Aquaculture 402–403 (2013) 113–118

Contents lists available at SciVerse ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*)

Anne Berit Skiftesvik *, Reidun M. Bjelland, Caroline M.F. Durif, Inger S. Johansen, Howard I. Browman

Institute of Marine Research, Austevoll Research Station, 5392 Storebø, Norway

ARTICLE INFO

Article history:
Received 6 March 2013
Received in revised form 26 March 2013
Accepted 28 March 2013
Available online 10 April 2013

Keywords:
Integrated pest management
Green aquaculture
Lepeophtheirus salmonis
Sea lice control
Wrasse

ABSTRACT

The ectoparasitic salmon louse (*Lepeophtheirus salmonis*) is a serious problem in salmon aquaculture (*Salmo salar* and rainbow trout *Oncorhynchus mykiss*). These parasitic copepods attach to fish and feed on their mucus and tissue, reducing feed conversion efficiency and causing sores, thereby increasing farming costs and reducing the value of the product. Many non-pharmaceutical approaches to controlling sea lice are being developed. One such is to use cleaner fish (in this case, wrasse) in co-culture with salmon to remove salmon lice. The objectives of this study were to assess the efficiency of wrasse as delousing agents and to compare the relative efficiency of wild vs. cultured individuals. Wrasse were extremely efficient in delousing salmon. At a ratio of 5% wrasse to salmon, the mean number of mobile lice life history stages on salmon was maintained at a level of less than one per fish. Intensively cultured wrasse were as efficient as wild wrasse at removing lice. The presence of wrasse did not affect the growth of salmon. This study demonstrates that wrasse, including intensively cultured ballan wrasse naïve of either salmon or salmon lice, can be introduced into sea cages on salmon farms and keep salmon lice loads at very low levels.

1. Introduction

The ectoparasitic salmon louse (*Lepeophtheirus salmonis*) is responsible for enormous economic losses to the salmon aquaculture industry (*Salmo salar* and rainbow trout *Oncorhynchus mykiss*) costing hundreds of millions of Euros annually (Costello, 2009). These parasitic copepods attach to fish and feed on their mucus and tissue, reducing feed conversion efficiency and causing sores, thereby increasing farming costs and reducing the value of the product. Many pharmaceuticals have been used to control sea lice, typically administered using bath treatments or by addition to feed (Burka et al., 1997; Burridge et al., 2010; Roth et al., 1993). However, overuse of pharmaceuticals has resulted in the development of resistant strains of salmon lice (Fallang et al., 2004; Jones et al., 2008; Lees et al., 2008; Sevatdal et al., 2005; Treasurer et al., 2000; Tully and McFadden, 2000).

The detrimental effects of using chemicals to delouse salmon, on the environment and to the fish themselves (Davies et al., 2001; Mayor et al., 2008), have stimulated the pursuit of several non-pharmaceutical approaches to controlling sea lice (e.g. Browman et al., 2004; Dempster et al., 2011; Flamarique et al., 2009; Robertson et al., 2009; Treasurer, 2002). One such is to use cleaner fish in co-culture with salmon to remove salmon lice (Costello, 1993, 1996; Kvenseth and Kvenseth, 1997; Sayer et al., 1996a; Treasurer, 2002; Tully et al., 1996). This approach was first tested in laboratory trials in 1988, followed by experiments in sea cages (Bjordal, 1988, 1990, 1992; Deady et al., 1995; Treasurer, 1994). The results of those trials were promising and, following from that, commercial fisheries for wrasse, to be used as cleanerfish on salmon farms, began - in 1988 for goldsinny wrasse (*Ctenolabrus rupestris*) (Bjordal, 1991), and later for corkwing wrasse (*Symphodus melops*), rock cook (*Centrolabrus exoletus*) and juvenile ballan wrasse (*Labrus bergylta*). In 2007 and 2008, salmon lice in several geographic areas developed resistance to a popular delousing pharmaceutical, Slice® (Nilsen, 2008). Consequently, the estimated use of wrasse (several species) in Norway surpassed 10 million fish in 2010 (Norwegian Directorate of Fisheries statistics).

Of the four wrasse species used as cleanerfish, only two grow large enough to be used to delouse salmon during their second year in net pens - corkwing and ballan wrasse. Ballan wrasse is the largest and hardiest of these two species and, therefore, the one having the highest value for the industry. However, the ballan wrasse is the least abundant of the wrasse species used and the fishery is insufficient to meet the needs of salmon farms (Skiftesvik et al., in press). As a result, intensive culture of ballan wrasse has been developed over the past decade to relieve the fishing pressure on wild stocks and to provide a consistent supply to salmon farms (D'Arcy et al., 2012; Skiftesvik et al., 1996; Stone, 1996). Ballan wrasse is the only wrasse species currently cultured, but whether these intensively cultured cleaner fish are as efficient as wild ones is not known.

The objectives of this study were to assess the prevalence of salmon lice on Atlantic salmon in sea cages, in the presence or absence of wrasse, thereby testing their efficiency as delousing agents. The relative efficiency of wild-caught vs. cultured ballan wrasse was also assessed.

* Corresponding author. Tel.: +47 911 87 639.
E-mail address: anne.berit.skiftesvik@imr.no (A.B. Skiftesvik).

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http://dx.doi.org/10.1016/j.aquaculture.2013.03.032

2. Material and methods

2.1. Fish and experimental design

Experiments were conducted in September–October 2012. Salmon were obtained from Fitjar laks and on-grown in sea cages for two months before the experiments. The health status of the fish was assessed regularly during routine inspections by a veterinarian. Salmon were deloused using Salmosan® in mid-July, before they were introduced into the sea cages. All of the salmon were inspected to be sure that only fish without any visible damage and with a normal condition factor were used in the experiment. The dimensions of the experimental sea cages were 5.5 × 5.5 × 7 m.

Wrasse were obtained from three sources: 1. Marine Harvest (MH) (ballan wrasse), 2. Institute of Marine Research (IMR) (ballan wrasse), 3. Wild-caught (ballan and corkwing wrasse) obtained from a local fisher. The cleaner fish (25 fish per sea cage) were set out on 30.08.12 and the salmon (500 fish per sea cage, mean weight 429 ± 115 g) one week later. This is about the ratio of wrasse/salmon that is currently in use on commercial salmon farms in Norway. All of the wrasse were visually inspected to be sure that only fish without any visible skin damage and with a normal condition factor were used in the experiment. However, some of the ballan wrasse from Marine Harvest had truncated pectoral fins. Lice were counted before distribution and every week until 25.10.12.

There were four replicates and five treatments: control (no wrasse), IMR ballan wrasse, MH ballan wrasse, wild ballan wrasse, and a mix of wild corkwing + IMR ballan wrasse (corkwing was used in this treatment because of an insufficient number of ballan wrasse). Treatments were randomly distributed over the floating

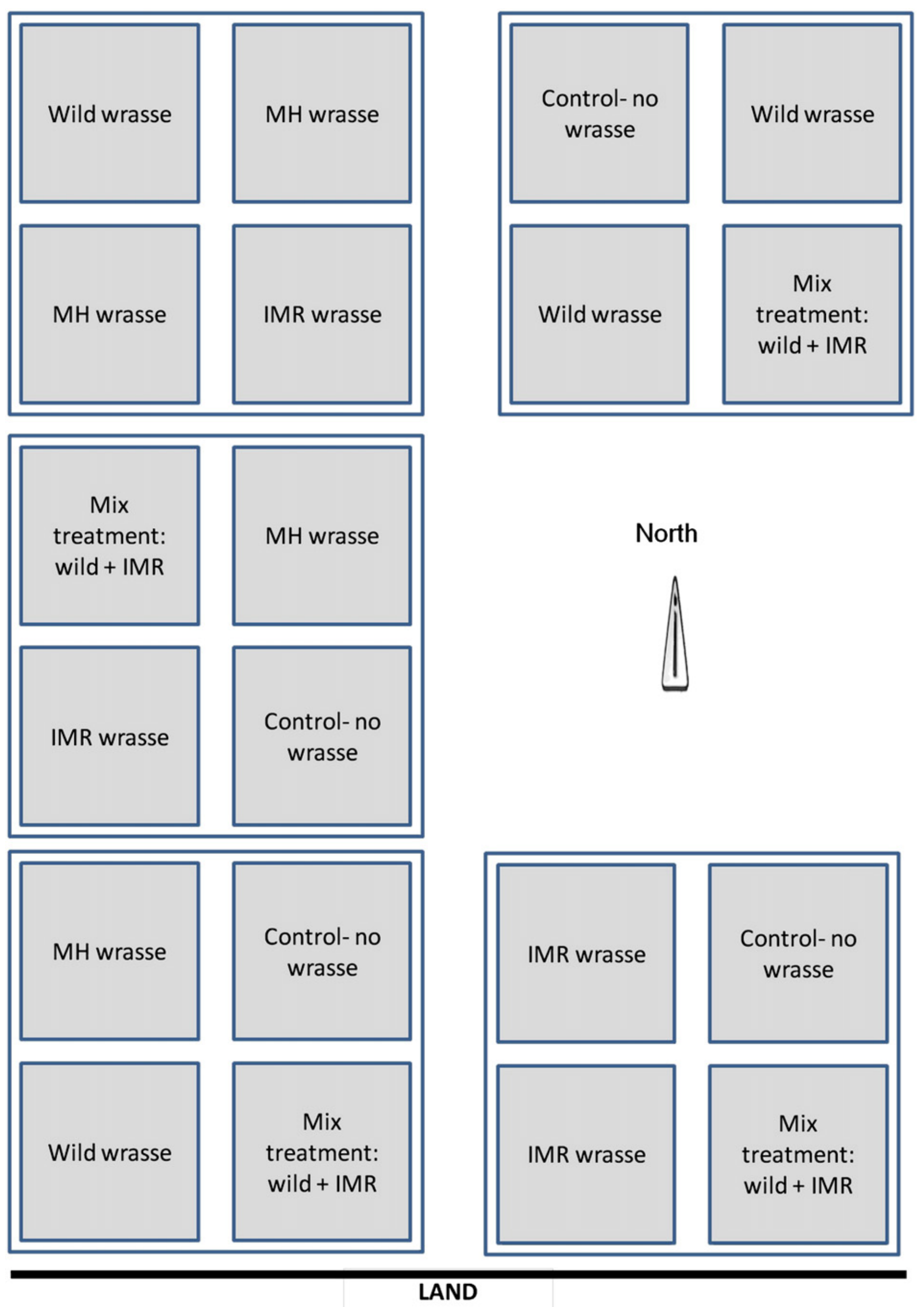

Fig. 1. Schematic of the sea cages and location of control and treatment fish.

experimental sea cage facility (Fig. 1). Artificial shelters - dark plastic "kelp" - were placed in the nets as shelter for the wrasse. Control treatments had no wrasse. All other treatments had 25 wrasse each.

Every week, ten salmon were randomly collected from each of the sea cages and anesthetized (MS-222). Lice were counted and classified into three life history stages: chalimus, pre-adult and adult. The general status of these fish was visually assessed and classified into four categories 1- no damage; 2- skin damage most likely due to handling, 3- fin and gill bites by the wrasse, 4- both types of damage (skin damage and bites). After lice counting, the fish were released back into the sea cage from which they had been collected. At the end of the experiment (week 6) length and weight were measured on ten salmon per sea cage. To minimize the load of fouling organisms colonizing the sea cage nets (and available as food for the wrasse), the nets were changed mid-way through the experiment.

2.2. Statistical analysis

Two-way ANOVA was used to evaluate the effect of time, treatment, and the interaction between time and treatment on the number of lice. Holm–Sidak method was used for further multiple comparisons. A non-parametric Kruskal–Wallis ANOVA was used when normality and homoscedasticity assumptions were not met. The same procedure was also used to compare condition factors of salmon and wrasse. The proportions of damaged salmon in control vs. treatments were compared by Chi-square tests.

Table 1
Number of sea lice (*Lepeophtheirus salmonis*) (maximum and mean ± sd) per salmon (*Salmo salar*) for the control and treatment nets.

Salmon louse	Control - no cleaner fish		Treatment - with cleaner fish	
	Max nb	Mean ± sd	Max	Mean ± sd
Chalimus	13	1.7 ± 2.0	12	1.3 ± 1.6
Pre-adults	25	6.3 ± 4.9	7	0.6 ± 1.1
Adults	13	3.0 ± 2.4	5	0.1 ± 0.5
Total	44	11.0 ± 6.9	12	2.06 ± 2.0

3. Results

3.1. Salmon louse prevalence

The number of chalimus-stage lice increased significantly throughout the experiment (Kruskal–Wallis ANOVA, $H_5 = 226.154$, $P < 0.001$) and reached a maximum after 6 weeks (Fig. 2A). There was no significant difference in the number of chalimus across treatments (Kruskal–Wallis ANOVA, $H_3 = 5.057$, $P = 0.168$).

The number of pre-adult and adult lice increased every week in the control treatment (Two way ANOVA, $F(5, 1170) = 27.398$, $P < 0.001$) (Fig. 2B and C). Differences between control vs. cleaner fish treatments were significant after 1 week (Multiple comparison procedure, $P < 0.001$) as pre-adult and adult lice remained low and stable in the sea cages with cleaner fish (Two way ANOVA, $P > 0.7$).

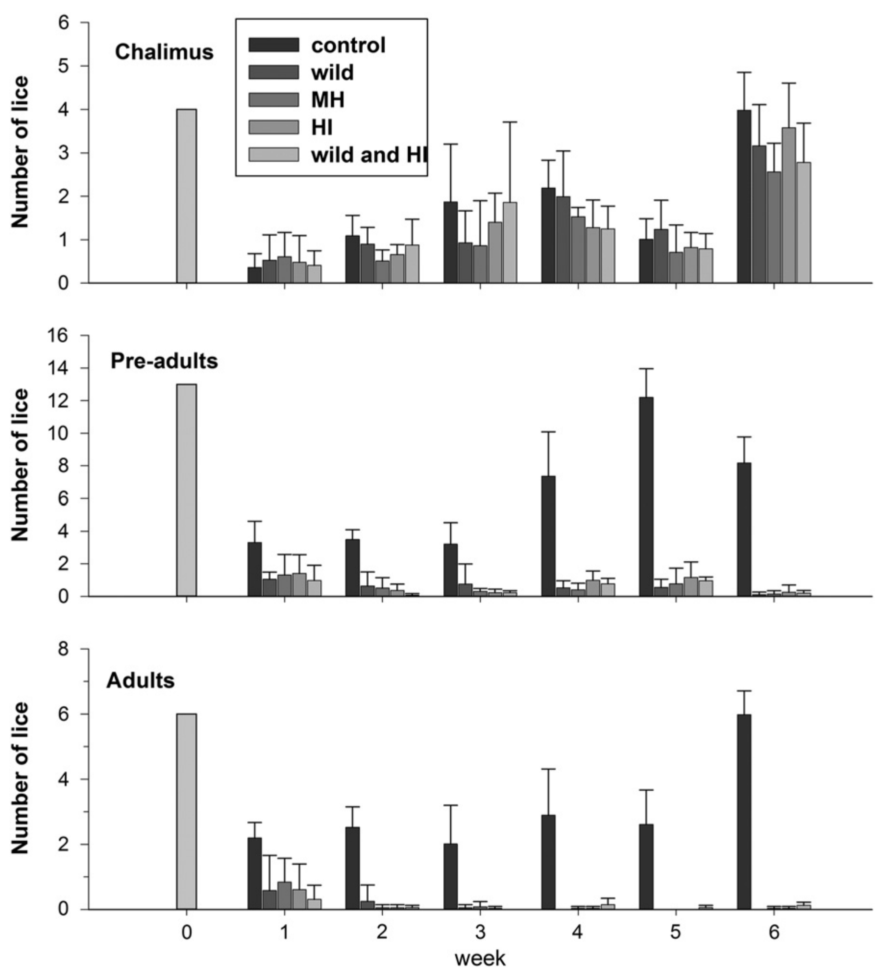

Fig. 2. Number of sea lice (*Lepeophtheirus salmonis*) per salmon (*Salmo salar*) counted once a week for each treatment. Each bar represents the mean ± sd. The bar at week 0 represents louse counts before the start of the experiment.

Table 2
Length, weight and condition factor (mean ± sd) of ballan wrasse (*Labrus bergylta*) from: Institute of Marine Research (IMR), Marine Harvest (MH), and wild fish at the start and end of the experiment. N represents the number of fish that were measured. At the end point, all cleaner fish were measured and thus N represents the total number of cleaner fish left at the end of the experiment.

Ballan wrasse	IMR		MH		Wild	
	Start	End	Start	End	Start	End
Length (cm)	13.9 ± 1.1 N = 25	13.9 ± 1.2 N = 71	11.5 ± 0.4 N = 25	12.0 ± 0.5 N = 84	14.4 ± 1.6 N = 53	14.5 ± 1.7 N = 86
Weight (g)	40.4 ± 11.2 N = 100	37.0 ± 10.5 N = 71	27.6 ± 3.7 N = 100	24.0 ± 4.1 N = 84	42.3 ± 16.0 N = 103	40.0 ± 14.8 N = 86
Condition factor	1.53 ± 0.11 N = 25	1.35 ± 0.12 N = 71	1.72 ± 0.16 N = 25	1.37 ± 0.17 N = 84	1.36 ± 0.14 N = 53	1.26 ± 0.13 N = 86

There were no significant differences in the number of lice (whatever their stage) between the different cleaner fish treatments: HI, MH, wild or mix, (Multiple comparison procedure, $P > 0.9$).

Overall, louse prevalence (pre-adult and adult stages) decreased from 9 lice on average per fish in the controls to less than 1 in the sea cages with wrasse (Table 1). In total, for all treatments and replicates, wrasse consumed approximately 4000 lice in seven days. This represents a minimum consumption rate of 23 lice per wrasse per day.

3.2. Condition factor of fish

Ballan wrasse from all treatments had significantly lower condition factor after 6 weeks (t-tests, $t_{(94)}$, $t_{(107)}$ and $t_{(137)} < 0.0001$). Maximum body mass reduction occurred in the MH wrasse (6%), while IMR and wild wrasse decreased by respectively 3.6 and 2.5% (Table 2, Fig. 3). Corkwing wrasse (only present in the mixed treatment) also decreased in condition factor; from 1.45 to 1.36 (t-test, $t_{(59)} = 2.074$, $P = 0.0424$).

The weight of salmon increased from 413 g to 672 g on average at the end of the experiment. Final weight was not significantly different between treatments (Table 3, Kruskal–Wallis ANOVA, $H_4 = 7.628$ $p = 0.106$). The condition factor of salmon was not significantly different between treatments (Kruskal–Wallis ANOVA, $H_4 = 8.088$, $P = 0.088$).

3.3. Health status and mortality

Damaged salmon represented 15% of the control fish, and from 23 to 43% of the treatment fish (Chi-square, $X^2_2 = 4.895$, $P = 0.027$),

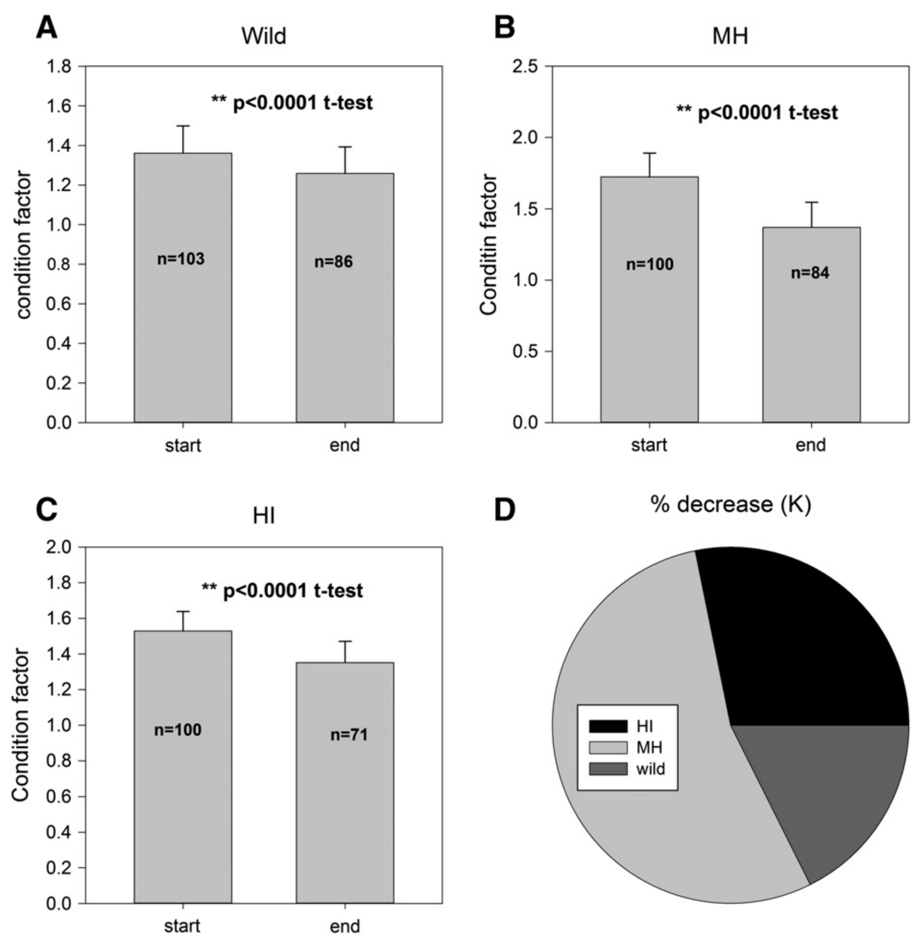

Fig. 3. Condition factor (mean ± sd) of ballan wrasse (*Labrus bergylta*) from IMR, MH and wild individuals at the beginning and at the end of the experiment. Panel D shows the decrease in percentage according to origin.

Table 3
Weight (mean ± sd) of Atlantic salmon (*Salmo salar*) at the start and at the end of the experiment for each treatment.

Atlantic salmon	Start	Control	IMR	MH	Wild	Wild + IMR
Weight (g)	429 ± 115	722 ± 166	680 ± 243	680 ± 166	632 ± 140	646 ± 173

(Fig. 4). Among these, skin damage was in equivalent proportions (9–13% of total; Chi-square, $X^2_2 = 2.157$, $P = 0.142$) in control and treatments (Fig. 4). Fin bites were present only in treatment salmon, representing 8 to 16% of the total. The number of individuals with fin bites significantly differed between treatments (Chi-square, $X^2_2 = 9.017$, $P = 0.029$) and were highest in the mixed treatment (wild + IMR). Fish that had both types of damage were also highest in the mixed treatment (19%). No other type of bite-related damage was observed. Mortality in salmon (310 individuals died over the 6-week study period) was not significantly different between treatments (Chi-square, $X^2_2 = 3.820$, $P = 0.431$) (Fig. 5). There were only 13 mortalities, however, when the wrasse were counted at the end of the experiment, a total of 59 individuals had disappeared (died or escaped) (IMR: 29; MH: 16; Wild: 14), (Fig. 5).

4. Discussion

Wrasse were extremely effective at delousing salmon. Although the wrasse were of different origin, there was no difference in delousing efficiency between the different groups. Intensively cultured wrasse were as effective as wild wrasse at removing lice, within just one week, and despite having had no prior contact with salmon or salmon lice. In a previous study, louse numbers remained below a mean of five mobile stages per fish when wrasse (goldsinny and corkwing) were introduced at a ratio of 1 wrasse per 250 salmon (0.4% wrasse) (Deady et al., 1995). We used a higher ratio (5% wrasse) - the ratio recommended for commercial farms - resulting in a mean number of louse mobile stages below 1 in all of the net pens containing cleaner fish. In Norwegian aquaculture, the established limit by the authorities is 6 lice (1 adult and 5 mobile stages) per salmon, for the time period (September–December) during which we carried out our experiments.

Cultured wrasse did not require prior experience with cleaner fish to be effective, as has been suggested from observations of the cleaning behavior of wrasse in the wild (Henriques and Almada, 1997). Thus, our study demonstrates that intensively cultured ballan wrasse can be introduced into sea cages on salmon farms, naïve of either salmon or salmon lice, where they will delouse the salmon and keep salmon lice loads at very low levels.

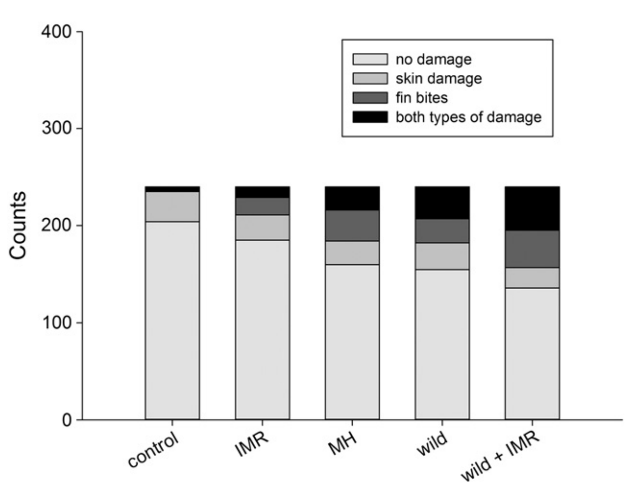

Fig. 4. Damage to salmon (*Salmo salar*) over the experimental period (6 weeks).

All wrasse groups efficiently removed pre-adult and adult lice from the salmon, but not chalimus stage lice. The number of sea lice chalimus stages on the salmon increased throughout the experiment. However, the wrasse effectively reduced the older lice stages and, therefore, it would only be a matter of time until these chalimus stage lice developed into life stages that the wrasse remove.

Wrasse appear to clean other fish only during the day (Deady et al., 1995), indicating that they probably use vision to identify the lice. It is possible that sea lice chalimus were not removed by the wrasse because they could not see them on the sides of the salmon, either because they were too small and/or because of low target contrast. Although mature female lice were much more visible because of the egg strings than the pre-adults, these were equally consumed. The target contrast between the salmon (silver) and the louse (brown), rather than the shape of the target (e.g. egg strings protruding off of the female louse), is probably what attracts the wrasse. The exoskeleton of adult sea lice refracts light differentially with wavelength and also creates patterns of polarized light (HB, unpublished), thereby potentially increasing their target contrast.

Wrasse lost weight throughout the experiment. This indicates that 25 wrasse per 500 salmon may be too high a ratio and argues for a more systematic assessment of the wrasse:salmon ratio and monitoring of the wrasse welfare so that they can be fed if necessary. Ballan wrasse in this experiment consumed a mean of 23 lice per wrasse per day. In an analogous experiment, a smaller species of wrasse (goldsinny) consumed almost twice as many lice per fish (Deady et al., 1995). Goldsinny consumes approximately 3 to 6% of its body weight on a daily basis when fed blue mussels (*Mytilus edulis*) (Garforth et al., 1996). If normalized to the mean weight of ballan wrasse in our experiment (36 g), this would represent a gross estimate of 2 g of rations per fish per day. In the wild, wrasse are opportunistic feeders and their diet varies seasonally. Amphipods, copepods, barnacles, polychaetes, hydrozoans, and mollusks (bivalves and gastropods) are the dominant food categories found in their stomach (Sayer et al., 1996b). If wrasse are fed too much, they will not eat salmon lice. Wrasse tend to ignore the salmon and feed on items that they find on the nets when these are heavily fouled (Deady et al., 1995). Intermittent dietary supplements might have improved their condition. Future research should assess procedures to supplement their food without impeding their delousing efficiency. The low food availability (also due to the absence of fouling on the nets) in the experiment reported here, probably caused the ballan wrasse to nibble on the fins and opercula of the salmon. However, Deady et al. (1995) reported that this does not occur when there is sufficient food for the wrasse.

Damage to salmon was highest in the sea cage with corkwing wrasse indicating that this species may be more aggressive than ballan wrasse, that its food requirements are higher and/or that its dietary breadth is narrower. The inter-species dynamics that might result from introducing more than one species of wrasse to the sea cages simultaneously is a topic for future research.

The presence of wrasse did not affect the growth of salmon and salmon grew similarly in all treatment/replicate sea cages. Overall, they increased their body mass by 63% over 6 weeks. This is an acceptable growth rate compared to salmon growth in aquaculture (Kvenseth pers. comm.). Mortality remained relatively low - at least over the experimental period: 6 weeks - and equivalent regardless of treatment.

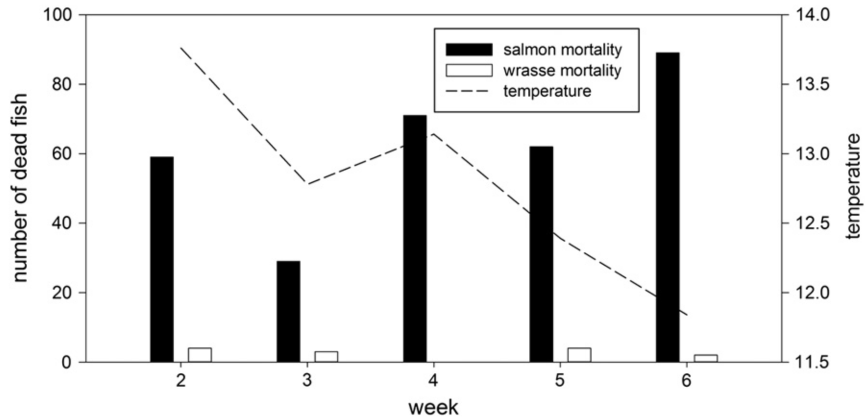

Fig. 5. Number of dead fish (ballan wrasse (*Labrus bergylta*) and Atlantic salmon (*Salmo salar*)) found in the net pens during the 6-week study period.

Acknowledgments

This research was supported by the Norwegian Seafood Research Fund (Project # 900554). We thank all of the support staff working on the sea cages at IMR Austevoll.

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