Behavioral and depth preference for wrasse (*Labridae*) used as cleaner fish in salmon aquaculture

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Abstract

Wild and farmed wrasse are used as cleaner fish in Norwegian salmon aquaculture as a biological remedy against the ectoparasite salmon lice. For wrasse to be a well-functioning cleaner fish, it is essential that the species can coexist with salmon in the cage environment. There have been reports of high mortality of wrasse in cages with salmon, and there is limited information about wrasse depth and behavior preference in a sea cage environment. Therefore, a trial of behavior and depth preference of wild-caught corkwing (*Symphodus melops*), goldsinny (*Ctenolabrus rupestris*), and wild-caught and farmed ballan wrasse (*Labrus berggylta*) was done in cages with and without Atlantic salmon (*Salmo salar*) present.

The trial consisted of nine sea cages (12 x 12 m square) divided into three replicates. Three sea cages with salmon and wild-caught wrasse, three with only wild-caught wrasse, and three with salmon and farmed ballan wrasse during summer-autumn 2022. Behavioral and depth observations were done with underwater cameras three times a week for ten weeks during daylight. The cages were supplied with artificial kelp and feeding stations for wrasse with feeding blocks, pellets, and shrimp at three depths (3, 6, and 9 m).

There was a clear difference in behavior for all wrasse species. Wrasse exhibited a greater variety of behaviors, and more observations was recorded in cages without salmon than in cages with salmon. In cages without salmon, goldsinny used the entire depth range, while in cages with salmon, goldsinny was most dominant at deeper depths. Corkwing was observed at the same depths in cages with and without salmon. Ballan was observed in the upper water layer, in cages with and without salmon, but slightly deeper in the cages with salmon, however this was based on very limited data. Farmed ballan wrasse was dominant at the bottom of the cages and was mainly observed resting, most likely due to poor fin welfare. Wrasse in cages with salmon had a twice as high mortality rate than wrasse in cages without salmon.

The findings of the present study show that goldsinny and salmon have a mismatch in depth preference, so the possibility of performing cleaning behavior could be minimized. Corkwing and ballan wrasse share the same depth as salmon and are therefore more likely to perform cleaning behavior. The farmed ballan wrasse struggled to perform well in the sea cages due to poor fin welfare, therefore the chance of performing cleaning behavior was minimized.

1. Introduction

1.1 Norwegian aquaculture

Norway has a long history as a fisheries nation, and fisheries have contributed to sustaining Norway with economic growth and nutritious food for centuries (Solhaug, 1983). In the 1960s, some pioneers started experimenting with farming Atlantic salmon (*Salmo salar*) along the Norwegian coast. The salmon farming adventure in Norway took its leap in the 1970s, and since then, it has contributed to an even further economic upswing and value creation for Norway (Olaussen, 2018).

In 2022, fish worth 146.6 billion NOK were exported, an increase of 25.7% from 2021, and farmed Atlantic salmon accounted for 70 % of the export value (SSB, 2023). Norwegian salmon aquaculture has overgone both biological and technological improvements, making the production go from extensive to intensive (Bergheim, 2012). It makes Norway world-leading in the salmon aquaculture industry (Blanco Gonzalez and de Boer, 2017). The production of salmon is highly profitable. From 01.01.2023, the Norwegian government has enforced a base rate tax on salmon aquaculture to regulate the industry to achieve sustainable development, balance economic growth, disease control, and fair competition (NOU 2019: 18). However, many believe it might limit further growth and expansion of the industry.

The ocean is an essential resource for the future of food production, and the growing middle class and world population increase the need for animal protein (Olafsen et al., 2012). This makes the Norwegian aquaculture industry an essential part of the future and reaching the UN's sustainable development goals. Salmon has a relatively low feed factor compared with other animal proteins and is a good source of protein, essential amino acids, lipids, and micronutrients (Béné et al., 2015), making salmon aquaculture central in reaching food security (SDG 2.1), nutritional needs (SDG 2.2) and good health and well-being (SDG 3) in both developed and developing countries (Thilsted et al., 2016).

The Norwegian Ministry of Fisheries and Coastal Affairs aims for Norway to produce 5 million tons of salmon by 2050 (Sandvik et al., 2020). However, environmental threats need to be solved to continue sustainable development in agreement with the UN's sustainable development goals and further growth of the salmon aquaculture industry in Norway. The ectoparasite salmon louse (*Lepeophtherius salmonis*) has been listed as one of the greatest environmental threats to the further growth of salmon aquaculture (Taranger et al., 2015; Forseth et al., 2017; Sandvik et al., 2021).

1.2 Atlantic salmon

Atlantic salmon (order Salmoniformes, family Salmonidae) is a ray-finned fish. Atlantic salmon has an anadromous lifecycle consisting of reproduction in freshwater and growth in saltwater (Aas et al., 2011). Sexually mature adults spawn in the fall in freshwater, where the fertilized eggs incubate in the river gravel until spring, when they are developed to fry (Hansen and Quinn, 1998). They continue to grow in freshwater as parr for 2–4 years before undergoing the journey to the ocean for further growth for 1–4 years before returning to their natal river to mate (Aas et al., 2011). Atlantic salmon aquaculture production is based on the salmon’s natural lifecycle. It consists of four steps: 1) the production of broodstock and roe, 2) the production of fry, 3) the production of smolts, and 4) the grow-out phase at sea until harvest (Asche and Bjorndal, 2011). Intensive production of farmed Atlantic salmon does not come without consequences. One issue with salmon aquaculture is the increase of available hosts for salmon lice all year round (Heuch and Mo, 2001; Johnson et al., 2004; Vollset et al., 2014). Consequently, both farmed and wild salmon are more prone to lice infestations. Especially wild salmon post-smolts migrating from rivers to the ocean are at risk of harmful lice infestation (Johnsen et al., 2021).

1.3 Salmon lice

Salmon lice is a copepod ectoparasite belonging to the Caligidae family. The lice comprise of eight stages separated by molting (Figure 1), two free-living nauplii larvae, one infective copepodite stage, two attached chalimus stages, two pre-adult stages, and one final adult stage (Hamre et al., 2009). The eight development stages can be divided into three categories: free-living, sedentary, and mobile. The salmon lice are host specific for anadromous salmonid fish (Olaussen, 2018). The development of the lice is determined by several environmental measurements such as salinity, temperature, light, and host species (Grimnes and Jakobsen, 1996).

The salmon lice feed on the salmon’s mucus, skin, and blood (Hamre et al., 2009). As a result, the salmon might experience increased cortisol levels and osmoregulatory issues, which increases the risk of secondary infections (Wagner et al., 2003). If the injuries caused by the salmon lice are big enough, they can lead to mortality (Wootten et al., 1982; Bjørn and Finstad, 2011).

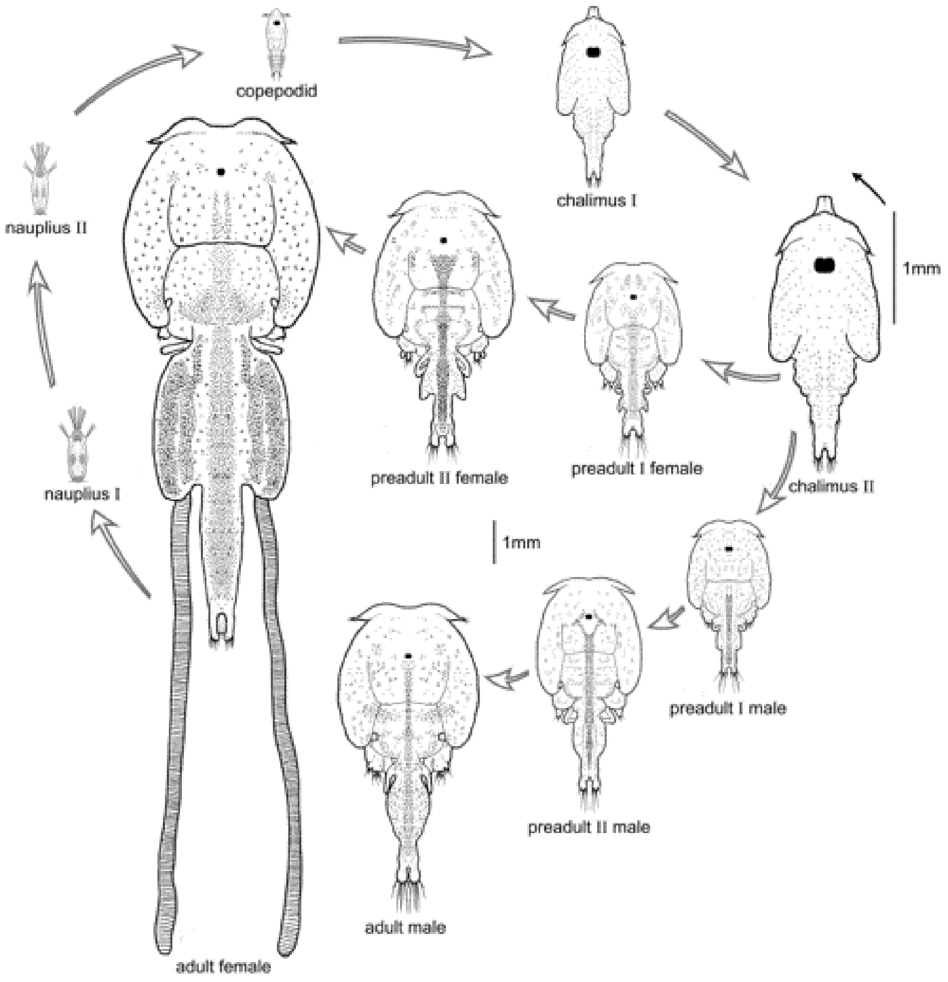


Figure 1: Life-cycle salmon louse (Lepeophtherius salmonis). Free-living: napulis 1, napulis 2 and copepodid. Sedentary: chalimus 1, chalimus 2. Mobile: preadult 1, preadult 2 and adult. Illustration: (Sea Lice Reaserch Centre, 2020).

1.4 Traffic light system

The traffic light system in Norway regulates the production volume of farmed salmonoids and lice treatments (Grefsrud et al., 2022). This ensures predictable and environmentally friendly growth in the industry. There are 13 production areas from the south to the north of Norway (Hersoug, 2022). Each region is given a green, yellow, or red light based on professional assessments of how lice affected wild salmon in the area in the previous years (Sandvik et al., 2021). Furthermore, delousing treatments must be done if there are 0.5 adult female lice per fish. In weeks 16–21, when the wild salmon migrates to the sea, there can only be 0.2 adult female lice per fish before delousing treatment must be done (Heuch et al., 2005).

1.5 Delousing

The Norwegian animal welfare act protects fish and promotes good animal welfare and respect for animals (Dyrevelferdsloven, 2009). The salmon industry experiences several welfare issues related to salmon lice regarding the parasite itself and its effect on the host. But also, several delousing methods pose a welfare threat to the salmon, especially thermal and mechanical delousing (Hjeltnes et al., 2019).

The most used remedy against salmon lice used to be chemical therapeutics, such as hydrogen peroxide, azamethiphos, and diflubenzuron (FHI, 2022). However, because of limited chemical agents, some have been in use for over a decade, making the salmon lice resistant to many of the chemicals (Aaen et al., 2015; Carmona-Antoñanzas et al., 2016; Sviland Walde et al., 2021), rendering the treatments less effective. The frequent use of chemical therapeutics can quicken the evolution of salmon louse (Coates et al., 2021). Furthermore, salmon lice therapeutics can negatively impact sensitive non-target species, such as crustaceans and bivalves (Johnson et al., 2004; Hamoutene et al., 2023), since the therapeutics are released into the environment, and lack specificity to only target the salmon lice (Burridge et al., 2010).

Mechanical delousing removes lice with brushing, flushing, and turbulence (Østevik et al., 2022). Handling related to mechanical delousing can lead to stress, hypoxia, and injuries for the fish (Noble et al., 2018; Østevik et al., 2022). Thermal delousing is a process where salmon is exposed to warm water (28–34 ºC) for 30 seconds before being transported back to the sea cages (Noble et al., 2018). Thermal delousing with warm water has become the most used non-medical delousing in Norwegian aquaculture (Sommerset et al., 2022). However, thermal delousing has been shown to promote poor fish welfare, where salmon have been observed with gill and brain bleeding and skin wounds (Gismervik et al., 2019). From 2012–2017 thermal delousing resulted in a 31% increase in salmon mortality (Overton et al., 2019). Furthermore, salmon exposed to warm water react with abnormal behavior, such as jumping, colliding, and sudden swimming behavior (Nilsson et al., 2019).

Because of welfare issues for Atlantic salmon related to these delousing methods, cleaner fish have become one of the more used options for delousing (Barrett et al., 2020).

1.6 Cleaner fish

Biological control is defined as controlling pets and weeds by using other living organisms (Henderson and Lawrence, 2016), and cleaner fish are used as biological control to remove salmon lice from salmon (Treasurer, 2018). In 2021, 45.5 million wild and farmed cleaner fish were stocked in Norwegian aquaculture (Directorate of Fisheries, 2022). It is mainly wrasse (*Labridae*) and lumpfish (*Cyclopterus lumpus*) used as cleaner fish. The use of cleaner fish started in the late 1980s after cleaning symbiosis was observed between salmon and wrasse (Bjordal, 1988).

Lumpfish is a semi-pelagic fish found across the North Atlantic, often observed around floating seaweed (Daborn and Gregory, 1983; Geitung et al., 2020). Lumpfish is the most used cleaner fish in Norway (Directorate of Fisheries, 2022) and is suggested as a biological cleaner when the water temperature is low (Jónsdóttir et al., 2018). As a result, lumpfish is the preferred cleaner during fall and winter and in the northern parts of Norway (Erkinharju et al., 2021). Even though lumpfish is an important cleaner fish, the rest of this thesis will focus on wrasse.

Wrasse (order labriformes, family Labridae) (Figure 2) is a marine fish habituating rocky reefs alongside the coast of the Atlantic, Pacific, and Indian oceans (Erkinharju et al., 2021). Wrasse have browsing and grazing feeding behavior (Treasurer, 2018). With their thick protruding lips, solid teeth, and protractile mouths, wrasse are specialized to feed on invertebrates, for instance, hard-shelled crustaceans (Treasurer, 2018). Cleaner wrasse used in aquaculture have a spawning season from spring until late summer (Blanco Gonzalez and de Boer, 2017). Wrasse used as cleaner fish in aquaculture are temperature sensitive and are therefore not recommended to be used at temperatures lower than 6 ^{o}C as they become inactive at such low temperatures (Imsland et al., 2014a; Yuen et al., 2019).

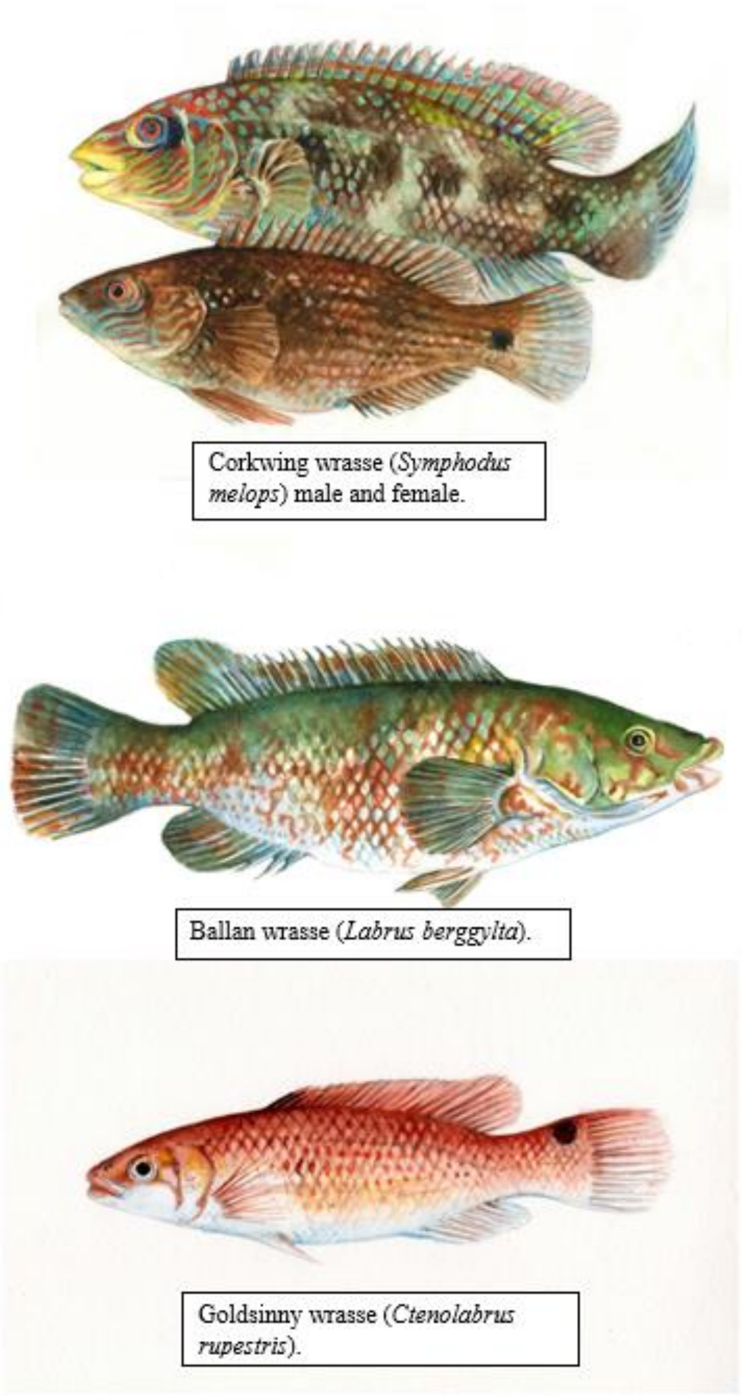


Figure 2. Wrasse species used in the trial. Corkwing wrasse (*Symphodus melops*), ballan wrasse (*Labrus berggylta*), and goldsinny wrasse (*Ctenolabrus rupestris*). Illustration: (Stein Mortensen, 2015).

Ballan wrasse (*Labrus berggylta*) (Figure 2) is an inshore species found around rocks, boulder slopes, and offshore reefs (Treasurer, 2018). They are a diurnal species that are active during the day and go into hiding in rock crevices and seaweed beds at night (Davie et al., 2018). Ballan wrasse is one of the bigger wrasse species and can obtain a length of up to 65.9 cm (Ottesen et al., 2012). They have a lifespan of up to 29 years (Davie et al., 2018). Ballan wrasse is a preferred cleaner fish in the salmon industry because of its size, hardiness, and grazing effectiveness (Erkinharju et al., 2021).

Goldsinny wrasse (*Ctenolabrus rupestris*) (Figure 2) is a small wrasse species (10–14 cm) found around rocky shores and seeks shelter in rock holes (Treasurer, 2018). Goldsinny are usually found in the intertidal zone (Treasurer et al., 2018b). The average lifespan for females is 20 years, and for males, 14 years, where they reach maturity after 1–2 years (Blanco Gonzalez and de Boer, 2017). Goldsinny is a territorial fish, and males have been observed defending an area up to 0.5–2 m^2 (Hilldén, 1981)

Corkwings (*Symphodus melops*) (Figure 2) have a lifespan of up to 9 years, reaching maturity after 1–3 years, and can reach a length of 28 cm (Blanco Gonzalez and de Boer, 2017). The species show a sexual size dimorphism, where the total length of nesting males is usually larger than females and sneaker males (Blanco Gonzalez and de Boer, 2017).

Cleaner fish are considered a more environmental option than other delousing methods and create a less stressful environment for salmon than chemical and mechanical delousing (Powell et al., 2018). However, in the last couple of years, there have been raised several ethical concerns about using cleaner fish (Overton et al., 2020). A survey on behalf of the Norwegian Food Authority shows that the median mortality of cleaner fish is 42% independent of species, and it is most likely higher since this only accounts for the registered losses (Stien et al., 2020).

With the use of wild-caught wrasse, there is an increasing concern for the abundance of the wild stocks since there is high fishing pressure (Sayer et al., 1996; Blanco Gonzalez and de Boer, 2017). Consequently, the wrasse fisheries are strictly regulated, and the fishing quota for wrasse in Norway for 2022 was set to 18 million (Directorate of Fisheries, 2021). Intensive fisheries on goldsinny have shown a decrease in the stock abundance of the species (Jansson et al., 2020). In addition, the corkwing and goldsinny population structure are most likely being altered by fisheries, primarily through the removal of larger fish (Darwall et al., 1992). Wrasse are being transported long distances to be used as cleaner fish, and corkwing in the north of Norway have shown evidence of gene flow from southern populations (Faust, 2021).

Furthermore, there is a higher risk of biosecurity by using wild wrasse. Wrasse can be a reservoir for disease and be a route of infection for Atlantic salmon in a sea cage environment, for instance, amoebic gill disease (Karlsbakk et al., 2013; Vaughan et al., 2017).

Farmed cleaner fish is becoming more common to obtain the need for cleaner fish and reduce the risk of biosecurity with the use of wild fish and concern for wild stocks. Ballan wrasse and lumpfish are the only cleaner fish that are farmed on a commercial scale, and 29.9 million farmed cleaner fish were sold in 2021 (Directorate of Fisheries, 2022). For cleaner fish to succeed as active cleaners in a sea cage environment, key elements need to be in place, such as keeping the nets clean and providing shelter in the form of hides and additional feed for the cleaner fish (Sveier and Breck, 2018). The hides are crucial to give the cleaner fish protection and a place to rest (Sveier and Breck, 2018).

The depth preference and behavior of salmon is well documented (Juell, 1995; Johansson et al., 2006, 2009; Oppedal et al., 2011). Wrasse distribution in the wild is also well documented (Skiftesvik et al., 2015; Treasurer, 2018). However, less information about wrasse depth and behavior preferences in sea cages are available. For instance, the majority of studies reporting that wrasse exhibits cleaning behavior are in small scale tank trials (Overton et al., 2020). In the context of aquaculture, there is minimal information on depth preference for corkwing, whereas goldsinny has been reported to be positioned close to the bottom of the tank (Tully et al., 1996; Imsland et al., 2016a), and ballan wrasse at depths below 16 m in sea cages (Leclercq et al., 2018). There has yet to be investigated in more detail if salmon alters the behavior and depth preference of wrasse in salmon aquaculture.

1.7 Objective

For cleaner fish to function as a remedy against salmon lice, the cleaner species and salmon must coexist in the cage environment, and especially given the welfare issues related to the use of cleaner fish, more information about how and if salmon and cleaner fish coexist in a cage environment is crucial. This study aimed to investigate if the presence of salmon impacts the behavior and depth preference of wrasse and survival in the sea cage, as well as looking at behavior and depth preference between the different species, using various species of wild wrasse and farmed ballan wrasse.

To be able to answer the aim of the study, the following hypotheses were investigated:

$H0_1$: Salmon does not impact the depth preference of wrasse.

HA_1: Salmon impacts the depth preference of wrasse.

$H0_2$: Salmon does not impact the behavior of wrasse.

HA_2: Salmon impacts the behavior of wrasse.

$H0_3$: The mortality of wrasse is not impacted by the presence of salmon.

HA_3: The mortality of wrasse is impacted by the presence of salmon.

$H0_4$: There is no variation in depth preference between the different wrasse species.

HA_4: There is variation in depth preference between the different wrasse species.

2. Materials and methods

2.1 Study site and experimental fish

The trial was conducted at the Institute of Marine Research sea cage facility, Solheim (60.9º N, 5.5º E) in Masfjorden from 3 August 2022 to 27 October 2022 (86 days). The facility comprised of ten sea cages (12 x 12 m square), and nine were used for this trial. The facility is located at the end of a long fjord, providing shelter from the rough sea (Figure 3).

Figure 3. Map of the location of the facility Solheim (60. 9º N, 5.5º E) marked in red.

Atlantic salmon (Mowi strain, 20G) vaccinated with micro-6/ micro 1 PD delivered by Nesfossen Smolt AS were stocked three months before the trial, with about 9.000 salmon per cage and a mean weight of 74 g. Wild caught wrasse (corkwing, goldsinny, and ballan wrasse) was caught locally in Masfjorden and delivered by a fisherman on 3. August 2022. They were sorted and counted into separate transportation tanks (1.000 L) to ensure a similar and random distribution of each species of wild wrasse in the sea cages and to know the exact number of each species per cage. Despite this, the species distribution varied in the different cages (Table 1) because the second delivery from the fisherman consisted mainly of corkwing wrasse and in an attempt to minimalize the handling of the fish, they were not sorted a second time to even out the numbers. In the holding and transportation tanks, there was an oxygen stone to maintain good oxygen (>85%) level during sorting. Fisheries of wrasse are regulated in Norway.

Consequently, wrasse that do not meet the maximum and minimum length requirements cannot be caught. Ballan wrasse (max length: 28 cm, min length: 22 cm), corkwing wrasse (min length: 12 cm), and goldsinny (min length: 11 cm) (Blanco Gonzalez and de Boer, 2017; Grefsrud et al., 2022). To certify that the length of the fish was within the minimum and maximum requirements of wrasse fisheries in Norway, the length of all the ballan wrasse and the fish that seemed shorter than the minimum length was measured. In the cages with wrasse, hides were put in the middle of the cage. Four straps of artificial kelp (Krantare™, NorseAqua, Terråk, Norway) with a length of 8 m were used as hide in each cage with a 1x1 m distance, creating a hide corridor (Figure 4). The wild wrasse was slowly introduced to the sea cages close to the hides with the help of a crane.

The farmed ballan wrasse originated from Mowi Rensefisk Øygarden (Ljøsøyvegen 5357 Rogn, Norway) and was deployed 5. September 2022. The ballan wrasse was transported directly from the Mowi Øygarden site to Solheim in a vehicle with an oxygenated holding tank. The oxygen tank had some minor technical issues during transport, resulting in high oxygen levels in the transport tank for a short period. The fish were counted and placed into three separate holding tanks. The farmed ballan wrasse were slowly introduced to the sea cages close to the hides with the help of a crane.

Table 1. Wrasse species distribution per cage at Solheim.

	Cage								
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Corkwing wrasse	430			263	407	279	257	405	
Goldsinny wrasse	106			273	120	256	278	130	
Ballan wrasse	4			4	13	4	5	5	
Farmed ballan wrasse		531	540						540
Total count	**540**	**531**	**540**	**540**	**540**	**539**	**540**	**540**	**540**

2.2 Experimental design

The trial consisted of nine sea cages divided into three replicates within each treatment group at the start, with a change of one group halfway. Three sea cages were stocked with only wild-caught wrasse (cages 4, 5, 8) and three with wild-caught wrasse and Atlantic salmon (cages 1, 6, 7). Furthermore, there were three cages with only salmon for the first four weeks of the trial (cages 2, 3, 9) before farmed ballan wrasse were deployed four weeks later. These three sea cages consisted of salmon and farmed ballan wrasse for the rest of the trial. This created four experimental groups with triplicates and was set up as a factorial design (Figure 4). In the cages with wrasse, there was put out a feeding station at three different depths (3, 6, and 9 m) in the middle of the hides. A camera was placed in the middle of the cage and could move through the hides (Figure 4).

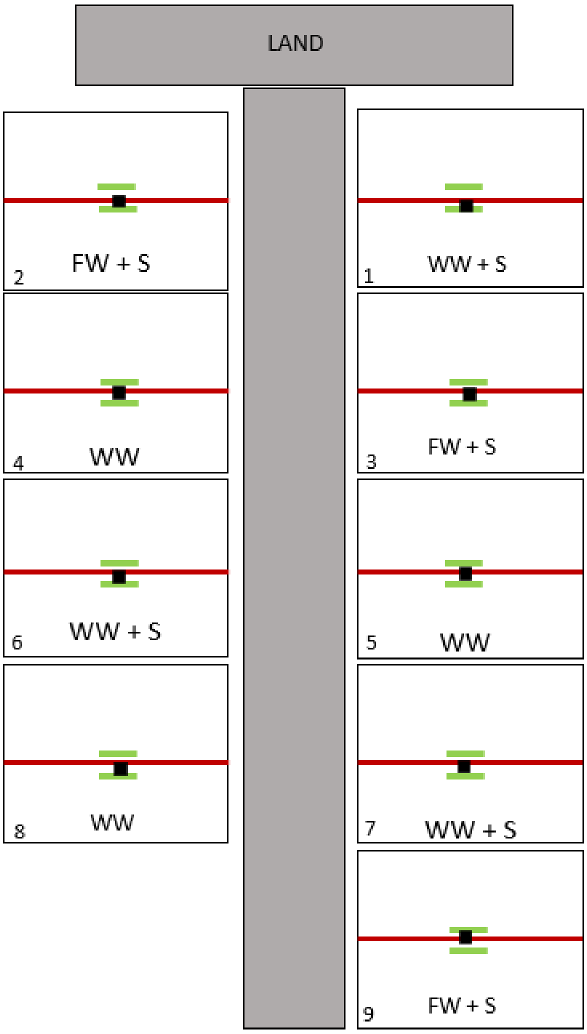


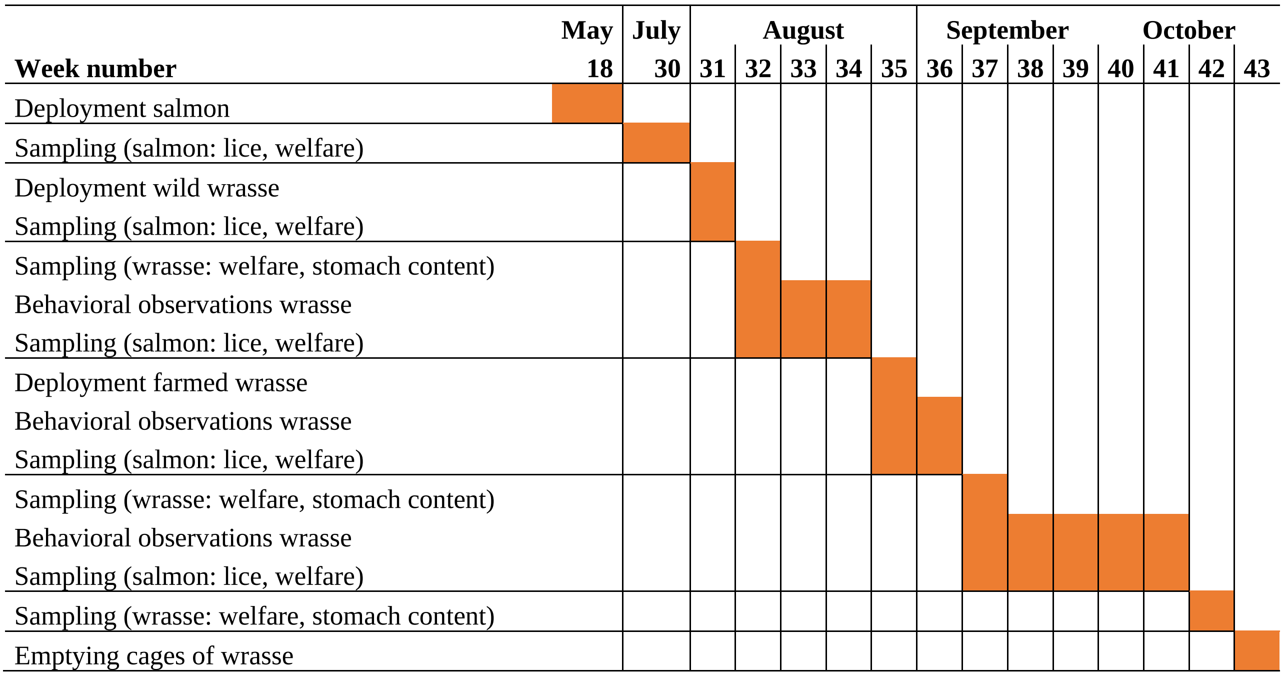
Figure 4. Overview of cage setup. The red lines indicate where the camera is placed and can move. The green lines indicate the placement of the hides. The black square indicates the placement of the feeding station. FW= farmed wrasse, WW= wild-caught wrasse, S= salmon.

The facility followed the standard husbandry protocol during the trial, including daily feeding, daily environmental depth profile recorded manually by a CTD (conductivity, temperature, and depth) instrument (SD204, SAIV AS, Norway), and dead fish registration and removal conducted by the facility workers. Atlantic salmon were fed pellets (Supreme Plus, Skretting, Norway) with an automatic feeder. Wrasse were fed manually with feeding blocks (VAF

Wrasse Feed Blocks, PTAqua, Ireland), pellets (CLEAN Labrus Soft, Skretting, Norway), and shrimp at three depths (3, 6, and 9 m). The feed was refilled every second day.

2.3 Behavioral and depth observations

Throughout the trial, behavioral and depth observations of cleaner fish were done three times a week (Monday, Tuesday, and Friday) (Figure 5).



	May	**July**	**August**					**September**				**October**			
Week number	**18**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**
Deployment salmon	■														
Sampling (salmon: lice, welfare)		■													
Deployment wild wrasse			■												
Sampling (salmon: lice, welfare)			■												
Sampling (wrasse: welfare, stomach content)				■											
Behavioral observations wrasse				■	■	■									
Sampling (salmon: lice, welfare)				■	■	■									
Deployment farmed wrasse							■								
Behavioral observations wrasse							■	■							
Sampling (salmon: lice, welfare)							■	■							
Sampling (wrasse: welfare, stomach content)									■						
Behavioral observations wrasse									■	■	■	■	■		
Sampling (salmon: lice, welfare)									■	■	■	■	■		
Sampling (wrasse: welfare, stomach content)														■	
Emptying cages of wrasse															■

Figure 5. Timeline of sampling during the trial period.

Before the behavioral and depth observations, the visibility in the water was measured manually using a Secchi disk. Furthermore, the wave meters on the day of the observations were checked using the weather forecast site YR (www.yr.no) by searching for Solheim, Masfjorden, Norway, and clicking on "Costal forecast". Behavioral observations were done using underwater cameras (Imenco Gemini Aquaculture camera, Imenco AS, Norway). The cameras were placed in the sea cage, with the ability to be moved up and down and rotated 360 degrees in the horizontal plane with the help of a winch. Observations were done every second meter from the top of the water column down to the sea cage bottom/ lift-up (2, 3, 5, 7, 9, 11, 13, and 17 m). The observations were done once in the middle of the hides and once in the open of the sea cage outside of the hides for every observation day in every cage. The observations were done when daylight was present (08:00 AM – 5:30 PM). One observation took 45–60 minutes. What cage the observations started at was chosen randomly every day but sometimes adjusted so that the observations were not always done at the same time in one cage.

At every depth investigated, the camera was rotated 360 degrees, and the number of wrasse, behavior, and species observed were registered in every cage. Furthermore, it was noted how many salmon were present in the cages with salmon and grouped by a) many, b) few, and c) NA – not applicable. The behavior observed was classified into 19 different behaviors modified from Imsland et al. (2014b, 2016b) and Geitung et al. (2020), as shown in Table 2.

Camera observations were also done at the feeding stations in each cage with wrasse. Camera observations were done for one minute each at the three depths to observe at what depth the different species fed and what type of feed was preferred. Feeding observations in one cage took 10–15 minutes. Feeding observations were primarily done in the morning before the behavioral observations unless the feed needed to be refilled. If so, the feeding observations were done after the facility workers had refilled the feed.

Table 2. Different types of behavior of wrasse in a sea cage environment are modified from Imsland et al. (2014b, 2016b) and Geitung et al. (2020).

Behavior	**Explanation**
a) Cleaning salmon (CS)	Observed actively cleaning *L. salmonis* of salmon.
b) Inspecting salmon (IS)	Observed inspecting behavior towards salmon.
c) Hovering (H)	Observed almost motionless near the hide area.
d) Swimming amongst hide (SWH)	Observed swimming around artificial kelp in the hide corridor.
e) Resting (hide) (RH)	Observed resting on artificial kelp.
f) Resting (net) (RN)	Observed resting on the net folds and corners.
g) Resting (other) (RO)	Observed resting on other objects, etc., lift-up.
h) Swimming along net (SWN)	Observed swimming up, down, or around the net.
i) Swimming in hide corridor (SWHC)	Observed swimming inside of the hide corridor.
j) Swimming in open (SWO)	Observed swimming in the sea cage outside of the hides.
f) Swimming among salmon (SWAS)	Observed swimming in the sea cage with salmon.
g) Eating pellets away from salmon (EPAFS)	Observed eating salmon feed away from salmon.
h) Competing for pellets (CFP)	Observed competing for feed with another wrasse.
i) Feeding on rope fouling (FRF)	Observed feeding on biofouling on ropes in the sea cage.
j) Feeding on net fouling (FNF)	Observed feeding on biofouling on the net.
p) Feeding on floating organisms (FFO)	Observed feeding on floating organisms in the sea cage.
q) Aggression wrasse (AGW)	Observed showing aggression towards another wrasse.
r) Aggression salmon (AGS)	Observed showing aggression towards salmon.
s) Other (O)	Observed showing other types of behavior, etc., swimming amongst lift-up.

2.4 Sampling

Sampling of wrasse were done three times during the trial period to score the welfare of the fish and check the stomach content (Figure 5). The sampling was done over two days, from morning to afternoon, with four weeks in between each sampling (weeks 32, 37, and 42). Ten wrasse from each cage were captured using traps containing feed in closed containers that were put out 20–40 minutes before fish collection. Wrasse were humanely euthanized using an overdose of Finquel (FINQUEL® vet. 1000 mg/g). Length and weight were measured on the sampled wrasse. Welfare scoring was based on Noble et al. (2019) RENSWEL OWI fact sheet series and Gutierrez Rabadan et al. (2021) (Table 3). Additionally, the pectoral fins of all the dead farmed ballan wrasse were scored due to poor fin welfare.

Furthermore, the stomach content in every wrasse from the esophagus to the rectum was identified. The stomach content was grouped by a) cleaner fish feed, b) salmon feed, c) different types of biofoulings, d) different types of zooplankton, e) fish scales, f) salmon lice, g) empty, and h) unidentifiable.

The three sea cages with only wild wrasse stood empty for several months until the wrasse was stocked, accumulating wild fish such as haddock (*Melanogrammus aeglefinus*), cod (*Gadus morhua*), pollock (*Pollachius virens*), Atlantic pollock (*Pollachius pollachius*) and European hake (*Merluccius merluccius*). Similar instances have been documented by Fjelldal et al. (2018, 2021). Consequently, wild fish were removed on 17–18 August 2022 because of negative interference with behavioral observations. Removal was done by lifting the sea cage and netting out the wild fish with minor handling of the wrasse. Wild fish were also present in the cages with salmon, but not as many to negatively influence the behavioral observations. Therefore, the wild fish were not removed here to reduce the handling of the fish.

At the end of the trial, the cages were emptied for wrasse to check if the remaining number of fish corresponded with what was initially stocked in the cage minus dead fish (Figure 5). This was done by lifting the cages and netting out every wrasse. Each fish was counted, identified to species level, and put in a holding tank for further use. The farmed ballan wrasse were humanly euthanized due to poor fin condition.

Table 3. Scores and definitions of welfare indicators based on the RENSWEL OWI fact sheet (Noble et al., 2019) and (Gutierrez Rabadan et al., 2021).

Physical condition	**Score**	**Definition**
Fins	0	No splitting or erosion
	1	Mild damage, shallow splitting, or erosion
	2	Moderate damage, moderate splitting, or erosion
	3	Severe damage, deep splitting to bays of rays, or severe erosion
Skin	0	No lesions or damage
	1	Mild damage with a moderate area of scale loss
	2	Moderate damage with minor active wounds or lesions
	3	Severe damage with large wounds or lesions
Eye	0	Good eye condition
	1	Mild damage to one or two eyes is not likely to cause blindness.
	2	Moderate damage or cataracts likely to cause blindness in one eye.
	3	Severe damage or cataracts likely to cause blindness in both eyes.
Mouth deformity	0	No deformities
	1	Mild deformity not likely to cause much impairment
	2	Moderate deformity causing some impairment
	3	Severe deformity
Opercula	0	No damage
	1	Mild damage on both sides or moderate damage on one side
	2	Moderate damage on both sides or severe damage on one side
	3	Severe damage on both sides

Following standard guidelines during the rearing phase, weekly lice counting was done every Tuesday during the trial period (Figure 5). The lice were classified by life stage (copepodite, chalimus I, chalimus II, preadult I, preadult II male, preadult II female, adult male, adult female, adult female with egg strings). In addition to lice counting, weight and length were measured, and welfare scoring was done according to SWIM (salmon welfare index model) based on (Noble et al., 2018). 20 salmon from each cage were captured using a haw on the facility boat. Feeding was turned off the day before sampling to use pellets to attract the fish. Salmon were humanely euthanized using an overdose of Finquel (FINQUEL® vet. 1000 mg/g) or Benzocaine (Benzoak vet. 200 mg).

2.5 Statistical analysis

All figures and the statistical analysis of the collected data were performed using R software (R v. 4.2.2) and RStudio v. (RStudio, Inc, Boston, USA). Data are presented as mean with standard error, if not else is stated. A significance level of $p<0.05$ was used for all the statistical models and further divided into blocks of $p<0.01$, $p<0.001$, and $p<<0.001$ to indicate how significant the results were. A zero-inflated binominal (z-inb) GLM was used on the count data for depth and behavior to deal with overdispersion and excess zeros. The depth range in the cages was divided into four different depth levels, upper (2 and 3m), upper-mid (5 and 7 m), mid (9 and 11 m), and bottom (13 and 17 m). A zero inflated negative binominal GLM was done to compare differences in depth preferences between the different species in cages with and without salmon (Appendix II, Table 2) and differences in depth preferences between the different treatment groups and species (Appendix II, Table 1, 2). To compare the feeding depth preferences in the different treatment groups, a zero-inflated negative binomial GLM was used (Appendix II, Table 3). A zero-inflated negative binominal GLM was preformed to compare behavior preferences in the different treatment groups (Appendix II, Table 4–9 and 14) and to compare the differences in behavior preference of corkwing and goldsinny in cages with and without salmon (Appendix II, Table 10–13). To compare the differences in mortality between the treatment groups, the percentage values were arcsine transformed before a one-way ANOVA was done (Appendix II, Tables 15–16). A one-way ANOVA was performed to compare the differences in welfare scoring of fins (Appendix II, Tables 17–18). Significant differences revealed with the one-way ANOVA were followed by a Tukey HSD post-hoc test to determine differences between experimental groups.

3. Results

3.1 Environment

At the beginning of the trial, the temperature was 14–16 ºC in the upper water layer and 12 ºC in the deeper water layer, whereas at the end of the trial, the water temperature in the upper layer was 8–10 ºC and 12–14 ºC in deeper water layers (Figure 6A). The highest recorded temperature during the trial was 16 ºC in September. The salinity during the trial period mainly consisted of brackish water (<16 ppt) between 0 and 6 m (Figure 6B).

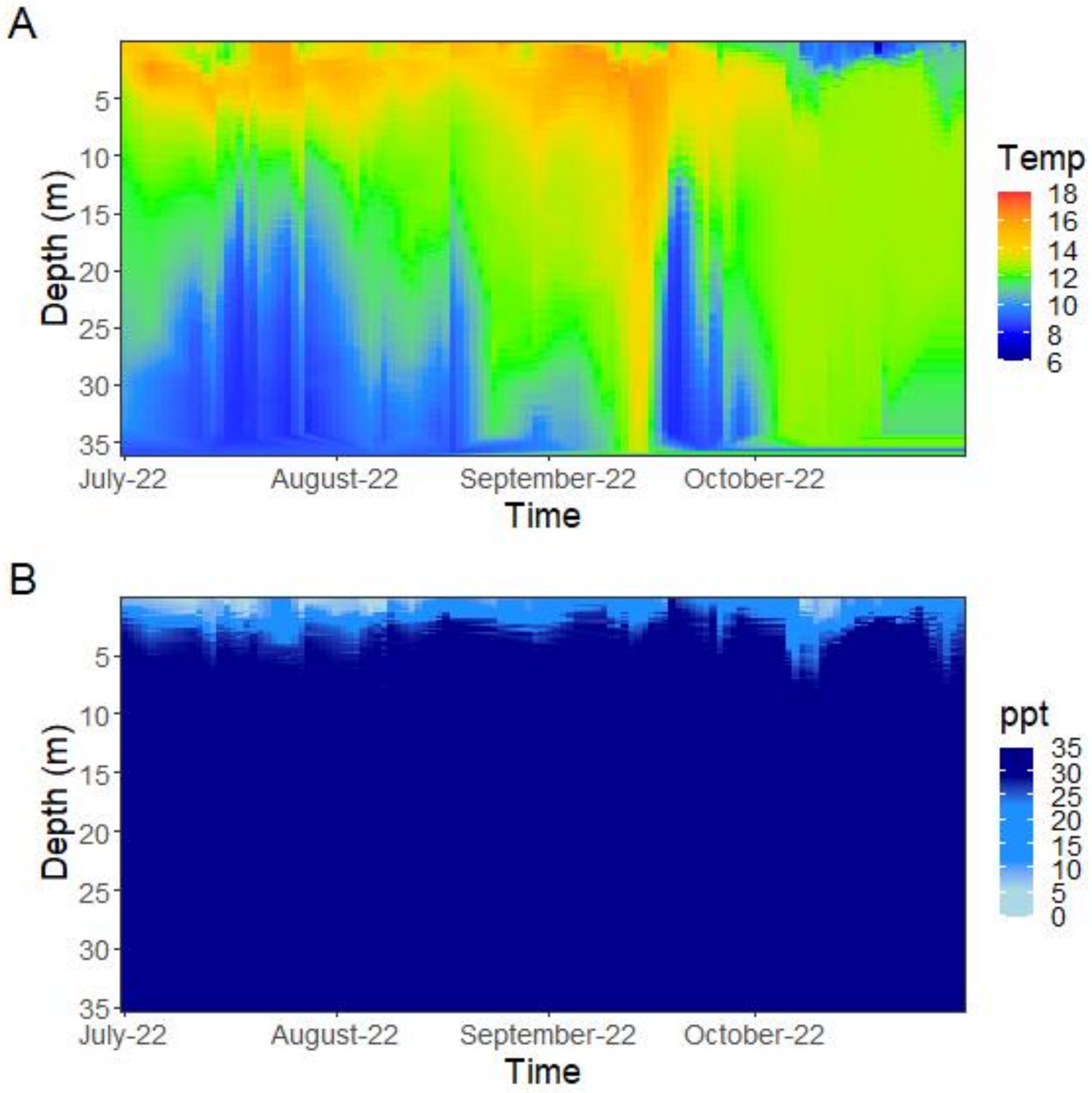


Figure 6. Daily profiles from depth 0 – 35 m of A) temperature and B) salinity from a reference point at the end of the farm at Solheim.

3.2 Depth

The wild ballan wrasse was most dominant at a depth of 7 m (zero-inflated negative binominal (z-inb) GLM, z-value=-23.629, p<<0.001) (Figure 7A), but this was based on few data points because there were few wild ballan wrasse used in the trial (mean = 5.2 ± 1.4 per cage). In the cages with Atlantic salmon, corkwing was most dominant in the upper layer of the water column from 2–7 m (z-inb GLM, z-value=2.458, p<0.05) (Figure 7B). In contrast, farmed ballan wrasse was mainly observed at depths between 9–17 m (z-inb GLM, z-value=3.382, p<0.001) (Figure 7C). The same applied to goldsinny, which was most dominant in the deeper part of the water column (z-inb GLM, z-value=-8.493, p<<0.001) (Figure 7D).

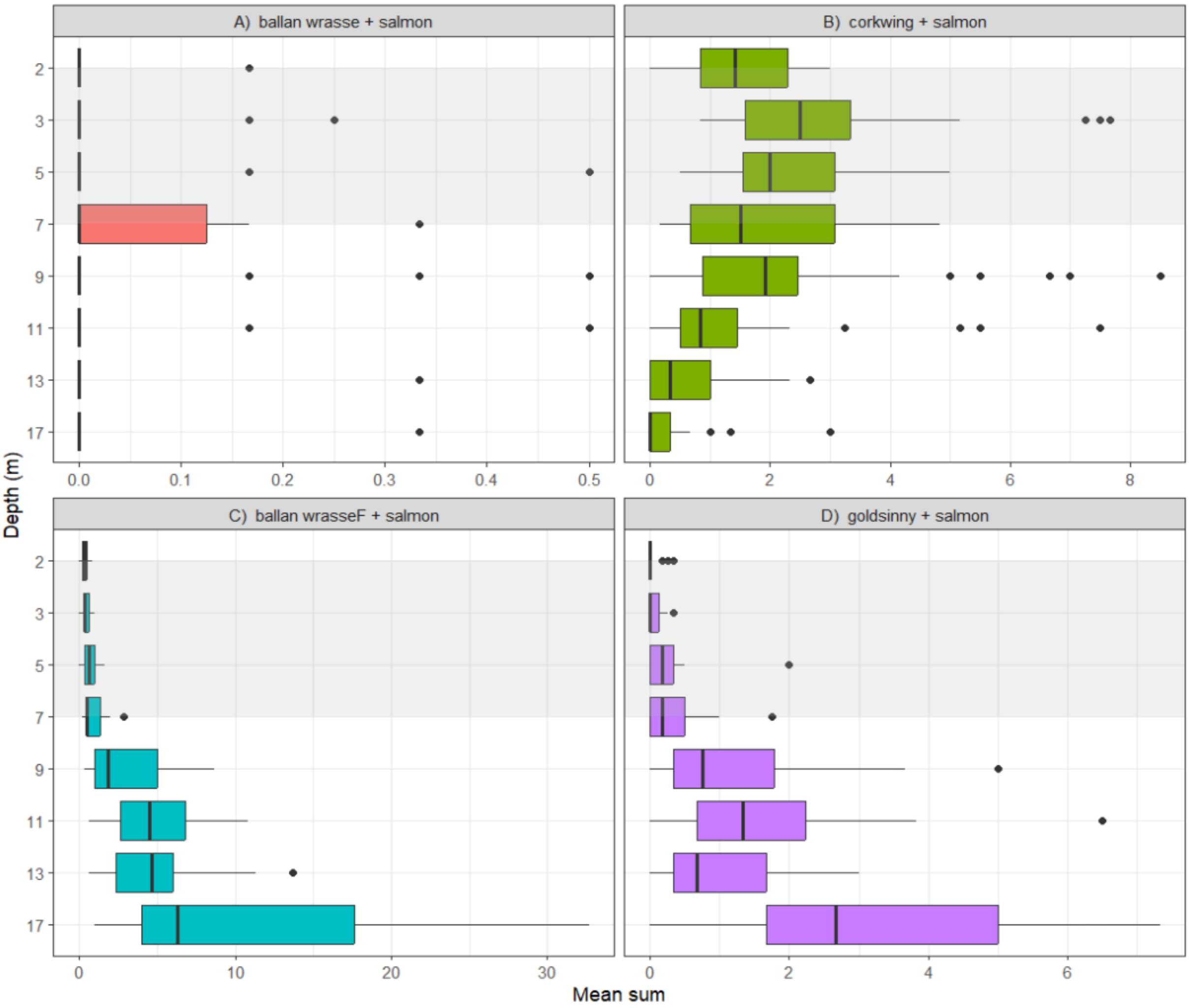


Figure 7. Mean daily depth for ballan wrasse, corkwing, farmed ballan wrasse, and goldsinny in cages with salmon. The grey background indicates where salmon was present. Black dots represent outliers in the dataset. The x-axis on each boxplot is adjusted for the number of observations of each species, and therefore slightly different between the species.

There were limited observations of wild ballan wrasse, but differences between cages with and without salmon were observed. Wild ballan wrasse was observed most often at 2 and 5 m in cages without salmon (z-inb GLM, z-value =-22.156, p<<0.001) (Figure 8D), and at 7 m when salmon were present (z-inb GLM, z-value =2.209, p<0.05) (Figure 8A). Corkwing were present at the same depths in the sea cages with and without salmon (Figure 8B, 8E), but a higher number of corkwings were observed in cages without salmon (mean= 6 ± 6 with salmon vs. 1.7 ± 2 without salmon). In cages with salmon, goldsinny was observed deeper in cages at depths from 9–17 m (z-inb GLM, Z-value =-8.493, p<<0.001), below the area where salmon was mainly observed (depths 2–7 m). However, in cages without salmon, goldsinny used the entire water column and was present from 2–7 m as well (z-inb GLM, z-value=-1.459, p<0.001) (Figure 8F). Farmed ballan wrasse was dominant in the lower depths of the sea cage (z-inb GLM, z-value=3.382, p<0.001) (Figure 8G). Wild and farmed ballan wrasse show different depth preferences in cages with salmon, respectively 7 m (z-inb GLM, z-value=-23.629, p<<0.001) (Figure 8A) and 9–17 m (z-inb GLM, z-value=3.382, p<0.001) (Figure 8G).

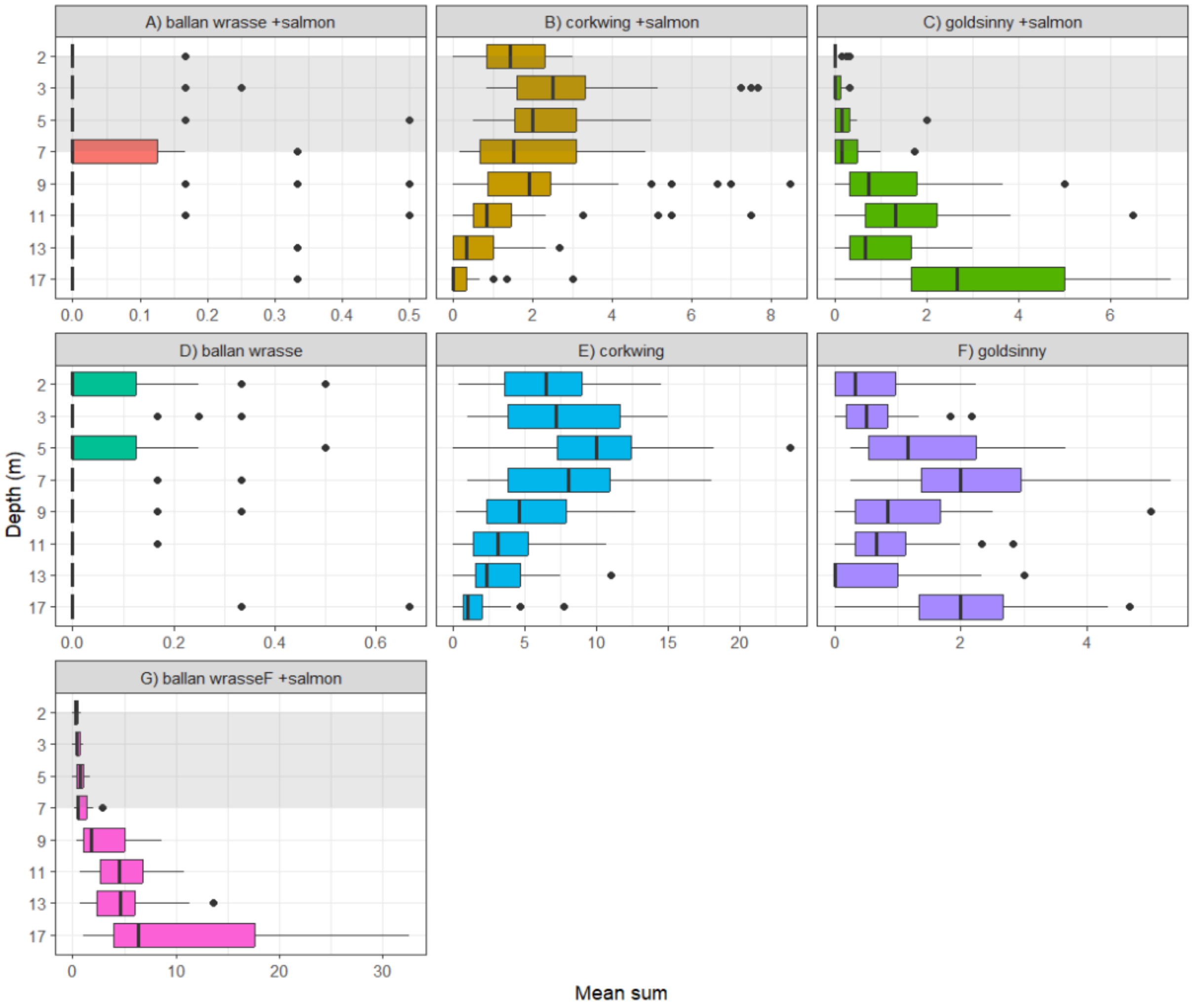


Figure 8. Boxplot of daily mean depth for ballan wrasse, corkwing, goldsinny, and farmed ballan wrasse in the three different treatment groups. The grey background indicates where salmon was present. The black dots are outliers in the dataset. The x-axis on each boxplot is adjusted for the number of observations of each species and therefore different, which should be considered when comparing cages with and without salmon.

In cages without salmon, both corkwing and goldsinny used the entire depth range (2–17 m) throughout the trial period (Figure 9A). In cages with salmon, corkwing used a greater variety of depths at the beginning of the trial. In contrast, towards the end of the trial, corkwing was mostly observed in the upper layer of the water column (Figure 9B) (z-inb GLM, z-value=2.458, $p<0.05$). Goldsinny was more frequently observed deeper in cages with salmon through the entire trial period (z-inb GLM, z-value=-7.217, $p<<0.001$) (Figure 9B). More observations were recorded of wrasse in cages without salmon than in cages with salmon (mean = 3.8 ± 6 without salmon vs. 1.3 ± 2 with salmon, independent of depth).

Figure 9. Dot plot of mean depth preference over time in cages without salmon (A) and cages with salmon (B). Species: corkwing (CW) and goldsinny (GS). Ballan wrasse is excluded from the figure because of very limited data. The black dotted line in B) indicates where salmon was present. The fish count represents the size of each dot in the figure.

Farmed ballan wrasse was mainly observed deep in the water column (z-inb GLM, z-value=3.382, p<0.001), where salmon was not present (Figure 10).

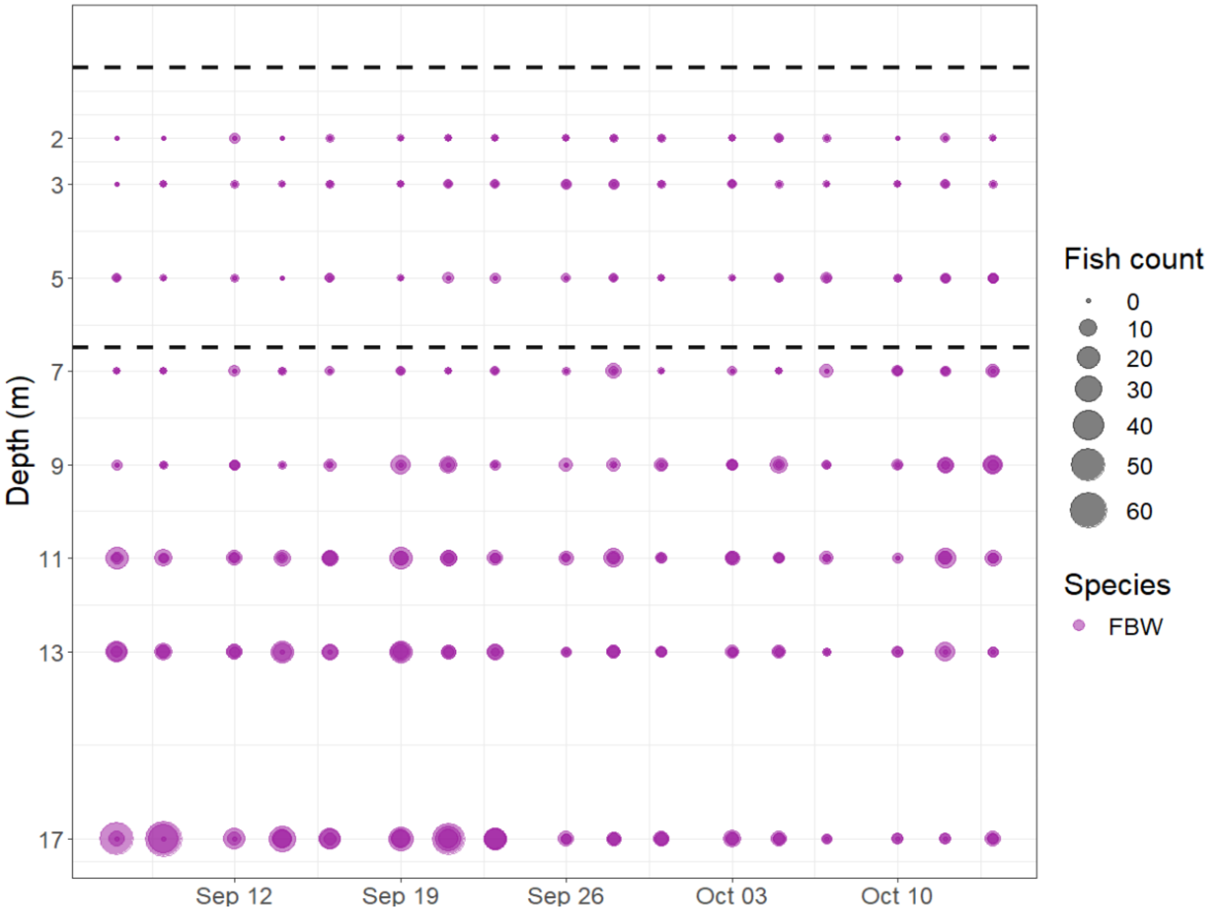


Figure 10. Dot plot of mean depth preference over time for farmed ballan wrasse in cages with salmon. The black dotted line indicates where salmon was present. Species: farmed ballan wrasse (FWB).

Wild wrasse in cages with salmon were often observed actively feeding at the shallowest feeding stations, 3 m (z-inb GLM, z-value=-4.651, p<<0.001) and 6 m (z-inb GLM, z-value= -4.970, p<<0.001), and only sometimes at the deepest feeding station at 9 m (z-inb GLM, z-value=-7.873, p<<0.001). Wild wrasse in cages without salmon was observed actively feeding at all depths (Figure 11). Wild wrasse was more often observed actively feeding at the feeding stations in cages without salmon, compared to with salmon (mean = 0.6 ± 0.9 with salmon, 2.5 ± 2.6 without salmon). Farmed ballan wrasse was rarely observed feeding at the feeding stations.

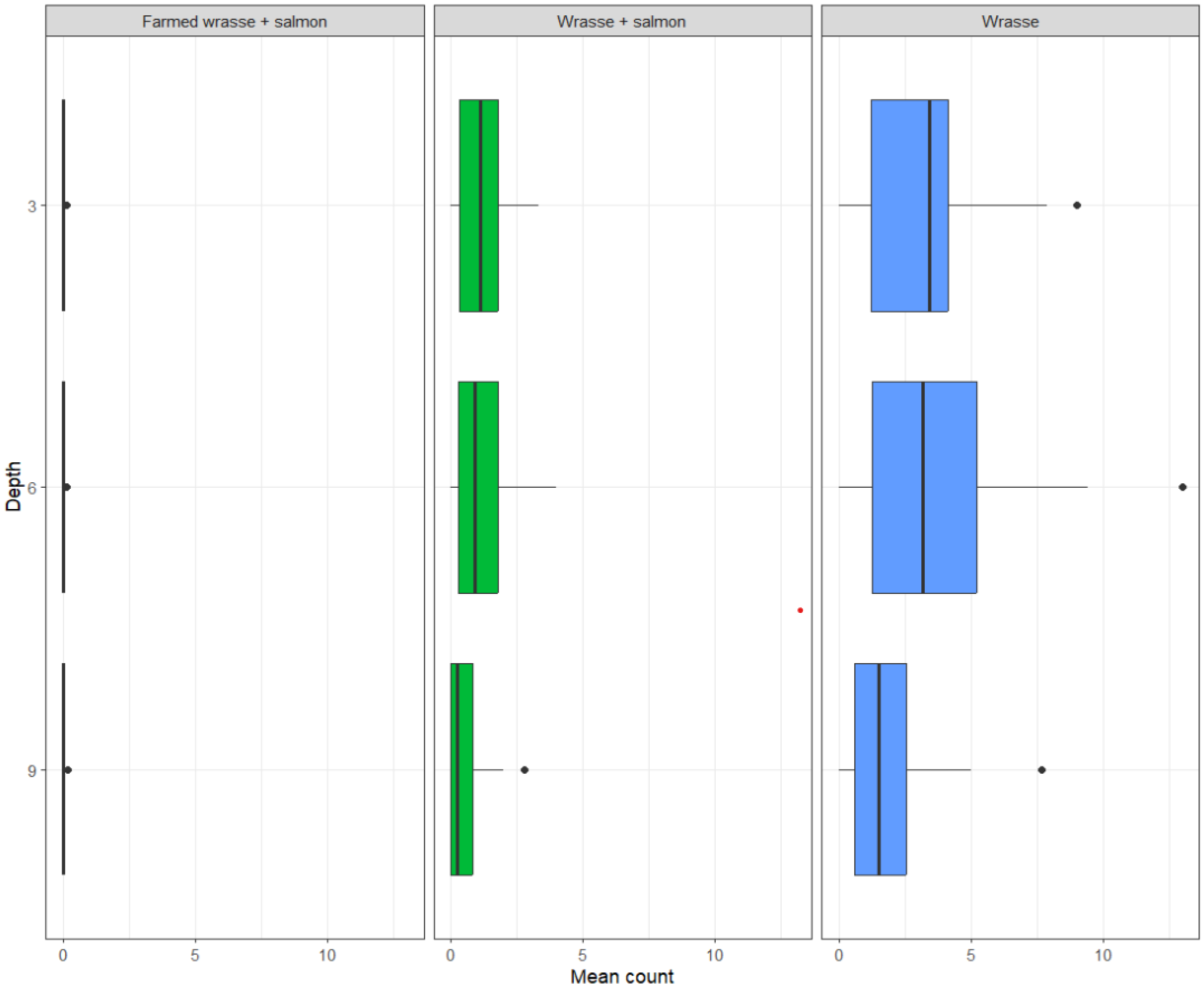


Figure 11. Boxplot of daily mean feeding depth for the three different treatment groups: farmed ballan wrasse + salmon, wild wrasse + salmon, and wild wrasse, recorded at feeding stations at depths 3, 6, and 9 m. The black dots are outliers in the dataset. Deployment of farmed ballan wrasse was one month later than wild wrasse, hence one month less with feeding observations.

3.3 Behavior

Swimming amongst hide, swimming along net, swimming in open, swimming in hide corridor, and resting other accounted for 87% percent of the total recorded behavior for the three treatment groups (Figure 12). There was a limited recording of the remaining behaviors, and some were not observed during the entire trial period, such as inspecting salmon, cleaning salmon, and aggression towards salmon (Figure 12) (Appendix II. Table 19).

The most observed behavior for wild-caught wrasse in cages with salmon was swimming along net (z-inb GLM, z-value=3.488, p-value<0.001), whereas, in cages without salmon, it was swimming in hide (z-inb GLM, z-value=23.835, p<<0.001). The most observed behavior for farmed ballan wrasse, on the other hand, were resting behaviors, with resting net on top (z-inb GLM, z-value=19.518, p<<0.001), followed by resting other (zero-inflated negative binominal GLM, z-value=5.264, p<<0.001) and resting hide (Figure 12). In comparison, these resting behaviors were rarely observed for wild wrasse in cages with (z-inb GLM, z-value=-27.028, p<<0.001) or without (z-inb GLM, z-value=-9.767, p<<0.001) salmon. The resting behaviors were reflected in the depth preference for farmed ballan wrasse (Figure 8G, Figure 10).

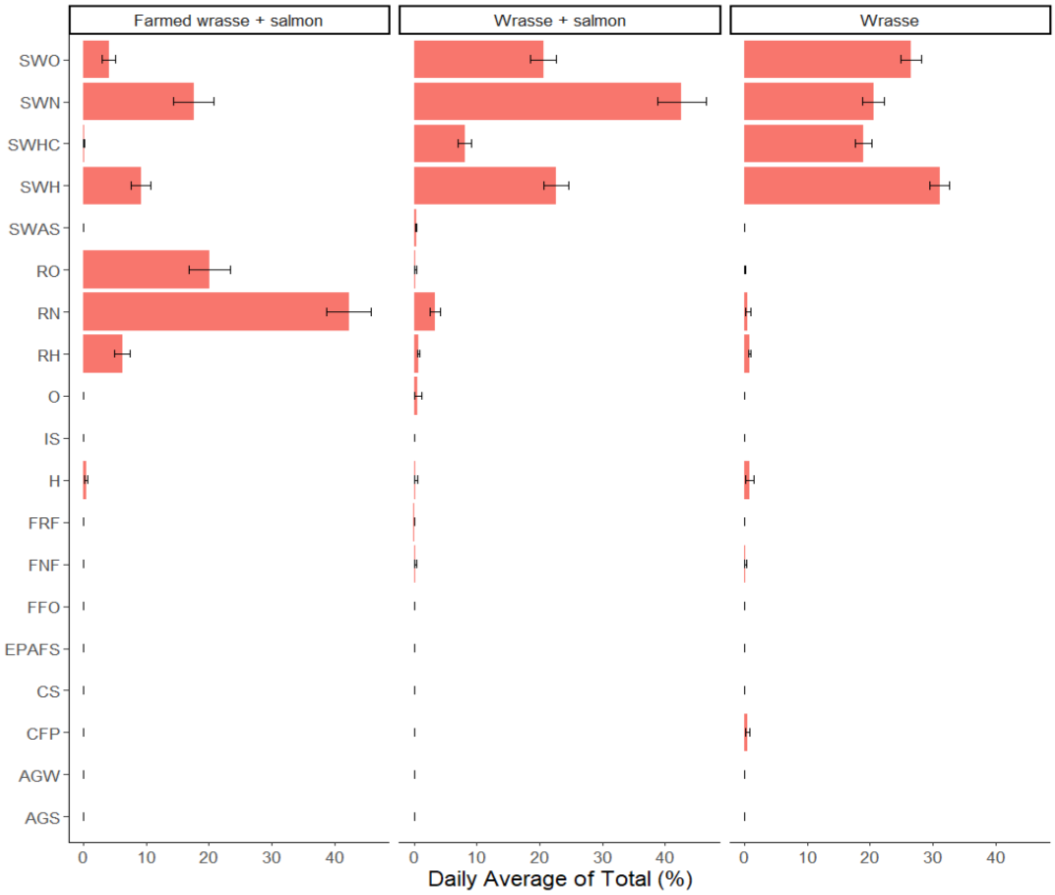


Figure 12. Daily average of total recorded behavior (±SE) in cages with farmed wrasse and salmon, wild wrasse and salmon, and wild wrasse without salmon. For an explanation of abbreviations for behavior, see Table 2.

The most observed behavior for corkwing in cages with salmon was swimming along net z-inb GLM, z-value=6.435, p<<0.001) (Figure 13A), while in cages without salmon the most observed behavior for corkwing was swimming in open (z-inb GLM, z-value=10.790, p<<0.001) and swimming in hide (z-inb GLM, z-value=12.689, p<<0.001) (Figure 13B). Corkwing was observed exhibiting a greater variety of behaviors in cages without salmon, from swimming in open, swimming along net, and swimming in hide and hide corridor (Figure 13B). In cages with salmon, goldsinny was mainly observed swimming along the net (z-inb GLM, z-value=-4.584, p<<0.001) (Figure 13A). While in the cages without salmon, they were most often observed swimming amongst hide (z-inb GLM, z-value=2.486, p<0.05) (Figure 13B). Goldsinny was observed exhibiting several different behaviors in cages without salmon (Figure 13B) compared to with salmon (Figure 13A).

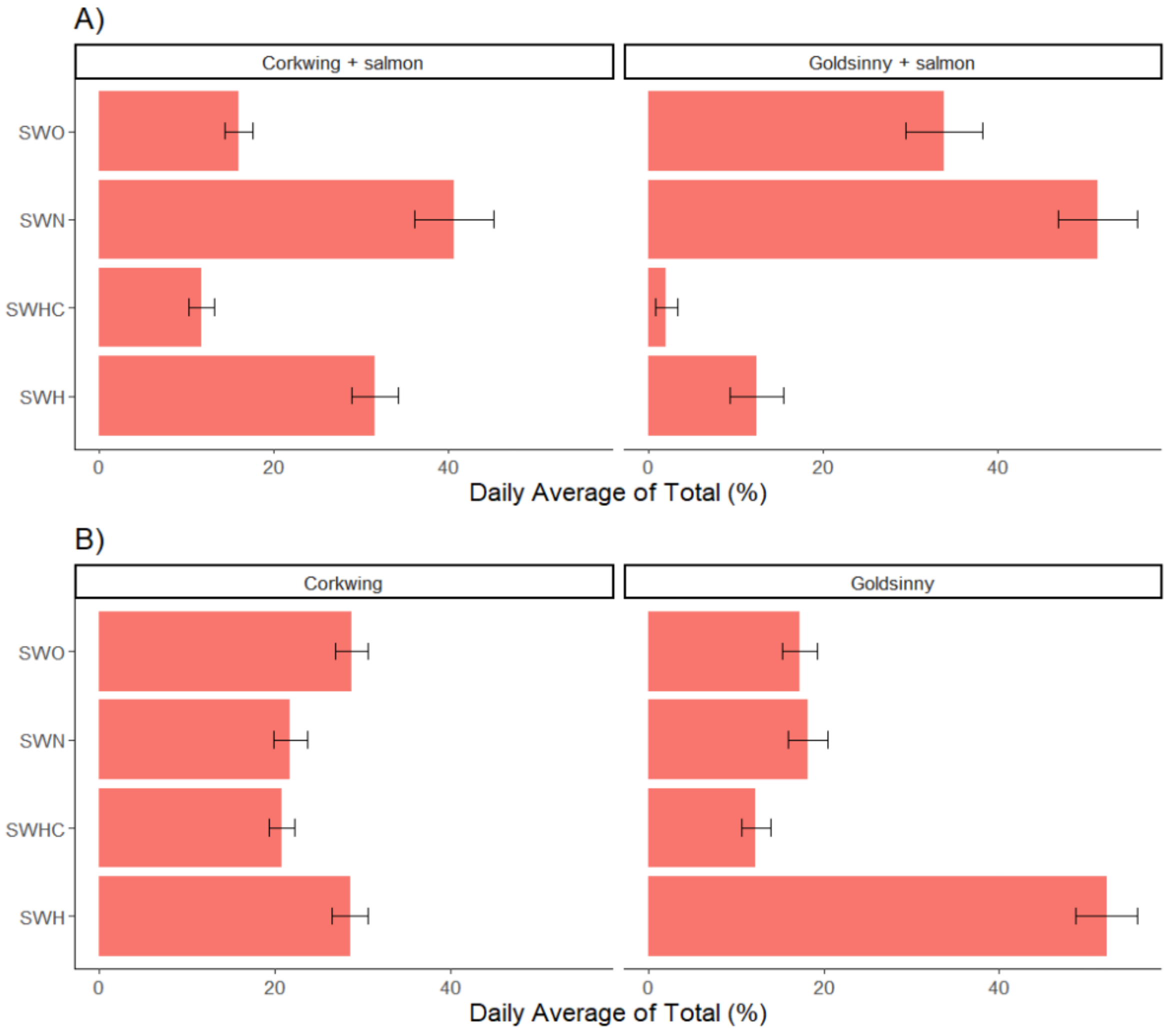


Figure 13. Daily average of total recorded behavior (±SE) of corkwing and goldsinny in cages with salmon (A) and corkwing and goldsinny in cages without salmon (B). Wild ballan wrasse is excluded from the figure because of limited data points. For an explanation of behavior abbreviations, see Table 2.

3.4 Mortality and welfare

Wrasse in cages without salmon had a registered mortality of 16 ± 2% and a daily mortality of 0.9 ± 0.1%. In contrast, wrasse in cages with salmon had a higher mortality of 33 ± 10% and a daily mortality of 2 ± 0.5% (one-way ANOVA, $F=34_{2,207}$, $p<<0.001$) (Figure 14). If the unregistered mortality were added (found when the remaining cleaner fish were counted at the end of the trial) to the registered mortality, wrasse in cages without salmon had a total loss of 38 ± 5%, and wrasse in cages with salmon had a total loss of 54 ± 4%. Farmed ballan wrasse had a registered mortality of 19 ± 3% (Figure 14), a total loss of 23 ± 3%, and a daily mortality of 1.9 ± 0.2%.

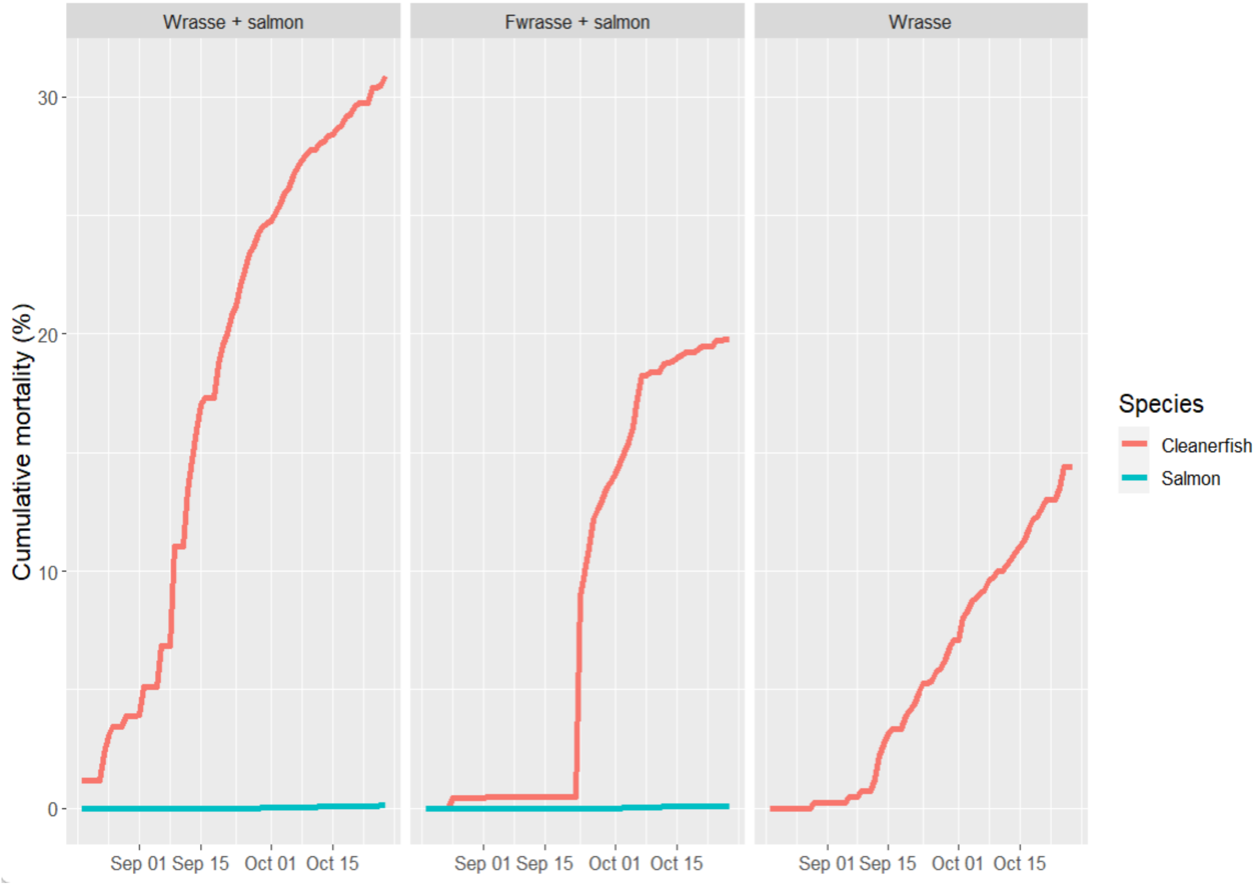


Figure 14. Overview of mean registered mortality for the three different treatment groups. Wild wrasse + salmon, farmed wrasse + salmon, and wild wrasse. The mortality of all the wrasse in the different treatment groups is added together and called cleaner fish due to some incorrect registration of the different species during the trial. Farmed ballan wrasse was deployed one month later than wild wrasse. In the cages with farmed ballan wrasse, the lift-up was not run until three weeks after deployment because live wrasse was resting on the lift-up. Consequently, there was a high spark in mortality at the end of September because this was the first time the lift-up was run after deployment of farmed ballan wrasse.

There were differences in welfare scoring of fins between the treatment groups (one-way ANOVA, $F=5513_{2,10}$, p<<0,001). Farmed ballan wrasse generally had a much higher fin score than wild wrasse in the two other treatment groups, with 45% of the fish receiving a score of 2 and 55% with a score of 3 (Figure 15). Whereas wild wrasse in both cages with and without salmon had generally good fin welfare (Figure 15), with 86 % and 90 % received a score of 1, respectively. Only 15% of wrasse in cages with salmon and 10% of wrasse in cages without salmon got a score of 2 (Figure 15).

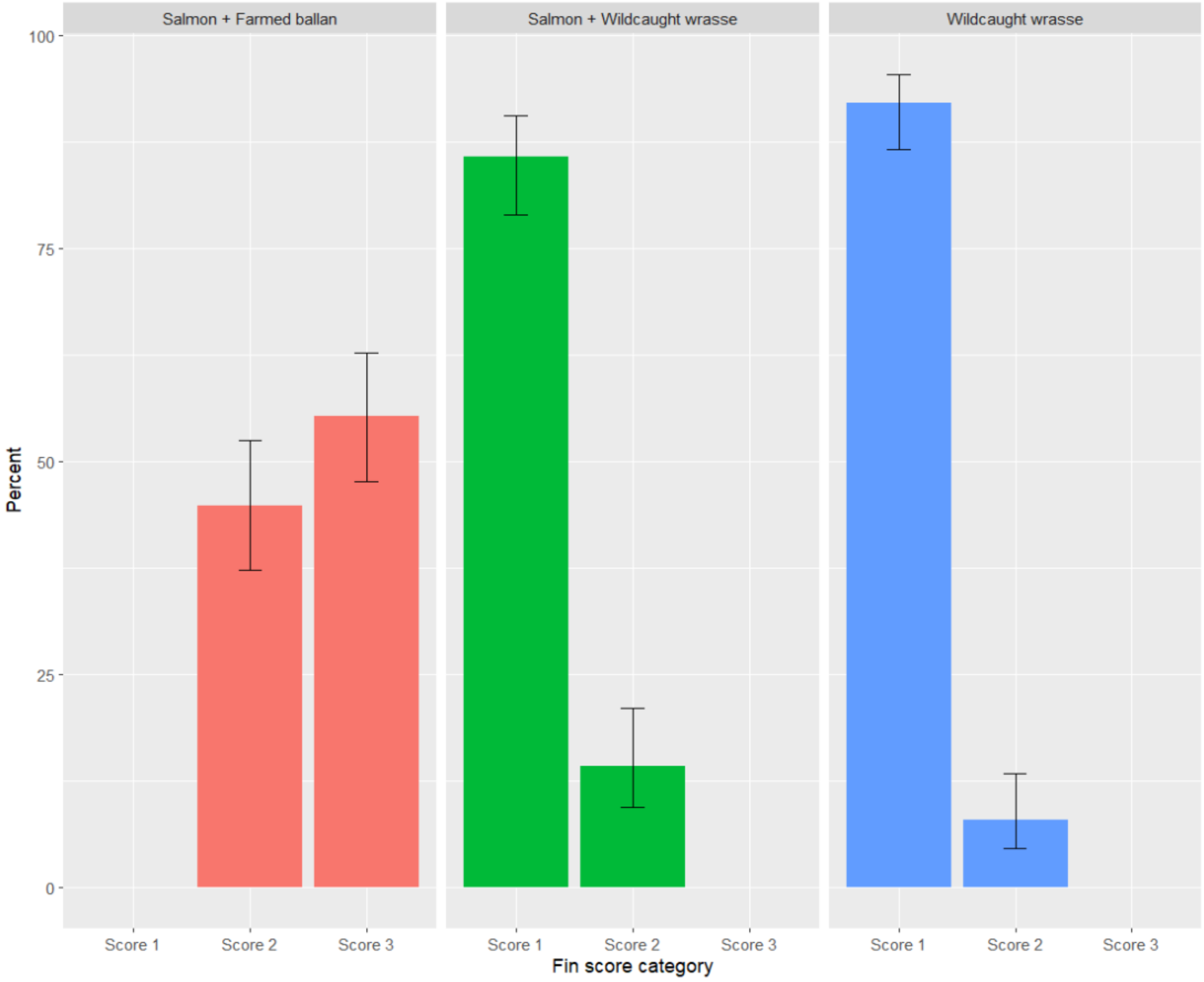


Figure 15. Welfare scoring of fins in the different treatment groups from the last sample in the trial. The fin score category is based on RENSWELL OWI factsheet (Figure 3). In the figure, score 1 = 0, score 2 = 1 and 2, and score 3 = 3. The fin score categories are presented with a 95% confidence interval.

Lice numbers were low throughout the entire trial period in all treatment groups, and delousing was not necessary (Figure 16).

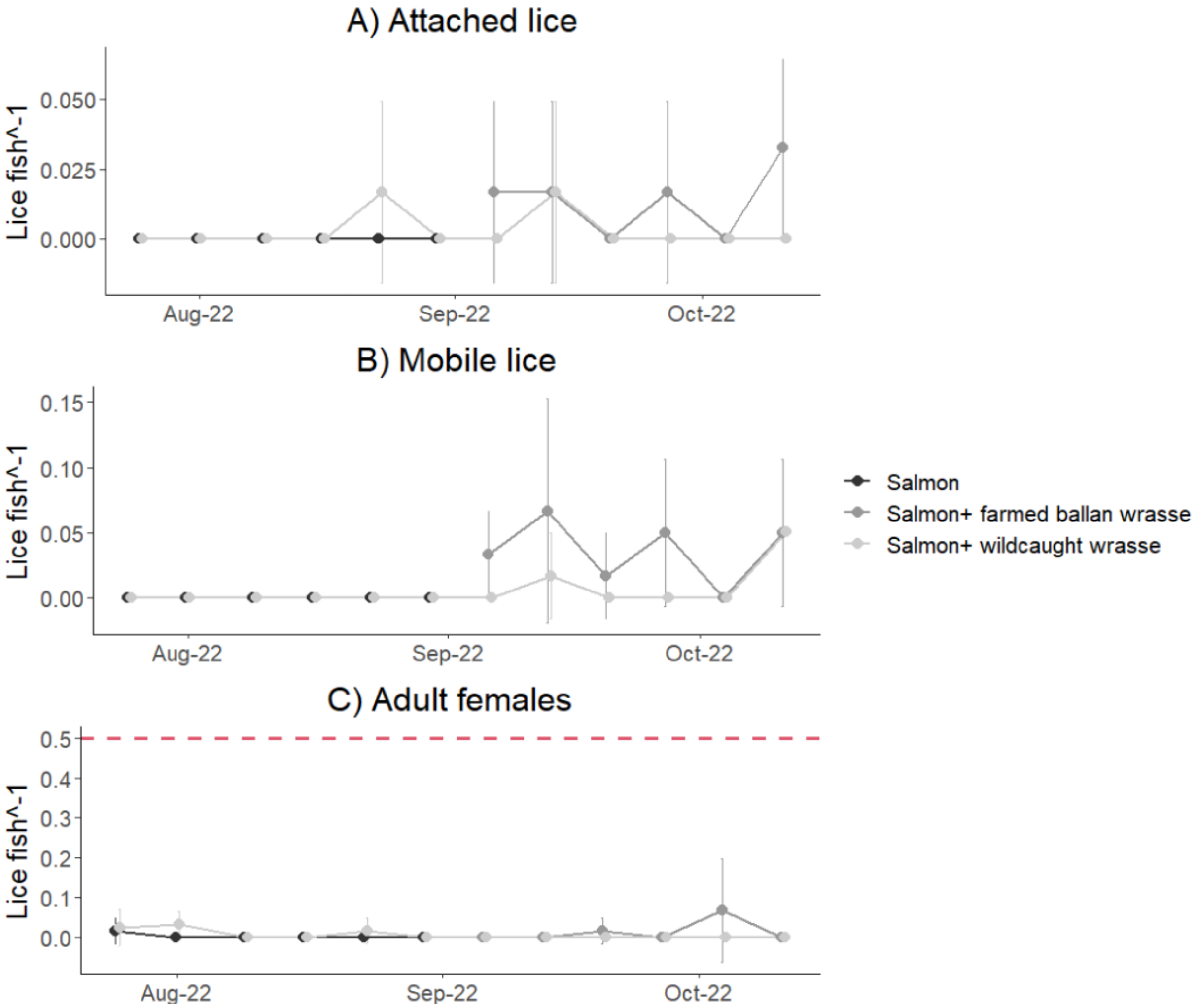


Figure 16. Mean lice count (±SE) of attached lice (copepodit, chalimus 1, chalimus 2), mobile lice (preadult 1, preadult 2 male, preadult 2 female, adult male), and adult females in cages with only salmon (25.07.22 – 2.09.22), salmon and farmed ballan wrasse (03.09.22 – 11-10.22) and salmon with wild caught wrasse (25.07.22 – 11.10.22) during the trial period. The broken line in Figure 16C indicates the Norwegian regulation lice treatment threshold of 0.5 gravid female lice/salmon.

When stomach content was examined, most corkwing had eaten CF feed (Table 4). There was one louse found in one corkwing. For goldsinny, the majority had eaten biofouling or fish scales, and most of the farmed ballan wrasse were empty. The fish scales category included both wrasse and salmon scales. However, the majority had a silver coating around them (J. Kleppe, pers. Obs.), indicating that they were salmon scales.

Table 4. Listing of observed stomach content from sampling of 339 corkwing, 73 goldsinny, and 180 farmed ballan wrasse.

Feed category	Corkwing	Goldsinny	Farmed ballan wrasse
Cleaner fish feed	152	27	9
Salmon feed	3	0	0
Biofouling	46	34	39
Zooplankton	54	11	0
Fish scales	55	30	7
Salmon lice	1	0	0
Empty	81	7	112
Unidentifiable	43	16	27

4. Discussion

4.1 Depth

Salmon influenced the depth preference of goldsinny and ballan wrasse, but not corkwing wrasse. In cages with salmon, goldsinny used less of the depth range and stayed mainly near the bottom of the cage (9–17 m), where salmon were not present. However, in cages without salmon, goldsinny used the entire depth range (2–17m). To be a well-functioning cleaner fish, it is essential that the species can coexist. In this trial, the presence of salmon seemed to “push” goldsinny to stay at a deeper depth away from salmon. Consequently, the question arises whether this can affect the cleaning ability of goldsinny. Goldsinny is one of the smallest cleaner fish used, usually 10–14cm (Treasurer, 2018). Thus, it can be suggested that goldsinny is potentially intimidated by bigger fish, such as salmon, and therefore prefer to stay at a depth where salmon is not present. When observations were done at the feeding stations, goldsinny kept a distance and was not feeding when ballan wrasse was feeding (J. Kleppe, pers. obs.). In a study by Imsland et al. (2016a) which investigated behavioral interactions between goldsinny and lumpfish in land-based tanks, goldsinny was found to be positioned near the bottom of the tank. There was also reported aggression from lumpfish towards goldsinny, and the authors suggest that this might be a contributing factor as to why goldsinny was positioned close to the bottom of the tank. Tully et al. (1996) also reported that wrasse was positioned at the bottom of the tank where salmon was not present. This strengthens the suggestion that goldsinny can be intimidated by bigger fish. Nevertheless, when stomach content was sampled on 73 goldsinny, fish scales were found in 30 of them. Most of the scales were salmon scales, which indicates that goldsinny have some sort of interaction with salmon. From this, it could be inferred that goldsinny prefers to stay at depths where salmon is not present but occasionally swim to depths where salmon is present to potentially forage for salmon lice and act as an opportunistic feeder. In addition, the salmon scales in the stomach content might indicate that goldsinny bite salmon even though there are no lice or do not necessarily eat the salmon lice even though it is present, which could potentially harm the skin of salmon (Kaland et al., 2023). Another possible reason why goldsinny showed a different depth preference in the two treatment groups could be due to the brackish water layer present from 0–6 m during most of the trial. Sayer et al. (1993) performed a field study on the distribution and density of goldsinny populations on the west coast of Scotland. The study reported that goldsinny was largely absent in one of the study areas, where freshwater runoffs likely affected the depths (Sayer et al., 1993). In addition, it is reported that goldsinny did not tolerate reduced salinity levels in Scottish salmon farms (Sayer et al., 1993)

For corkwing, on the other hand, salmon did not have an impact on the depth preference, as corkwing stayed at the same depths in cages with and without salmon (2–11 m). However, there were recorded fewer observations of corkwing in the cages with salmon, indicating that salmon might affect the activity level to some degree. Since corkwing and salmon stayed at the same depth in this study, one can argue that corkwing is the wrasse species that would be best suited as a cleaner fish and is a well-functioning species in the sea cage environment. In addition, corkwing has been reported as the most abundant wrasse species (Skiftesvik et al., 2015) and might be less susceptible to overfishing when the fisheries are well regulated. During sampling of the stomach content of 339 corkwings, salmon lice were found in the stomach content of one corkwing, a preadult 1, which confirms that corkwings exhibit cleaning behavior. Findings of lice in the stomach content of corkwing have also been reported by Gentry et al. (2019), but this was only in 11% of the fish examined (Gentry et al., 2019). There were also found salmon scales in the stomach content of 55 corkwings. As mentioned earlier, the salmon scales might indicate that corkwing bite salmon even though there are no lice or do not necessarily eat the salmon lice even though it is present, which could potentially harm the skin of salmon (Kaland et al., 2023). Despite sharing the same depth preference as salmon, a study by Gentry et al. (2019) reported corkwing as an ineffective cleaner in a full farm setting with high mortality rates. Higher mortality rates for corkwing are also reported by Nilsen et al. (2014), and that study reported that the use of corkwing in the industry is most likely because there are not enough ballan wrasse and goldsinny available. In addition, Nilsen et al. (2014) stated that the use of corkwing should be re-evaluated because of poor survival rates. The reported high mortality of corkwing is consistent with the findings in this study, but this is most likely since corkwing was the dominant wrasse species used in the trial. A study by Treasurer and Feledi (2014) reported corkwing as one of the wrasse species used as cleaner fish most prone to physical damage under aquaculture conditions, which could negatively affect lice grazing and mortality rates.

Wild ballan wrasse was observed in the upper water layer, in cages both with and without salmon, but slightly deeper in the cages with salmon. This indicates that salmon does not affect the depth preference of wild ballan wrasse to any large extent in this study. However, this is based on a few data points, so it is difficult to draw any firm conclusions. A study by Leclercq et al. (2018) showed different findings. Ballan wrasse was found 60% of the time at depths below 15 m, however, this was in larger cages (24 x 24 square m). Ballan wrasse is a large and robust wrasse and is a preferred cleaner fish in the industry (Erkinharju et al., 2021), but it is difficult to obtain the demand. Skiftesvik et al. (2015) conducted a trial on the species richness of wrasse, and the trial showed that ballan wrasse was represented in <2% of the catches. This is reflected in this trial, where only 35 of 3779 wild wrasse were ballan wrasse. It is recommended to have a 5% of salmon number stocking rate (Brooker et al., 2018), which is not possible if only ballan wrasse are used.

Farmed ballan wrasse have shown effective cleaning performance in tank environments (Leclercq et al., 2014) and in small scale studies (Skiftesvik et al., 2013). However, in this study, farmed ballan wrasse were observed mostly at the bottom of the cage (11–17 m), where salmon was not present. Consequently, in this study, the farmed ballan wrasse would be less likely to act as a cleaner in the sea cage environment. This is in line with the findings of Brooker et al. (2020). However, in that study, farmed ballan wrasse started to exhibit a similar depth preference as wild ballan wrasse after one week of acclimatization (Brooker et al., 2020). This indicates the importance of ensuring that necessary acclimatization is carried out for this species in sea cages. Though acclimatization did not have an effect in this trial, the farmed ballan wrasse mainly stayed at the bottom of the cage throughout the entire trial period. Farmed ballan wrasse is known to have a varying effect as cleaner fish in a sea cage environment, as the transition from a tank environment to a sea cage environment can be difficult for the cleaner (Brooker et al., 2020). However, in this study, the lack of desired cleaner fish performance was most likely due to poor fin welfare, especially the pectoral fins. Wrasse is a species that is highly dependent on the pectoral fins for swimming, as they generate thrust by oscillating (Walker and Westneat, 1997; Yuen et al., 2019). Consequently, without well-functioning pectoral fins, it is hard for the farmed ballan wrasse to swim and thereby also graze lice on a moving salmon.

Wrasse was actively feeding in cages with and without salmon, indicating that salmon does not have a negative impact on feeding for wrasse. The feeding stations were placed in the middle of the hides, away from where salmon was dominant in the sea cage. So, it figures that salmon influences the depth and behavior of wrasse but not the food intake. Food is essential for survival. Therefore, one can assume that is why salmon were not altering the feed intake of wrasse in sea cages.

4.2 Behavior

The behavioral observations showed that wrasse in cages with and without salmon exhibited different behaviors, which indicates that the presence of salmon can alter the behavior of all wrasse species tested in the present trial. In cages without salmon, the most observed behavior was swimming in hide. However, in the cages with salmon, the presence of salmon seemed to drive wrasse out towards the cage net and corners. Other studies show similar behavior, where ballan wrasse was often observed in the corners of the sea cage (Leclercq et al., 2018; Brooker et al., 2020; Geitung et al., 2020). Salmon is the dominant species in the cage environment due to its size and numbers and, consequently, could alter the preferred behavior of wrasse in the sea cage to some degree. The second most observed behavior of wrasse in cages with salmon was swimming in hide. Salmon were rarely observed swimming inside the hide corridor during the trial, supporting the theory that wrasse, to some extent, avoided areas where salmon was present and that the hides function as a refuge where wrasse can relax without any disturbance from salmon. For instance, Sayer et al. (1993) report the availably of suitable refuge as the main limiting factor for the distribution of goldsinny in the wild, as they were only observed closely related to rock surfaces. A study by Overton et al. (2020) reviewed the evidence of sea lice removal in salmon aquaculture, and the findings of that study reported that there are limited recordings of interactions between salmon and cleaner fish. The second most observed behavior was swimming along net, which aligns with the findings of Tully et al. (1996), who recorded goldsinny behavior in commercial scale sea cages with scuba divers. Indicating that swimming along the net is normal behavior for goldsinny in sea cages with salmon, as well as minor interactions with salmon.

Something wild wrasse had in common in both cages with and without salmon was exhibiting different swimming behaviors (SWO, SWN, SWHC, and SWH), while there were minimal recordings of resting behaviors (RN, RO, and RH). Farmed ballan wrasse, on the other hand, predominantly only exhibited resting behaviors (RN, RO, and RH). This was also reflected in the depth preference of farmed ballan wrasse, which was dominant in the deeper parts of the sea cage, either resting at the bottom of the cage or along the net. In this study, it was most likely because of the poor fin welfare of the farmed ballan wrasse. Good cleaner fish welfare is essential to promote natural behavior and lice grazing (Brooker et al., 2018). The result of this study is a clear example of how poor fin welfare can come at the expense of desired behaviors.

There were no observations of any wrasse species cleaning salmon or inspecting salmon during the entire trial period. An essential factor to justify using wrasse as cleaner fish is that they exhibit cleaning behavior and go foraging for salmon lice. Despite the findings in this study, several studies have reported lice eating (Bjordal, 1988; Deady et al., 1995; Tully et al., 1996; Skiftesvik et al., 2017). However, these are mainly in tank trials, and there are limited recordings of effective delousing of cleaner fish in commercial scale studies (Overton et al., 2020). The behavior observations are only a snapshot of what the fish is doing when the observations are done (Gutiérrez-Estrada et al., 2022), so there might have been incidents of wrasse eating salmon lice that were not recorded. Another explanation for why there was not observed cleaning behavior in this trial could be because the lice numbers were very low throughout the entire trial period. Furthermore, there was brackish water from depths 0–6 m throughout the entire trial period, which may have a connection to the low lice numbers since salmon lice prefer higher salinity (Coates et al., 2021).

When looking at behavior on species level, there was a difference in behavior between corkwing and goldsinny, which was also reflected in the depth preference of the two species. In the wild, corkwing and goldsinny inhabit the same rocky and inshore areas (Treasurer, 1994). Consequently, one can argue that it is beneficial to have the two species in sea cages to complete each other’s shortcomings related to cleaning behavior.

When corkwing was present in cages without salmon, it had a greater variation in behavior, indicating that salmon can influence the behavior of corkwing. A study by Norin et al. (2021) investigated the effects of predator presence on corkwing. The results showed that predators affect the behavior of prey species by being present in the environment (Norin et al., 2021). Even though corkwing and salmon do not have a prey-predator relationship, similar effects are shown in this trial. This could potentially be because the presence of salmon could be intimidating to corkwing.

4.3 Mortality and welfare

The conditions in the cage environment were arranged for the fish to thrive. All cages were equipped with hides, and the wrasse was fed three different feeds continuously. Yet there was still a 16% mortality of wrasse in cages without salmon. The question arises whether this is normal mortality for wrasse or if there are other causes for this. One explanation for some registered mortality could be the late effects of high cortisol levels due to physical stress from capture and handling. High cortisol levels can be associated with reduced growth rates (Mommsen et al., 1999; Treasurer et al., 2018a) and ultimately can lead to higher mortality (Finstad et al., 2003; Treasurer et al., 2018a). Another factor that could have contributed to the registered mortality is the wild fish, such as haddock, cod, pollock, and European hake, found in the cages. This could have been a perceived stressor for wrasse, which is defined as fear and presence of a predator (Treasurer et al., 2018a). Cannibalism is common for cod in both the wild and aquaculture (Puvanendran et al., 2008), and cod is categorized as a predator for wrasse (Norin et al., 2021). However, the cod were small and not likely to consume wrasse, given the size. Wild fish were dominant at the feeding stations, especially at 9 m (J. Kleppe, pers. obs.). The wild fish were removed from the cages after the trial had started. This was done by lifting the cages, with minor handling of wrasse, but this could have affected the mortality rates to some degree.

The registered mortality for wrasse in cages with salmon was 33%, which was a twice as high mortality rate compared to cages with only wrasse, indicating that salmon had a negative impact on the mortality of wrasse to some degree. In addition, when the daily mortality was compared for farmed ballan wrasse and wild wrasse in cages with salmon to those without salmon, there was a higher daily mortality in cages with salmon. Wild fish were also present in the cages with wrasse and salmon, which could have influenced the mortality of wrasse. However, there were a limited number of wild fish, as salmon seemed to hinder the wild fish from entering the cages to a large extent (J. Kleppe, pers. obs.). Several other studies have reported high mortality rates for wrasse used as cleaners in cages with salmon (Nilsen et al., 2014; Mo and Poppe, 2018; Geitung et al., 2020; Stien et al., 2020). The high mortality rates contribute to public concern about using cleaner fish (Mo and Poppe, 2018).

Farmed ballan wrasse had a registered mortality of 19%, but some of that mortality could most likely be a consequence of poor fin welfare and not so much the presence of salmon. Farmed ballan wrasse were rarely observed feeding. Severe erosion of the pectoral fins made it hard for the ballan wrasse to swim to the feeding stations. There were put out additional feeding stations and hides where the ballan wrasse was resting. Yet, the farmed ballan wrasse rarely touched the feed, which explains why some farmed ballan wrasse showed signs of emaciation (J. Kleppe, pers. obs.). When the stomach content of 180 famed ballan wrasse was checked, 112 fish had not eaten anything.

There was a high rate of unregistered loss at the end of the trial. Contributing to the fact that welfare is not necessarily optimal for wrasse in sea cages, even though the sea cage conditions followed standard guidelines, with hides and several types of feed available continuously. The unregistered losses could be because of predation from fish outside the cage (Dempster et al., 2009; Uglem et al., 2014) or the wild fish that were trapped in the cages. Another reason, given the small size of wrasse, could be that they decomposed before reaching the lift-up system, which happens quickly for cleaner fish in a sea cage environment (Nilsen et al., 2014) or being stuck in the cage corners. In addition, when the facility workers registered the daily mortality, there were some incorrect registrations of the wrasse species. Therefore, some of the missing wrasse were not necessarily gone, only registered as a different species. But there were still several fish that were missing. Thus, this is not a big part of the explanation. Several other studies have also reported unregistered losses when using wrasse as delousers in salmon aquaculture (Geitung et al. 2020; Stien et al. 2020).

Looking at the welfare scoring on fins, there was no significant difference between wild wrasse in cages with and without salmon. The fins were in good condition at deployment and stayed so throughout the trial. However, for farmed ballan wrasse, the welfare scoring on fins was not good. The majority of the farmed ballan wrasse that was examined got a fin score of 3, which is defined as severe damage with deep splitting to bays of rays or severe erosion (Noble et al., 2019; Gutierrez Rabadan et al., 2021). As mentioned earlier, this highly impacted both behavior and the depth preference for farmed ballan wrasse. The pectoral fins on most of the farmed ballan wrasse had severe erosion, making it hard for the farmed ballan wrasse to perform normal swimming behavior (J. Kleppe, pers. obs.). Poor fin welfare is an issue related to the farming of ballan wrasse and is often associated with clumping in the tanks during the larval stage (Lekva and Grøtan, 2018).

5. Conclusion

Overall, Atlantic salmon were found to alter the depth preferences and behavior of the tested wrasse species. Wrasse exhibited a greater variety of behaviors, and more observations were recorded in cages without salmon than in cages with salmon. Goldsinny did not share the same depth range as salmon, while corkwing shared the same depth range as salmon. Ballan wrasse seemingly had different depth preferences in cages with and without salmon, but was present in the depth range of salmon, however this was based on limited data. Farmed ballan wrasse was dominant at the bottom of the cage where salmon was not present and was mainly observed resting, most likely due to poor fin welfare. Wrasse in cages with salmon had twice as high mortality rate than wrasse in cages without salmon. From the results in this study, it appears that goldsinny and salmon have a mismatch in depth preference, so the likelihood of exhibiting cleaner behavior could be minimized. Corkwing and ballan wrasse share the same depth as salmon and are therefore more likely to exhibit cleaning behavior. The farmed ballan wrasse struggled to function optimal in the sea cages due to poor fin welfare, consequently the likelihood of exhibiting cleaner behavior was less likely.

Hypothesis **$H0_1$** which states that salmon does not impact the depth preference of wrasse **is rejected** in favor of HA_1, indicating that salmon has a significant impact on the depth preference of wrasse**.**

Hypothesis **$H0_2$** which states that salmon does not impact the behavior of wrasse **is rejected** in favor of HA_2, indicating that salmon has a significant impact on the behavior of wrasse.

Hypothesis **$H0_3$**, which states that the mortality of wrasse is not impacted by the presence of salmon, **is rejected** in favor of HA_3, indicating that the presence of salmon impacts the mortality of wrasse.

Hypothesi**s $H0_4$**, which states that there is no variation in depth preference between the different wrasse species, **is rejected** in favor of HA4, indicating a significant variation in depth preference between the different wrasse species.

6. Future perspective

This trial has given insightful information about the depth and behavior preference of wild and farmed wrasse in sea cages with and without salmon during summer-autumn. Salmon influenced the behavior of all wrasse species and altered the depth preference of goldsinny and ballan wrasse, but not corkwing wrasse. This trial was from July to October, so it would have been interesting to do this at another time of the year to investigate whether depth and behavior preferences could be seasonally based. In addition, the lice numbers were low throughout the entire trial, so it would have been interesting to do a new trial with the same setup when lice numbers are higher to investigate lice-eating behavior of the wrasse. Since the wrasse mortality was higher in the cages with salmon present, future trials should investigate how and why salmon influences the mortality and how it can be reduced. The farmed ballan wrasse struggled to perform well in the sea cages due to poor fin welfare. Consequentially, fin improvement is necessary through further research and improvement of rearing practices for ballan wrasse.

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Appendix I – Discussion of methods

Farmed ballan wrasse were deployed one month later than wild wrasse. It would have been optimal to have farmed ballan wrasse throughout the entire trial to have a better basis for comparison with wild wrasse. Though, this was not possible as the farmed ballan wrasse was not ready to be delivered until a month later. Furthermore, there was only a mean of 5.2 ± 1.4 wild ballan wrasse in each cage, out of a total of 540 wrasse in each cage. This gave the results of the depth and behavior preference of wild ballan wrasse less credibility since it was based on very limited data.

The lice numbers were low throughout the trial period, making it hard to record lice-eating behavior. However, regarding the welfare of salmon and the environment, it is good that the lice numbers were low. The focus of this trial was behavior and depth preference of wrasse; therefore, low lice numbers did not come at the expense of answering these research questions.

It is difficult to know if the wild fish, such as haddock, cod, pollock, and European hake, observed in the cages influenced the behavior and depth preference of wrasse because this has not been reported before. Nevertheless, the wild fish in the cages probably had some effect on the behavior of the wrasse. Therefore, most ideally, they should have been removed before deployment of wrasse to ensure no influence on behavior and depth preference as well as welfare and mortality of wrasse.

Depth preferences of wrasse was measured with underwater cameras. The depth gauge on some of the cameras was incorrect and needed service during the trial. Consequently, some of the registered depths could be wrong. When the camera showed the wrong depth, behavior and depth observations were not done in that cage until the depth gauge was fixed. Which gave fewer observations that day, because of this, a rope with each depth marked on was put out. This way, the observations could be done even if the cameras depth gauge was not working. Yet, due to the issues with the depth gauge, there might be some minor incorrect depth registrations. A way that could have limited this uncertainty is if the wrasse were pit tagged with tags that would record where the fish was present all the time, not only a snapshot of the day when the observations were done. This could also have recorded if the depth distribution was different during the night since the observations were only done when daylight was present.

Appendix II – Statistical results

Appendix II. Table 1. Test results of a zero-inflated negative binominal GLM on depth preference of wrasse in cages with and without salmon. The model includes count (the sum of all observed behaviors) as the dependent variable and depth level as the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The depth levels are divided into four different categories, upper (2 and 3m), upper-mid (5 and 7 m), mid (9 and 11 m), and bottom (13 and 17 m). The parameters that start with “CF” indicates that the variables consist of cages with only cleaner fish. The parameter that starts with “Salmon” indicates that the variables consist of cages with salmon and cleaner fish. The reference level is CF upper and without salmon. Cages with farmed ballan wrasse and salmon are excluded from the test because there are no cages with only farmed ballan wrasse. Significant effects ($P < 0.05$) are highlighted in red.

	Negative binominal part - depth		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	1.55955(0.086)	18.037	2E-16
CF bottom	-0.79308(0.127)	-6.223	4.88E-10
CF mid	-0.37162(0.098)	-3.783	0.000155
CF upper-mid	0.20867(0.094)	2.209	0.027152
Salmon bottom	-0.99801(0.138)	-7.217	5.33E-13
Salmon mid	-0.899712(0.102)	-8.764	2E-16
Salmon upper	-1.16587(0.109)	-10.640	2E-16
Salmon upper-mid	-1.13039(0.105)	-10.739	2E-16
Log (theta)	-0,70523(0.103)	-6.836	8.17e-12
	Zero-inflated part - depth		
	Estimate (SE)	Z - value	P - value
Intercept	-0.35462(0.119)	-2.956	0.00311
With salmon	0.25947(0.089)	2.912	0.00359

Appendix II. Table 2. Test results of a zero-inflated negative binominal GLM on depth preference of wrasse on species level in cages with and without salmon. The model includes count (the sum of all observed behaviors) as the dependent variable and species depth level as the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The depth levels are divided into four different categories, upper (2 and 3m), upper-mid (5 and 7 m), mid (9 and 11 m), and bottom (13 and 17 m). Species and depth levels are categorical variables. The reference level is CW upper and without salmon. Significant effects (P < 0.05) are highlighted in red.

	Negative binominal part – depth species		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	1.6503(0.059)	27.875	2,00E-16
Upper BW	-4,75162(0.212)	-22.357	2,00E-16
upper GS	-2.66402(0.108)	-24.629	2,00E-16
upper FBW	-2.28680(0.168)	-13.564	2,00E-16
uppermid BW	-4.42613(0.185)	-23.629	2,00E-16
uppermid CW	0.20271(0.082)	2.458	0.01399
uppermid FBW	-1.57627(0.149)	-10.547	2,00E-16
uppermid GS	-1.45959(0.091)	-15.890	2,00E-16
mid BW	-4.78826(0.216)	-22.156	2,00E-16
mid CW	-0.38354(0.084)	-4.538	5.68E-06
mid FBW	-0.08679(0.127)	-0.68	0.49662
mid GS	-1.36081(0.089)	-15.278	2,00E-16
bottom BW	-5.38423(0.421)	-12.772	2,00E-16
bottom CW	-1.14072(0.117)	-9.709	2,00E-16
bottom FBW	0.52140(0.154)	3.382	0.00072
bottom GS	-0.95672(0.112)	-8.493	2,00E-16
Log(theta)	-0.56817(0.040)	-14.044	2,00E-16
	Zero-inflated part – depth species		
	Estimate (SE)	Z - value	P - value
Intercept	-10.311(20.16))	-0.511	0.609
With salmon	9.148(20.16)	0.454	0.650

Appendix II. Table 3. Test result of a zero-inflated negative binominal GLM on feeding depth preference (3, 6, and 9 m) of wrasse in cages with and without salmon. The model included count (the sum of all observed feeding) as the dependent variable, and treatment group and depth level were the categorical variables in the model. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The parameters that start with “CF” indicates that the variables consist of cages with only wrasse. The parameter that starts with “Salmon” indicates that the variables consist of cages with salmon and wrasse. Significant effects ($P < 0.05$) are highlighted in red.

	Negative binominal part - feed		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	1.3783(0.233)	5.195	3.32E-09
CF 6m	0.0806(0.1918)	0.420	0.67438
CF 9m	-0.5296(0.192)	-2.756	0.00584
Salmon 3m	-0.9715(0.208)	-4.651	3.30E-06
Salmon 6m	-1.0107(0.211)	-4.790	1.67E-06
Salmon 9m	-1.7388(0.220)	-7.873	3.47E-15
Log (theta)	-1.2027(0.281)	-4.277	1.89E-05
	Zero-inflated part - feed		
	Estimate (SE)	Z - value	P - value
Intercept	-1.4161(0.978)	-1.448	0.148
With salmon	-0.4086(0.526)	-0.776	0.438

Appendix II. Table 4. Test results of a zero-inflated negative binominal GLM on behavior “swimming in open” in the different treatment groups. The model included SWO (the sum of all observed SWO behaviors) as the dependent variable, and treatment group was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The reference level is WW and without salmon. WW= wild wrasse, S_FW= salmon + farmed wrasse, and S_WW= salmon + wild wrasse. Significant effects ($P < 0.05$) are highlighted in red.

	Negative binominal part - SWO		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	0.9998(0.131)	7.633	2.29E-14
S_FW	-1.7884(0.228)	-7.835	4.68E-15
S_WW	-1.01516(0.119)	-8.819	2,00E-16
Log (theta)	-0.8952(0.223)	-4.014	5.97E-05
	Zero-inflated part - SWO		
	Estimate (SE)	Z - value	P - value
Intercept	1.0070(0.166)	6.067	1.30E-09
With salmon	0.4804(0.108)	4.436	9.16E-06

Appendix II. Table 5. Test results of a zero-inflated negative binominal GLM on behavior “swimming along the net” in the different treatment groups. The model included SWN (the sum of all observed SWN behaviors) as the dependent variable, and treatment group was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The reference level is WW and without salmon. WW= wild wrasse, S_FW= salmon + farmed wrasse, and S_WW= salmon + wild wrasse. Significant effects (P < 0.05) are highlighted in red.

	Negative binominal part - SWN		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	0.5635(0.191)	2.951	0.003171
S_FW	-0.3959(0.168)	-2.352	0.018664
S_WW	0.3878(0.111)	3.488	0.000487
Log (theta)	-1.2808(0.275)	-4.650	3.32E-06
	Zero-inflated part - SWN		
	Estimate (SE)	Z - value	P - value
Intercept	0.9086(0.254)	3.575	0.00035
With salmon	0.2451(0.109)	-2.231	0.02566

Appendix II. Table 6. Test results of a zero-inflated negative binominal GLM on behavior “swimming in hide corridor” in the different treatment groups. The model included SWHC (the sum of all observed SWHC behaviors) as the dependent variable, and treatment group was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The reference level is WW and without salmon. WW= wild wrasse, S_FW= salmon + farmed wrasse, and S_WW= salmon + wild wrasse. Significant effects (P < 0.05) are highlighted in red.

	Negative binominal part - SWHC		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	1.30386(0.070)	18.619	2,00E-16
S_FW	-4.33220(0.748)	-5.790	7.05E-09
S_WW	-1.20660(0.143)	-8.410	2,00E-16
Log (theta)	0.14576(0.1779	0.821	0.412
	Zero-inflated part - SWHC		
	Estimate (SE)	Z - value	P - value
Intercept	1.85077(0.075)	24.410	2,00E-16
With salmon	0.94105(0.135)	6.965	3.29E-12

Appendix II. Table 7. Test results of a zero-inflated negative binominal GLM on behavior “swimming in hide” in the different treatment groups. The model included SWH (the sum of all observed SWH behaviors) as the dependent variable, and treatment group was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The reference level is WW and without salmon. WW= wild wrasse, S_FW= salmon + farmed wrasse, and S_WW= salmon + wild wrasse. Significant effects ($P < 0.05$) are highlighted in red.

	Negative binominal part - SWH		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	1.32088(0.055)	23.835	2,00E-16
S_FW	-1.37177(0.138)	-9.899	2,00E-16
S_WW	-1.17588(0.091)	-12.823	2,00E-16
Log (theta)	0.14411(0.138)	1.043	0.297
	Zero-inflated part - SWH		
	Estimate (SE)	Z - value	P - value
Intercept	1.32168(0.064)	20.568	2,00E-16
With salmon	0.29938(0.088)	3.365	0.000766

Appendix II. Table 8. Test results of a zero-inflated negative binominal GLM on behavior “resting other” in the different treatment groups. The model included RO (the sum of all observed SRO behaviors) as the dependent variable, and treatment group was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The reference level is S_FW and without salmon. WW= wild wrasse, S_FW= salmon + farmed wrasse, and S_WW= salmon + wild wrasse. Significant effects ($P < 0.05$) are highlighted in red.

	Negative binominal part - RO		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	1.8270(0.347)	5.264	1.41E-07
WW	-8.5091(1.830)	-4.684	3.35E-06
S_WW	-6.5091(0.631)	-10.302	2,00E-16
Log (theta)	-0.9437(0.590)	-1.599	0.11
	Zero-inflated part - RO		
	Estimate (SE)	Z - value	P - value
Intercept	-3.588(64.18)	-0.056	0.955
With salmon	5.873(64.18)	0.091	0.927

Appendix II. Table 9. Test results of a zero-inflated negative binominal GLM on behavior "resting net" in the different treatment groups. The model included RN (the sum of all observed RN behaviors) as the dependent variable, and treatment group was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The reference level is S_WW and without salmon. WW= wild wrasse, S_FW= salmon + farmed wrasse, and S_WW= salmon + wild wrasse. Significant effects ($P < 0.05$) are highlighted in red.

	Negative binominal part - RN		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	-3.5766(0.120)	-29.603	2,00E-16
S_FW	3.7593(0.192)	19.518	2,00E-16
WW	2.0687(0.866)	2.389	0.0169
Log (theta)	-2.7251(0.105)	-25.889	2,00E-16
	Zero-inflated part - RN		
	Estimate (SE)	Z - value	P - value
Intercept	4.0851(0.633)	6.444	1.16E-10
With salmon	-13.3979(67.57)	-0.198	0.843

Appendix II. Table 10. Test results of a zero-inflated negative binominal GLM on behavior "swimming in open" between corkwing (CW) and goldsinny (GS) in cages with and without salmon. The model included SWO (the sum of all observed SWO behaviors) as the dependent variable, and treatment group on species level was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The parameters that start with "CF" indicates that the variables consist of cages with only wrasse. The parameter that starts with "Salmon" indicates that the variables consist of cages with salmon and wrasse. The reference level is Salmon CW and without salmon. Significant effects ($P < 0.05$) are highlighted in red.

	Negative binominal part - SWO		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	0.08863(0.128)	0.690	0.4901
Salmon GS	0.25594(0.165)	1.547	0.1218
CF CW	1.36317(0.126)	10.790	2,00E-16
CF GS	-0.82897(0.150)	-5.496	3.89E-08
Log (theta)	-0.44378(0.186)	-2.378	0.0174
	Zero-inflated part - SWO		
	Estimate (SE)	Z - value	P - value
Intercept	0.2316(0.148)	1.556	0.12
With salmon	1.0693(0.121)	8.831	2,00E-16

Appendix II. Table 11. Test results of a zero-inflated negative binominal GLM on behavior "swimming along the net" between corkwing (CW) and goldsinny (GS) in cages with and without salmon. The model included SWN (the sum of all observed SWN behaviors) as the dependent variable, and treatment group on species level was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The parameters that start with "CF" indicates that the variables consist of cages with only wrasse. The parameter that starts with "Salmon" indicates that the variables consist of cages with salmon and wrasse. The reference level is Salmon CW and without salmon. Significant effects ($P < 0.05$) are highlighted in red.

	Negative binominal part - SWN		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	0.7480(0.116)	6.435	1.23E-10
Salmon GS	-0.5672(0.124)	-4.584	5.41E-06
CF CW	0.5045(0.116)	4.336	1.45E-05
CF GS	-1.3942(0.142)	-9.782	2,00E-16
Log (theta)	-0.6299(0.194)	-3.240	0.00119
	Zero-inflated part - SWN		
	Estimate (SE)	Z - value	P - value
Intercept	0.53988(0.159)	3.380	0.000725
With salmon	0.04478(0.114)	0.391	0.695747

Appendix II. Table 12. Test results of a zero-inflated negative binominal GLM on behavior "swimming in hide corridor" between corkwing (CW) and goldsinny (GS) in cages with and without salmon. The model included SWHC (the sum of all observed SWHC behaviors) as the dependent variable, and treatment group on species level was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The parameters that start with "CF" indicates that the variables consist of cages with only wrasse. The parameter that starts with "Salmon" indicates that the variables consist of cages with salmon and wrasse. The reference level is Salmon CW and without salmon. Significant effects ($P < 0.05$) are highlighted in red.

	Negative binominal part - SWHC		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	0.3018(0.127)	2.376	0.017489
Salmon GS	-2.4833(0.306)	-8.104	5.30E-16
CF CW	1.3151(0.133)	9.818	2,00E-16
CF GS	-0.7091(0.170)	-4.172	3.01E-05
Log (theta)	0.5930	3.412	0.000645
	Zero-inflated part - SWHC		
	Estimate (SE)	Z - value	P - value
Intercept	1.12413(0.079)	14.130	2,00E-16
With salmon	0.85748(0.149)	5.754	8.7E-09

Appendix II. Table 13. Test results of a zero-inflated negative binominal GLM on behavior “swimming in hide” between corkwing (CW) and goldsinny (GS) in cages with and without salmon. The model included SWH (the sum of all observed SWH behaviors) as the dependent variable, and treatment group on species level was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The parameters that start with “CF” indicates that the variables consist of cages with only wrasse. The parameter that starts with “Salmon” indicates that the variables consist of cages with salmon and wrasse. The reference level is Salmon CW and without salmon. Significant effects (P < 0.05) are highlighted in red.

	Negative binominal part - SWH		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	0.57714(0.078)	7.329	1.45E-13
Salmon GS	-2.14851(0.160)	-13.349	2,00E-16
CF CW	1.12335(0.088)	12.689	2,00E-16
CF GS	0.24768(0.099)	2.486	0.0129
Log (theta)	0.63834(0.131)	4.860	1.17E-06
	Zero-inflated part - SWH		
	Estimate (SE)	Z - value	P - value
Intercept	0.87991(0.060)	14.465	2,00E-16
With salmon	0.14323(0.104	1.368	0.171

Appendix II. Table 14. Test results of a zero-inflated negative binominal GLM on resting behaviors (RH, RN, RO) between the different treatment groups. RH, RN, and RO were added together and called resting. The model included resting as the dependent variable, and treatment group on species level was the categorical variable. Cages without/with salmon were the categorical variables in the zero-inflated part of the model. The parameters that start with “CF” indicates that the variables consist of cages with only wrasse. The parameter that starts with “Salmon” indicates that the variables consist of cages with salmon and wrasse. The reference level is S_FW and without salmon. Significant effects (P < 0.05) are highlighted in red.

	Negative binominal part – (RH, RN, RO combined)		
Parameter	Estimate (SE)	Z - value	P - value
Intercept	0.64651(0.107)	6.041	1.53E-09
WW	-3.82880(0.392)	-9.767	2,00E-16
S_WW	-3.96405(0.146)	-27.028	2,00E-16
Log (theta)	-2.03508(0.084)	-23.964	2,00E-16
	Zero-inflated part – (RH, RN, RO combined)		
	Estimate (SE)	Z - value	P - value
Intercept	-3.402E-04(7.020E-01)	0.000	1.000
With salmon	-1.055E+01(6.161E+01)	-0.171	0.864

Appendix II. Table 15. Test results of a one-way ANOVA on mortality in the different treatment groups. Significant effects (P < 0.05) are highlighted in red.

	One-way ANOVA - mortality				
Effect	SS	DF	MS	F	P
Treatment group	1.792	2	0.8956	34.03	1.67E-13
Residuals	5.447	207	0.0263		

Appendix II. Table 16. Test results of Tukey HSD post-hoc test on mortality in the different treatment groups. Significant effects (P < 0.05) are highlighted in red.

POST-HOC	
Effect	P
WW- FWS	0.3153265
SWW- FWS	0.0000000
WW- SWW	0.0000000

Appendix II. Table 17. Test results of a one-way ANOVA on fin welfare scoring in the different treatment groups. Significant effects (P < 0.05) are highlighted in red.

	One-way ANOVA – fin scoring				
Effect	SS	DF	MS	F	P
Treatment group	2.5570	2	1.2785	5513	6.11E-16
Residuals	0.0023	10	0.0002		

Appendix II. Table 18. Tukey HSD post-hoc test results on fin welfare scoring in the different treatment groups. Significant effects (P < 0.05) are highlighted in red.

POST-HOC	
Effect	P
WW- FWS	0.0000000
SWW- FWS	0.0000000
WW- SWW	0.0011932

Appendix II. Table 19. Summary statistics for behavior variables, independent of species and treatment groups.

Variable	n	sum	mean	median	min	max	Sd.
SWN	77	3361	43.64	32	0	169	35.77
SWH	77	3360	43.63	24	0	200	45.58
SWO	77	3058	39.71	26	0	198	45.44
SWHC	77	1864	24.20	10	0	100	30.14
RN	77	967	12.55	1	0	144	26.06
RO	77	417	5.48	0	0	88	14.61
RH	77	174	2.25	1	0	16	2.80
CFP	77	57	0.74	0	0	30	4.42
H	77	43	0.55	0	0	12	1.93
FNF	77	14	0.18	0	0	4	0.57
O	77	14	0.18	0	0	11	1.27
SWAS	77	12	0.15	0	0	3	0.51
FEF	77	4	0.05	0	0	2	0.27
AGW	77	2	0.02	0	0	2	0.22
AGS	77	0	0	0	0	0	0
CS	77	0	0	0	0	0	0
EPAFS	77	0	0	0	0	0	0
FFO	77	0	0	0	0	0	0
IS	77	0	0	0	0	0	0

Appendix II. Table 20. Summary statistics for the different treatment groups.

	Treatment group		
	SWW	WW	SFW
n	3504	3277	714
sum	3066	8421	1863
mean	0.875	2.569	2.609
median	0	0	0
min	0	0	0
max	32	39	60
Sd.	2.165	5.277	5.720

Appendix II. Table 21. Summary statistics for corkwing, ballan wrasse, and goldsinny in treatment group: wild wrasse.

	Species		
	CW	BW	GS
n	624	623	623
sum	3750.5	26.0	745.5
mean	6.010	0.041	1.196
median	4.0	0.0	0.5
min	0	0	0
max	5	2.	5
Sd.	6.227	0.174	1.551

Appendix II. Table 22. Summary statistics for corkwing, ballan wrasse, and goldsinny in treatment group: salmon + wild wrasse.

	Species		
	BW	CW	GS
n	658	658	658
sum	1109.5	1109.5	595.5
mean	0.035	1.686	0.905
median	0	1	0
min	0	0	0
max	1.5	17.5	15.0
Sd.	0.155	2.306	1.980

Appendix II. Table 23. Summary statistics for farmed ballan wrasse in the treatment group: salmon + farmed ballan wrasse.

	Species
	FBW
n	408
sum	1344.5
mean	3.295
median	1
min	0
max	60
Sd.	6.486

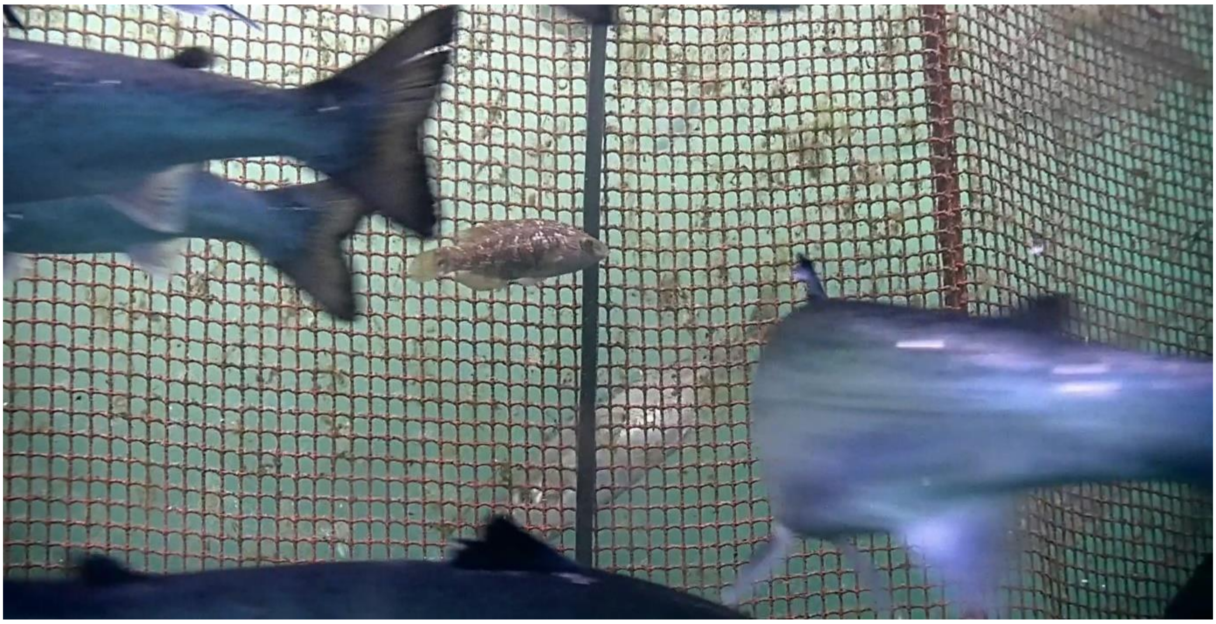
Appendix II. Table 24 Summary statistics for corkwing, goldsinny, and farmed ballan wrasse in the different treatment groups. Ballan wrasse is excluded in this table due to limited data points.

	Treatment group		
	SWW	WW	SFW
n	2336	2185	714
sum	3022	8372	1863
mean	1.293	3.831	2.609
median	0	1	0
min	0	0	0
max	32	39	60
Sd.	2.547	6.080	5.720

Appendix II. Table 25. Summary statistics for observations done at the three treatment groups feeding stations.

	Treatment group		
	SWW	WW	SFW
n	93	93	54
sum	87.0	279.0	0.5
mean	0.936	3.001	0.009
median	0.666	2.555	0.000
min	0	0	0
max	4.0	13.0	0.1
Sd.	0.945	2.619	0.033

Appendix III – Pictures



Appendix III. Picture 1. Picture of corkwing swimming along net in a cage with salmon.

Appendix III. Picture 2. Pictures of farmed ballan wrasse resting in different areas of the cage.

Appendix III. Picture 3. Wild fish inside of the cages with wrasse. Wild fish are dominating the feeding station.