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Effects of lumpfish size on foraging behaviour and co-existence with sea lice infected Atlantic salmon in sea cages

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ABSTRACT

To assess possible size effects of foraging of lumpfish and co-existence with Atlantic salmon with particular interest to the sea lice grazing efficiency, eight sea cages (5 × 5 × 5 m) were stocked with 150 Atlantic salmon with a mean (±SD) weight of 538 ± 14 g. Six of the cages were stocked with 15 lumpfish each (10% density), with two cages for each of three different size groups of lumpfish. Three duplicate groups of lumpfish with an initial mean (±SD) weight of 22.6 ± 0.7 g (small), 77.4 ± 3.6 g (medium) and 113.5 ± 2.1 g (large) were used in the study. Two cages without lumpfish acted as controls. Sea lice infestation levels were recorded at two to four week intervals for 159 days. To determine the diet preferences of lumpfish in the cages gastric lavage was performed at the same time intervals. Behaviour and growth of the lumpfish was assessed throughout the study period and mean weight of the Atlantic salmon measured at the start and end of the study period. From day 35 and onwards growth was higher for the small lumpfish group compared to the two other lumpfish size classes. Lumpfish from the smallest size class had a higher consumption of naturally occurring food items, including sea lice, compared to the other two size classes. Growth stimulation in salmon co-habiting the two smallest lumpfish size groups was observed. Signs of sexual maturation were found in the medium (13%) and the large (20%) size groups. Based on present data small lumpfish (initial size approx. 20 g) have a higher overall preference for natural food items compared to larger conspecifics. Although the sea lice infestation rate was low in the study (<-0.5 lice salmon^{-1}) final lice burden was 40% lower in salmon groups stocked with small lumpfish compared to the control group without lumpfish.

Statement of relevance: The data presented here are highly relevant for Aquaculture as the effective use of lumpfish for biological delousing of salmon is very important for commercial aquaculture of Atlantic salmon.

1. Introduction

The biological control of sea lice through the use of cleaner-fish has recently become a feasible option due to the increased occurrence of resistance towards medical treatments in salmon lice, *Lepeophtheirus salmonis* (Igboeli et al., 2012; Torrissen et al., 2013), the reduced public acceptance of chemotherapeutic use in food production and the urgent need for an effective and sustainable method of parasite control in Atlantic salmon aquaculture (Denholm et al., 2002; Treasurer, 2002). As a cold-water cleaner-fish alternative the common lumpfish (*Cyclopterus lumpus* L.) has been suggested. Initial results are very promising with up to 93–97% less sea lice infestation (adult female lice) in sea cages with lumpfish (Imsland et al., 2014a,b,c, 2015a) compared to salmon in sea cages without lumpfish present. The authors found clear signs of lumpfish grazing on sea lice, with significantly lower average numbers of pre-adult, mature male and female stages of lice per salmon. Overall, the results indicated that lumpfish is a suitable cold-water option for biological delousing of Atlantic salmon.

Production of lumpfish in Norway has increased dramatically over the last three years, but there exists challenges in optimizing its use as biological delousing agent. Previous studies with lumpfish have shown that the smaller juvenile stages (initial weight 54 g) exhibit a higher

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potential for lice grazing of Atlantic salmon compared to their larger (initial weight 360 g) conspecifics (Imsland et al., 2014c). This could be related to sexual maturation in the larger lumpfish that may have triggered adaptations in the feeding behaviour of the larger lumpfish as appetite may be suppressed (Davenport, 1985; Imsland et al., 2015a). Another widely used cleaner fish is the ballan wrasse, *Labrus bergylta* where a broad weight range (approx. 20–230 g) is used (Leclercq et al., 2013). Comparison of sea lice grazing efficiency of ballan wrasse between 23 and 75 g initial weight did not reveal any differences in sea lice grazing (Leclercq et al., 2013). However, it was recommended not to use ballan wrasse larger than 75 g when sea lice level were low (<0.5 lice salmon^{-1}) due to possible eye picking on the salmon.

Presently, salmon farmers are stocking lumpfish in commercial sea cages at an average size of approximately 20 g. Previous studies have shown good grazing potential of lumpfish stocked at 50–180 g (Imsland et al., 2014a,b), whereas sea lice grazing is suppressed in lumpfish larger than approx. 200–300 g (Imsland et al., 2014c). There is therefore a need to compare grazing effectiveness of the most commonly used sizes of lumpfish to help salmon producers to optimize the use of lumpfish. Hence this study aims at investigating the effect of lumpfish size on the grazing efficiency of sea lice on Atlantic salmon.

2. Materials and methods

2.1. Atlantic salmon

The Atlantic salmon ($N_{total} = 1200$) used in the study were underyearling (0+) 11G (eleventh generation of the Norwegian breeding program for Atlantic salmon) produced at Sundsfjord smolt AS and delivered to Gildeskål Research Station (GIFAS), Nordland, Norway in October 2014. The salmon were from the Aqua Gen strain and they were vaccinated with Pentium Forte Plus (Novartis Aqua, Oslo, Norway). All salmon originated from the same group of fish and shared the same genetic and environmental background. From October 2014 to January 2015 the salmon were reared at a sea pen facility at Langholmen, Nordland, Norway where the study was performed. The salmon were transferred to small-scale sea cages ($5 \times 5 \times 5$ m) in January 2015 and remained in those sea cages during the trial period. The salmon had an average initial mean ($\pm$SD) weight of 538 $\pm$ 14 g on 25 January 2015 when they were graded and the 1200 salmon were distributed among 8 sea cages (150 salmon in each sea cage). During the study period the salmon were fed a standard commercial diet (CPK 100, Biomar, Århus, Denmark) twice daily. A lice count was undertaken on the Atlantic salmon prior to transfer into the trial cage to assess the lice burden prior to treatment and thereafter every two to four weeks during the trial period. At each occasion 30 salmon in each sea cage ($N_{total} = 240$) were sedated and any lice present were recorded. Lice were registered in 5 categories: i) *Lepeophtheirus salmonis*: Adult female; ii) *Lepeophtheirus salmonis*: Adult male; iii) *Lepeophtheirus salmonis*: Pre-adult; iv) *Lepeophtheirus salmonis*: *Chalimus*; v) *Caligus elongatus*. As number of naturally occurring sea lice during the trial period was low comparisons were made on the combined total of all categories. In order to minimize inter-observer variation the same person categorized lice at each sampling.

2.2. Lumpfish

Sexually mature wild lumpfish (8 males and 7 females) were caught by gill nets in Sandnessundet outside Kraknes, Troms County, Norway during April–May 2014. Eggs were stripped, fertilized and incubated at 9–10 °C at Akvaplan-niva research station at Kraknes (APN-K) outside Tromsø, Norway. The juveniles were initially fed with Gemma Micro (150–500 μm, Skretting, Norway). After 30 days the juveniles were fed with 500–800 μm dry feed pellets (Gemma Wean Diamond, Skretting, Norway). On 7 October 2014 the juveniles (mean weight 7 g) were transferred from APN-K to GIFAS. On 7 December 2014 all lumpfish were anaesthetized (Benzoak® 80 mg l^{-1}) and tagged intraperitoneally with a Trovan® Passive Integrated Transponder. In addition a Floy tag was inserted slightly off centre at the highest vertical point of the dorsal array. A different coloured and/or numbered Floy tag was used for each lumpfish from each duplicate (two lumpfish with the same tag). All lumpfish were vaccinated with ALPHA MARINE micro 4 (Pharmaq AS, Oslo, Norway) on 7 December 2014.

2.3. Experimental set-up

At the start of the trial (25 January 2015), 1200 Atlantic salmon were individually weighed, counted and randomly (by using a systematic random assignment starting with a random number (between 1 and 8) and then take each individual salmon and put it into sea cage 4, then 5, 6, 7, 8, 1, 2, 3, and then repeat) distributed between eight cages of 125 m^3 ($5 \times 5 \times 5$ m), with 150 salmon in each cage. The experimental groups were thereafter assigned randomly (assigned by using random numbers) among predetermined duplicate distributions of the cages. There was one final weighing for Atlantic salmon in all eight cages at the end of the study period. Without prior starvation, all salmon in all cages were counted and individually weighed.

On 25 January three size classes of lumpfish were established by size grading with an initial mean ($\pm$SD) weight of 22.6 $\pm$ 0.7 g, 77.4 $\pm$ 3.6 g and 113.5 $\pm$ 2.1 g. These three groups are termed small, medium and large hereafter. Six sea cages were stocked with 15 lumpfish each (10% stocking density), with two sea cages for each size group and two cages without lumpfish as control group. Two submerged substrates of black 10 mm polyethylene (PE) plates (80 × 80 cm) (Helgeland Plast, Mo i Rana, Norway, see Imsland et al., 2015b) were sited in each of the eight cages prior to the lumpfish being transferred in order to provide shelters for the lumpfish. The study lasted for 159 days and was terminated on 5 July, 2015. Daily mean temperature in the sea cages increased from 4.5 °C on 25 January to 10.8 °C on the 5 July. Salinity ranged from 29.6 ppt. to 34.1 ppt. throughout the study period. Dissolved oxygen ranged between 9.0 mg l^{-1} and 12.4 mg l^{-1} during the trial period. Secchi depth in the sea cages varied from between 5 and 10 m. Individual pit-tag ID, weights and lengths of all the lumpfish were registered at the same dates when gastric lavage was performed.

Specific growth rate (SGR) of individual lumpfish and salmon was calculated according to the formula of Houde and Schekter (1981):

$$SGR = (e^{g} - 1) \times 100$$

where $g = (\ln(W_2) - \ln(W_1)/(t_2 - t_1)$ and W_2 and W_1 are weights on days t_2 and t_1, respectively.

2.4. Gastric lavage of lumpfish

During the trial period gastric lavage was performed at two to four week intervals to assess the feeding preferences of individual lumpfish. All samplings started at the same time in the morning. On each occasion, 20 lumpfish from each size group (i.e. 10 lumpfish from each sea cage) were anaesthetized (Benzoak® 80 mg l^{-1}) and a silicon tube (15 cm long and diameter of 4 mm) connected to a syringe filled with seawater was carefully inserted into the stomach cavity of the sedated lumpfish. Water was expelled from the syringe into the stomach cavity and the gut content was allowed to flow out of the stomach and into a container. After each lavage, the stomach contents were transferred to a clean Petri dish and identified under a dissecting scope. The food items identified by gastric lavage were categorized as: a) sea lice (all stages of *L. salmonis* and *Caligus elongatus*) b) formulated feed fragments, c) crustacean species (e.g. *Caprella* spp.), d) hydrozoan species, e) unidentified material or no contents found. After sampling, the lumpfish were placed into a recovery tank containing aerated seawater and allowed to recover before being placed back into their specific cages.