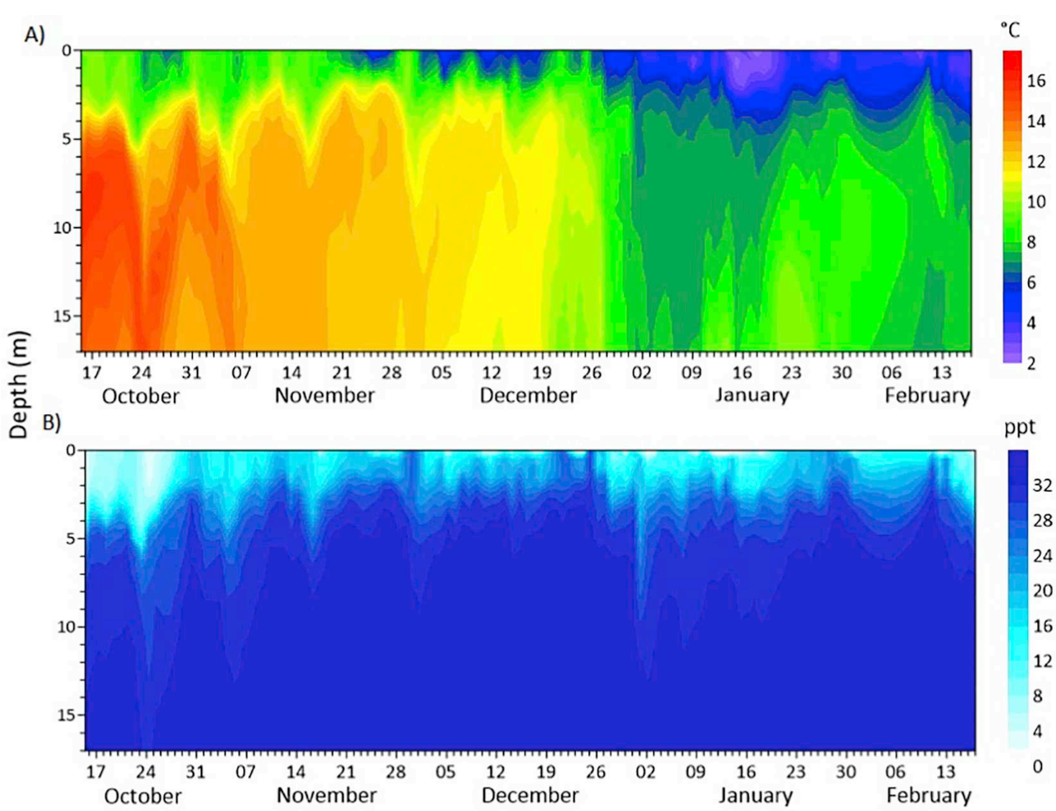

Fig. 1. Daily depth profiles between 0 and 17 m of a) temperature and b) salinity from a reference location at the outer end of the farm at Solheim, Norway.

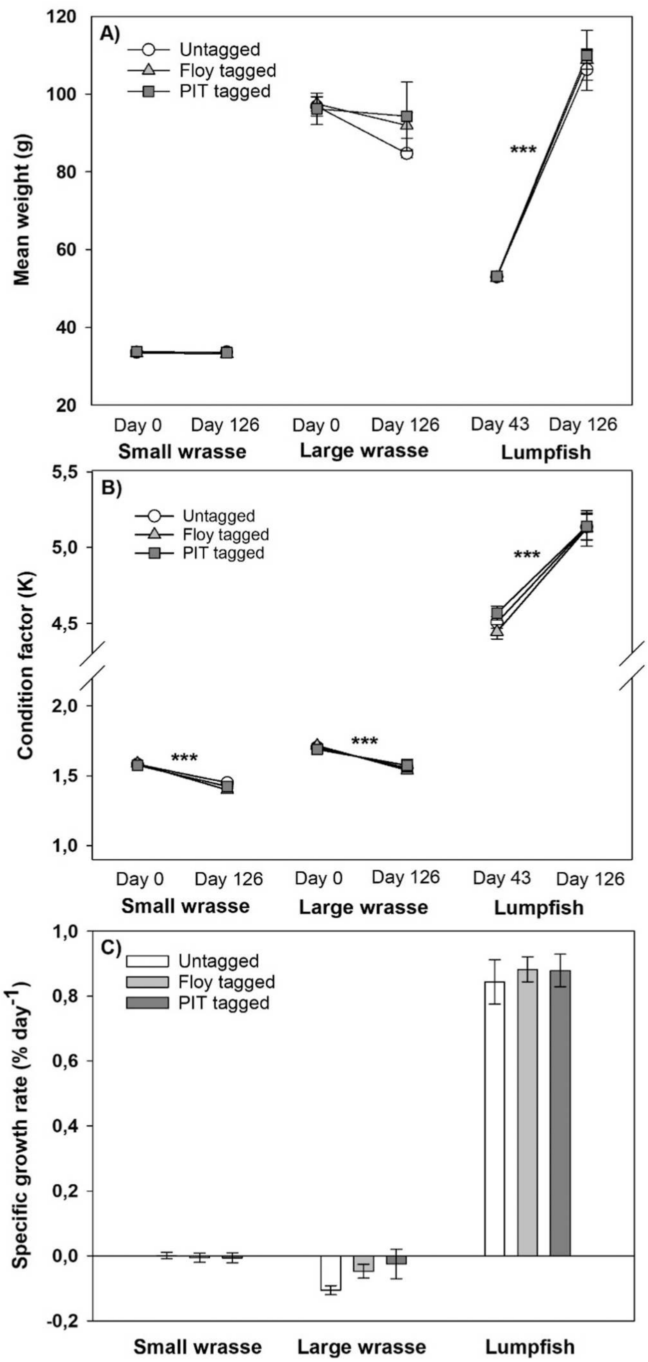

Fig. 2. Overview of a) mean weight (g); b) condition factor (K) and c) specific growth rate (% day^{-1}) from the initial and final sampling points for untagged, floy tagged and PIT tagged lumpfish, small and large ballan wrasse. ***$p < .001$.

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%, $F = 2.173$, $p = .148$).

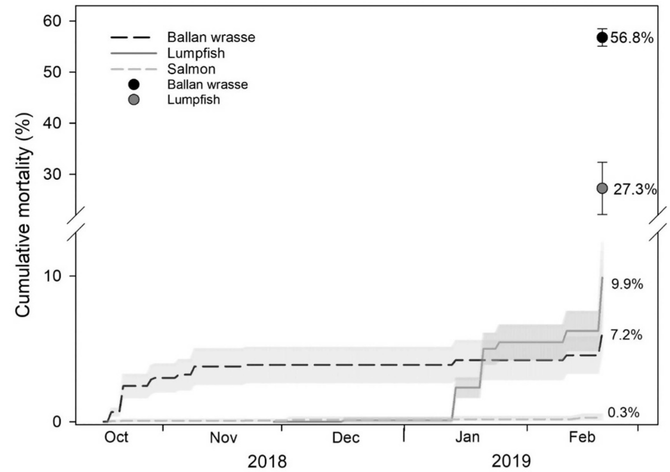

Fig. 3. Overview of mean (± SE) registered mortality (lines) for ballan wrasse, lumpfish and salmon as well as mean (± SE) total losses (dots) for ballan wrasse and lumpfish. Registered mortality is taken from daily mortality registrations while total losses was calculated at the end of the experiment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

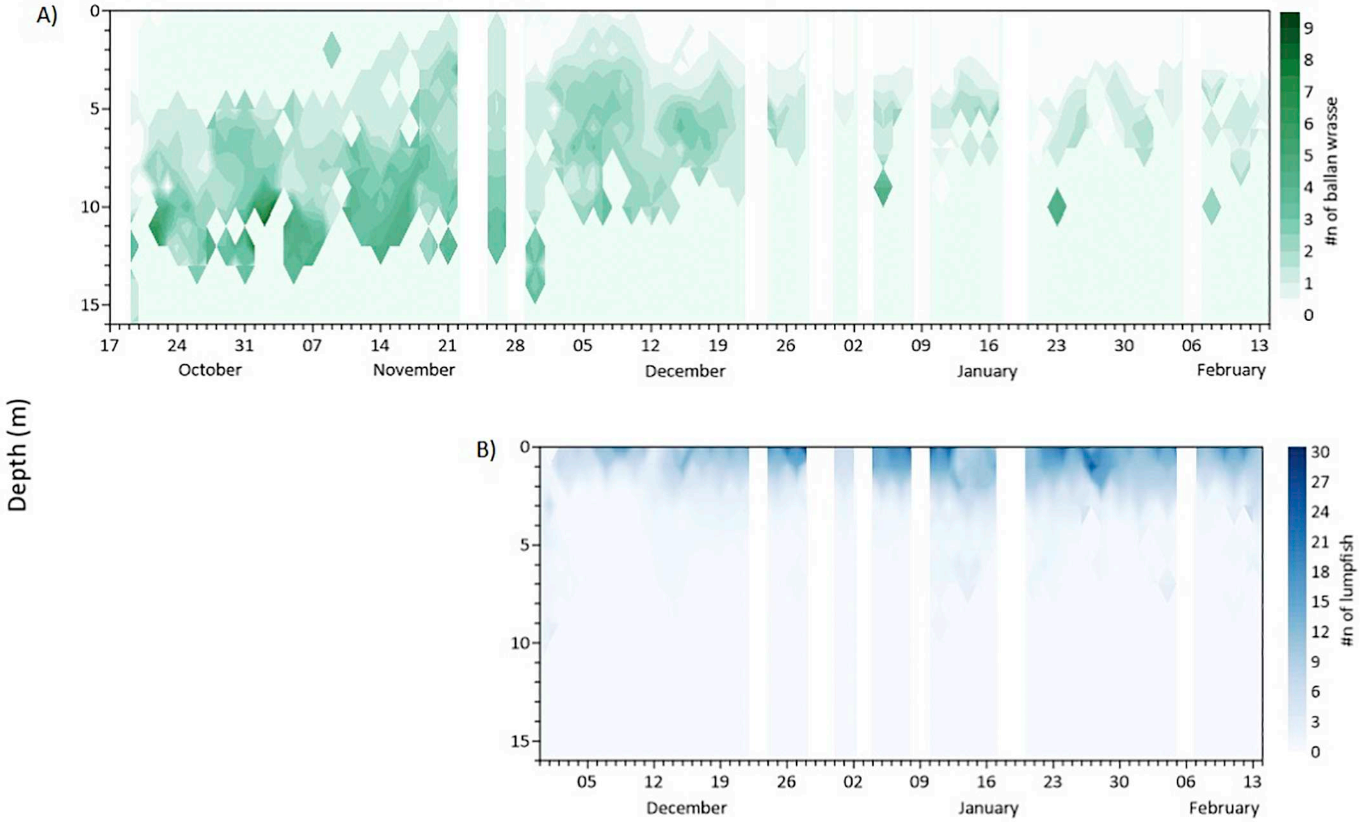

Fig. 4. Depth distribution of a) ballan wrasse and b) lumpfish from brief underwater camera observations at hides every 2–3 days with 1 min observation at every metre from 0 to 16 m depth. Lumpfish was added to the cages four weeks after ballan wrasse.

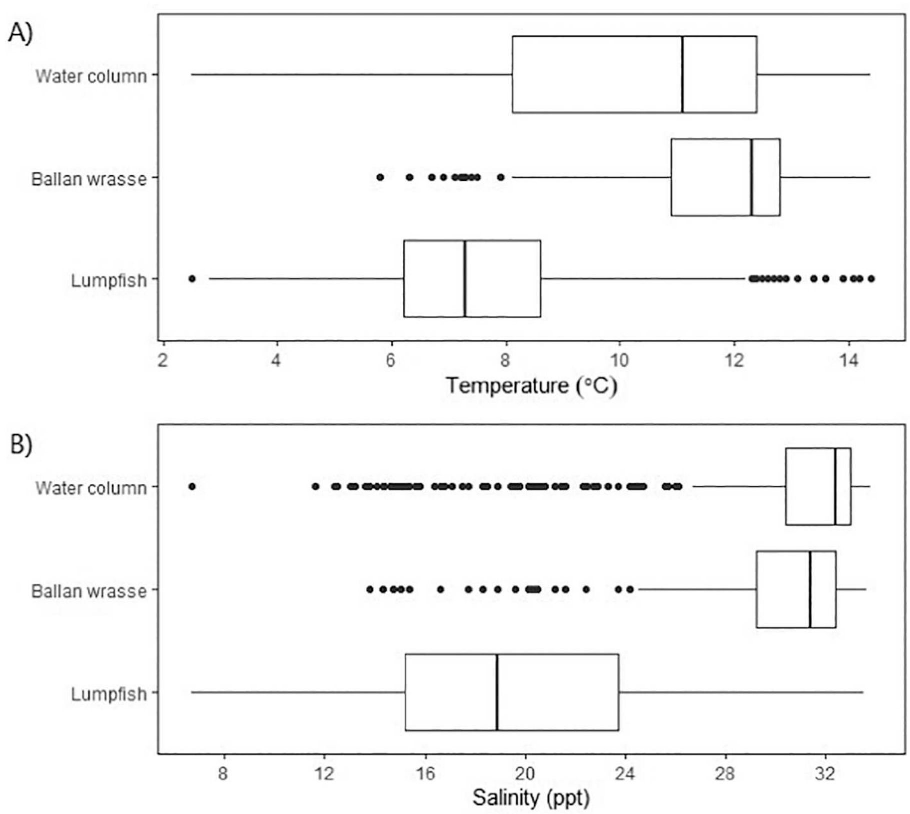

Fig. 5. Boxplots showing range of a) temperature and b) salinity values measured throughout the water column inside the cages as well as temperature and salinity conditions experienced by both ballan wrasse and lumpfish based on their depth from each observation day from the deployment of lumpfish to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