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Short communication

The cleaning efficacy of lumpfish (*Cyclopterus lumpus* L.) in Faroese salmon (*Salmo salar* L.) farming pens in relation to lumpfish size and seasonality

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ABSTRACT

Sea lice infestations causes major economic losses in the Atlantic salmon aquaculture industry, and cleaner fish, e.g. the lumpfish, *Cyclopterus lumpus* L., are therefore increasingly deployed as a biological control method. However, large variations in the cleaning efficacy have been observed, and in the Faroe Islands, the most prominent variation is seasonal. Over a period of approximately two years 5511 lumpfish stomachs were analysed. The stomach contents, where present, were identified and grouped as, a) sea lice, b) lumpfish feed, c) salmon feed, d) organisms associated with biofouling, e) zooplankton organisms and/or f) other. The presence of zooplankton organisms had a significant, and negative, influence on the cleaning efficacy, while the presence of organisms associated with biofouling had a more moderate, but positive, influence on the prevalence of sea lice in the lumpfish diet. Our findings indicate that biofouling, and the subsequent availability of alternative prey organisms, does not reduce the cleaning efficacy of lumpfish, while zooplankton does, i.e. reducing it by a factor of approximately five. The lumpfish size only seemed to play a minor role in the variation observed in the cleaning efficacy, while it had a significant influence on the proportion of empty stomachs, i.e. the smaller lumpfish (< 50 g) had the highest occurrence of empty stomachs.

This is convenient knowledge for implementation in the sea lice strategies of farming sites using lumpfish as cleaner fish in general, but especially in farming areas with large seasonal variations in the zooplankton abundance such as in the Faroes. The high occurrence of empty stomachs in small lumpfish highlights the necessity to adapt husbandry in the first period post lumpfish deployment, especially when the opportunity for naturally occurring food is sparse. Furthermore, our findings of biofouling having a positive influence on the grazing efficacy of lumpfish indicate that net cleaning might have a negative influence on lumpfish grazing efficacy.

1. Introduction

Sea lice have been a serious problem for the Atlantic salmon farming industry since the 1970s (Brandal and Egidius, 1977), and its economic impact is greater than that of any other parasite (Costello, 2009). The increased occurrence of resistance against medical treatments for sea lice has called for alternative and non-pharmaceutical methods (Browman et al., 2004; Dempster et al., 2011; Flamarique et al., 2009; Treasurer et al., 2002), and consequently the use of cleaner fish has emerged to be a robust method for controlling sea lice (Torrissen et al., 2013).

Several fish species have been identified as cleaners, particularly among the wrasses (Feder, 1966; Skiftesvik et al., 2013). However, the wrasse species currently in use for biological delousing are temperature sensitive, making them unfit for use at low temperatures (Sayer and Reader, 1996). As a cold-water alternative, the common lumpfish (*Cyclopterus lumpus*) can be used (Imsland et al., 2014a).

As of today, lumpfish mainly dominate the production of cleaner fish. The vastly expanding lumpfish production in Norway has increased from around 3.5 million individuals in 2014 to around 15 million in 2016. In 2016, lumpfish represented approximately 45% of the deployed cleaner fish in Norway (Norwegian Directorate of Fisheries, 2017). Due to relatively low temperatures, and the wrasse not being native, lumpfish is the only cleaner fish used in the Faroe Islands.

Despite its relatively small size (~10,000 km^2) the Faroe shelf (62°00′N 06°47′W) contains a distinct neritic ecosystem surrounded by an oceanic environment. The shelf water is relatively well separated from the open ocean by a persistent front that surrounds the shelf

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usually at between 100 and 130 m bottom depth (Gaard et al., 1998; Larsen et al., 2009). Due to strong tidal currents, the water column in the shallow parts of the shelf is mixed from surface to bottom throughout the year and no summer stratification occurs (Gaard, 1996; Gaard et al., 1998). The timing and intensity of the spring bloom on the Faroe shelf can vary considerably from one year to the next, but mainly occurs in the period from April to September (Gaard et al., 2002; Debes et al., 2008).

Lumpfish was for the first time introduced as a cleaner fish in the Faroese salmon farming industry in late 2014. Since then the numbers of farming sites using lumpfish has increased continuously, and of today, approximately half of the Faroese salmon farming sites are using lumpfish as cleaner fish. However, variations in the cleaning efficacy are frequently observed, especially seasonally, and the main aim of current paper is to clarify the influence of zooplankton and biofouling on the cleaning efficacy of lumpfish in the Faroe Islands.

2. Material and methods

2.1. Lumpfish

The lumpfish studied ($N_{Total} = 5511$) were from nine Faroese Atlantic salmon farming sites using lumpfish as cleaner fish in the period from May 2016 to July 2017. Lumpfish were sampled on 93 different sampling days divided on the nine farming sites. The lumpfish were sampled from the edge of the pen using a dip net. On average ± SE 60 ± 29.2 lumpfish were studied on each sampling date. After sampling from the salmon pens, the lumpfish were euthanized with an overdose of Finquel (Tjaldurs Apotek, Tórshavn, Faroe Islands).

The sampled lumpfish were weighed to the nearest gram. The weight of the lumpfish ranged from nine to 883 g. Due to lumpfish mortalities on site, the smaller sized lumpfish were heavily overrepresented (Fig. 1), and had an overall average weight ± SE of 97.9 ± 1.30 g.

2.2. Stomach content analysis

Where present, the stomach content, from oesophagus to pylorus, was identified and grouped as a) sea lice, b) lumpfish feed, c) salmon feed, d) organisms associated with biofouling, e) zooplankton organisms and/or f) other. Sea lice were *Lepeophtheirus salmonis*, *Caligus elongatus* and unspecified chalimus. The organisms associated with biofouling were mainly amphipods, *Caprella* spp., *Tubularia* spp. and seaweed, while the zooplankton organisms mainly were calanoid copepods, crustacean larvae, jellyfish, and fish larvae. “Other” was scale, insects, terrestrial plants, feathers, plastic, etc.

2.3. Statistical analysis

The effect of lumpfish size on likelihood of finding sea lice in the stomachs was analysed using a logistic regression with an untransformed “weight” predictor variable and a binary dependent variable (sea lice found or not). Due to the high variability in the number of sea lice found in the stomachs, and the many factors, which may affect the number of sea lice found, presence of sea lice was used instead of number. There was an outlier in a fish that weighed 545 g (the only fish larger than 361 g that had consumed sea lice), but leverage was negligible and did not affect the results. The analysis was carried out again excluding all fish larger than 200 g as the data is highly skewed with > 80% of all fish weighing < 200 g and the rest weighing between 200 g and 900 g. In this second analysis 680 fish were excluded resulting in 4831 fish. The results were not qualitatively different, so the final analysis includes all the data.

Stomach content was classified into five categories (see methods), and each stomach was coded as 0/1 for each food type and the data were tabulated for the purposes of analysis. A log-linear model was constructed by elimination of terms from a saturated model until no further terms could be eliminated without damaging the model fit. One three-way interaction was removed despite being borderline significant, because there were only two fish that had consumed all three food types, so any error in data collection would severely affect this

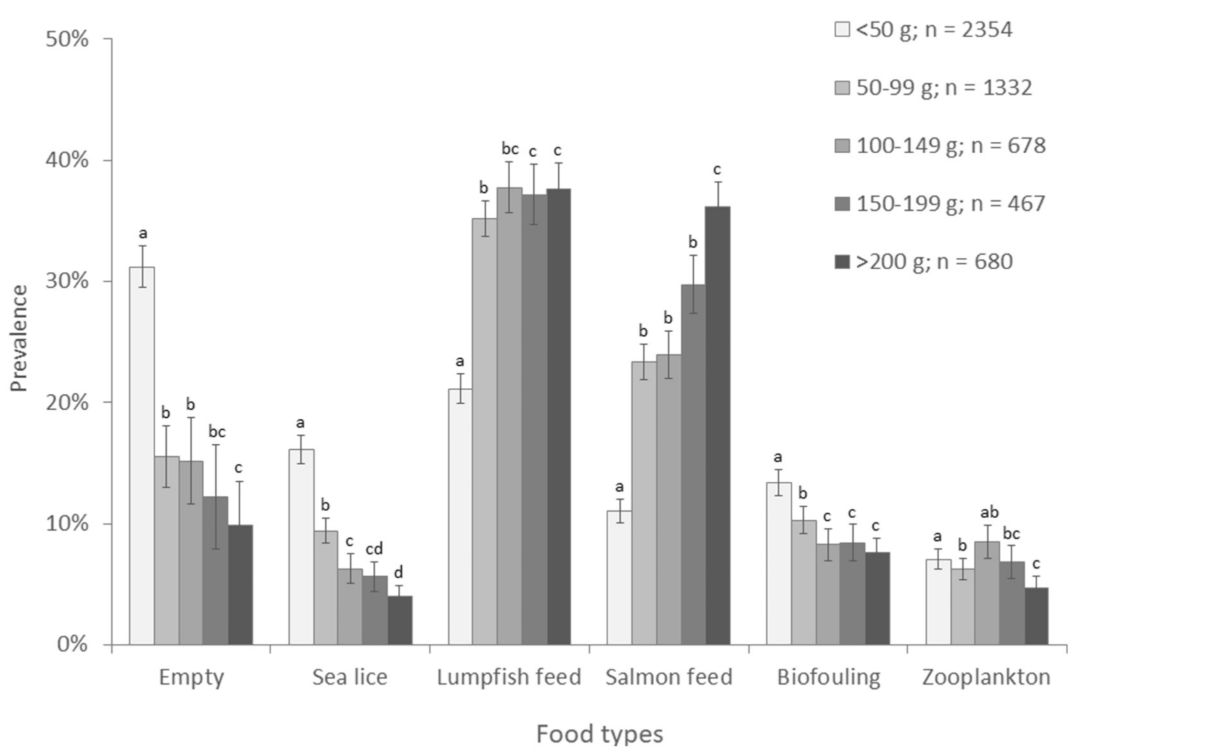

Fig. 1. Percentage values of empty stomachs and food choices for lumpfish at different sizes (g). Values are presented as means ± SE. Different letters indicate significant differences (Wald tests, $P < 0.05$) within the same food category at each size class.

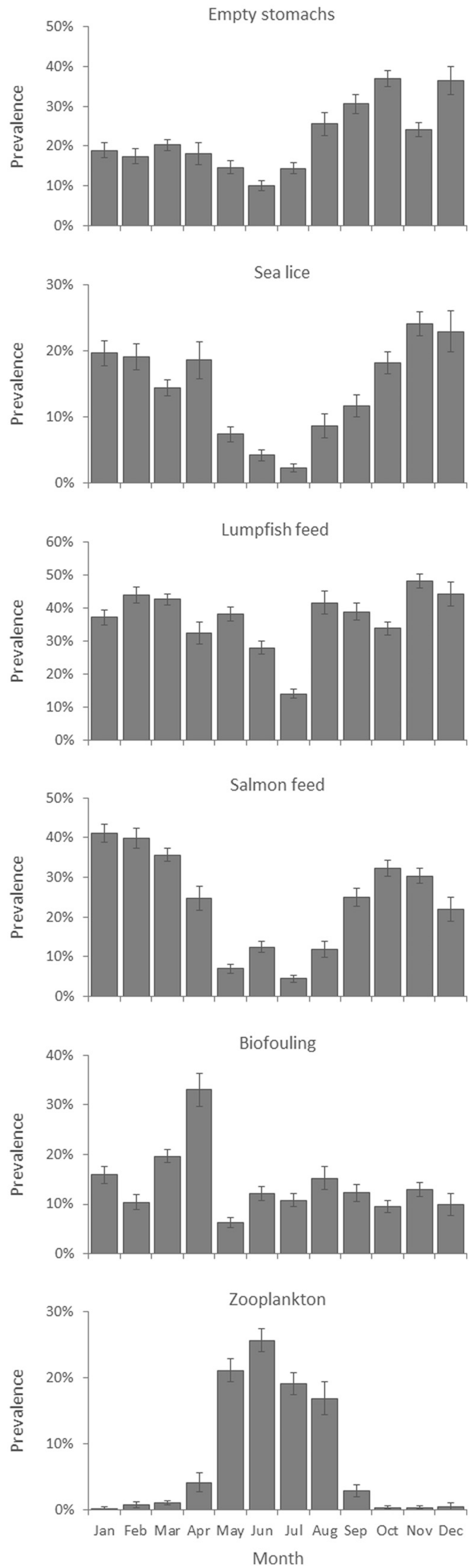

Fig. 2. Average monthly variation in the average prevalence of empty stomachs and in the average prevalence of food types in the lumpfish stomachs. Vertical bars indicate standard error (SE).

interaction. In the final model, only two-way interactions were included as all other higher order interactions did not significantly affect the model fit. For each type of prey, a binomial glm was used to ascertain whether frequency varied between size groups. This allowed for pooling all fish larger than 200 g and getting a more granular estimate of differences between smaller fish sizes. Therefore, fish were pooled into 50 g size spans up to 200 g and the analyses for each prey type were carried out with weight group as an independent factor and with a binary response variable (found, not found). All of the analyses yielded significant results, so the summary table (showing Wald tests) for each model was used to ascertain differences between factor levels (size groups) and are presented as significant differences in Fig. 1. This process was repeated with seasonal variation where factor levels represented months (Fig. 2). Summary tables for significant differences between individual months (Wald tests) are presented as supplementary information as these are not easily presented in the figure, but Likelihood Ratio Tests are presented in the text.

All analyses were carried out using R (R Core Team, 2017).

3. Results

3.1. Lumpfish size and food prevalence

Of the 5511 lumpfish examined, 743 lumpfish had sea lice in their stomachs that were visually detectable, i.e. 13.5%. The average weight (± SE) of the lumpfish without sea lice in their stomach was 103.7 ± 1.4 g, while the lumpfish with sea lice in their stomach were smaller, i.e. 61.2 ± 2.2 g. The smallest lumpfish examined weighed 9 g, while the smallest lumpfish with sea lice in its stomach weighed 13 g. The largest lumpfish examined weighed 883 g, while the largest lumpfish with sea lice in the stomach weighed 545 g.

Overall, the trend in the food choice of lumpfish was that the prevalence increased with size regarding lumpfish and salmon feed (Salmon feed; $Deviance_{4,5506} = 289.70$, $P < 0.001$, Lumpfish feed; $Deviance_{4,5506} = 185.04$, $P < 0.001$), and decreased with size regarding sea lice, organisms associated with biofouling and zooplankton (Sea lice; $Deviance_{4,5506} = 191.49$, $P < 0.001$, Biofouling organisms; $Deviance_{4,5506} = 53.40$, $P < 0.001$, Zooplankton; $Deviance_{4,5506} = 15.44$, $P = 0.004$). The likelihood of finding an empty stomach diminished significantly when the lumpfish weighed > 50 g ($Deviance_{4,5506} = 258.85$, $P < 0.001$).

3.2. Seasonal variation in food prevalence

Large seasonal variations were observed in the prevalence of empty stomachs ($Deviance_{11,5499} = 211.79$, $P < 0.001$) as well as in the prevalence of different food types in the lumpfish stomachs (Fig. 2). Most consistently, the proportions of empty stomachs and stomachs containing sea lice and salmon feed were lower in the summer months (Lice; $Deviance_{11,5499} = 269.06$, $P < 0.001$, Salmon feed; $Deviance_{11,5499} = 537.87$, $P < 0.001$), while the prevalence of zooplankton was at its highest during the summer months ($Deviance_{11,5499} = 726.69$, $P < 0.001$). The seasonal trend in the prevalence of lumpfish feed and organisms associated with biofouling was somewhat less evident, though there were significant differences (Lumpfish feed; $Deviance_{11,5499} = 230.70$, $P < 0.001$, Biofouling organisms; $Deviance_{11,5499} = 122.37$, $P < 0.001$).

3.3. Alternative food availability influencing lumpfish grazing efficacy

A large proportion of fish had consumed nothing at all (1916 out of 5511 fish) and there was no association between consumption of other food types in general and consumption of sea lice in specific ($X^2 = 0.573$, df = 1, $P = 0.449$). In other words, presence of any other

Table 1
Consumption of zooplankton was negatively associated with salmon feed, lumpfish feed, and sea lice. Positive associations were found between salmon feed and lumpfish feed as well as organisms associated with biofouling and sea lice. Estimate refers to Exp (Likelihood Ratio Estimate) for each interaction with negative values indicating a negative association and vice versa.

Food type 1 X	Food type 2	Estimate	Z statistic	*P*	Level of significance
Zooplankton	Biofouling	−0.08	−0.05	0.607	
	Salmon feed	−3.12	−7.54	< 0.001	***
	Lumpfish feed	−0.68	−5.34	< 0.001	***
	Sea lice	−0.96	−4.79	< 0.001	***
Biofouling	Salmon feed	−0.24	−2.42	0.016	*
	Lumpfish feed	+0.02	+0.29	0.773	
	Sea lice	+0.43	+4.07	< 0.001	***
Salmon feed	Lumpfish feed	+1.18	+17.92	< 0.001	***
	Sea lice	−0.18	−1.84	0.066	
Lumpfish feed	Sea lice	−0.07	−0.83	0.409	

food type in a stomach was not a good predictor of sea lice presence. In a log-linear model of interactions between the different food types, there were some clear connections between the consumption of zooplankton and other food types, where fish were less likely to have consumed salmon feed, lumpfish feed, and sea lice if they had consumed zooplankton (Table 1). On the contrary, lumpfish were more likely to have consumed sea lice if they had consumed organisms associated with biofouling (Table 1).

4. Discussion

Variations in the efficacy of cleaner fish has been observed beforehand and for several different reasons, e.g. size of the host fish, size of the cleaner fish and diurnal activity of the cleaner fish and availability of supplementary feed (Groner et al., 2013; Grutter, 1996; Grutter et al., 2002; Imsland et al., 2014b). In a review article on the use of lumpfish for sea lice control in salmon farming (Powell et al., 2017) one of the main knowledge gaps highlighted was the lack of understanding the seasonal variation in the grazing efficacy of lumpfish. Here, we enlighten the issue on the seasonal variation observed in the cleaning efficacy of lumpfish in large-scale salmon pens, hopefully making the gap somewhat smaller.

Like Imsland et al. (2014a) in a controlled study, observed larger lumpfish, i.e. with a mean (± SD) weight of 360 ± 30 g, being less efficient cleaners compared to smaller specimens, i.e. 54.0 ± 7.2 g, we also observed the average weight of the lumpfish that had consumed sea lice to be less than that of those who had not, nevertheless, lumpfish as large as 545 g were found to have ingested sea lice.

In a controlled study, Imsland et al. (2016) observed the smaller lumpfish (mean weight ± SD 22.6 ± 0.7 g) to have a higher consumption of naturally occurring food types, including sea lice, compared to larger lumpfish (mean weight ± SD 77.4 ± 3.6 g and 113.5 ± 2.1 g). This is in accordance with our findings were the proportion of stomachs containing sea lice and organisms associated with biofouling was significantly higher regarding the smallest lumpfish (< 50 g, Fig. 1). However, regarding zooplankton the trend was somewhat less consistent, indicating that foraging for zooplankton is a less size specific behavioural trait (Fig. 1).

Another size related feature was the proportion of empty stomachs. The percentage of empty stomachs reduced continuously from the smallest lumpfish size class (< 50 g, ~30%, Fig. 1) to the largest size class (> 200 g, ~10%, Fig. 1), which highlights the extreme importance of good husbandry, including an adequate feeding program, in the initial phase of lumpfish deployment in salmon farms, especially when the opportunity for naturally occurring food is sparse.

As lumpfish are let in to the Faroese salmon pens almost continuously throughout the year, the seasonal variation in the weight of the lumpfish is expected to be limited. On the contrary, all biofouling communities vary temporally. Major temporal changes are driven by seasonality in marine invertebrate populations, where the arrival of new recruits, periods of intense growth, or times of dormancy and regression, all influence community development at different times of the year (Fitridge et al., 2012; Greene and Grizzle, 2007). Surprisingly, a positive association between the prevalence of organisms associated with biofouling and sea lice was found (Fig. 2 and Table 1), indicating, that the occurrence of alternative food opportunities in shape of biofouling organisms, has a positive influence on the cleaning efficacy, perhaps due to a more active foraging behaviour or a more sheltered environment resulting in a better welfare. Around March, the lumpfish start to ingest organisms associated with biofouling; however, this does not seem to influence the sea lice grazing. On the contrary, in April/May there is a distinct shift in the foraging behaviour from organisms associated with biofouling and sea lice to dominantly zooplankton organisms (Fig. 2). There is no basis for assuming that sea lice numbers or biofouling decreases during this period and hence there seems to be a clear favouritism of zooplankton prey when available.

Although there are no direct measurements of secondary production, i.e. zooplankton, on the Faroe shelf, several studies have shown that the seasonal development in primary production typically is followed by a subsequent and similar development in the secondary producers (Niehoff et al., 1999). This is also the case on the Faroe shelf, where the primary production, and the subsequent secondary production, mainly is restricted to the period from April to September (Gaard, 1999; Debes and Eliasen, 2006). The availability of zooplankton prey organisms during the productive months on the Faroe shelf seems to have an expressed and negative influence on the cleaning efficacy of the local lumpfish (Table 1).

The organisms associated with biofouling found in the stomachs were highly dominated by crustaceans, i.e. species of Gamarus and Caprella, with occasional appearances of hydrozoans. On the contrary, the diversity of the zooplankton prey organisms was high and seemed to reflect the current zooplankton community and its succession, i.e. calanoid copepods, jellyfish, fish larvae, euphausiids, zoea, megalopa, and small juvenile crabs, similar to that described by Gaard (1999).

Our findings on the cleaning efficacy of lumpfish being seasonally influenced by zooplankton organisms, but not by organisms associated with biofouling, indicates that the foraging behaviour observed by Ingólfsson and Kristjánsson (2002), were the juvenile lumpfish appeared to ignore sessile and slow-moving animals, might be a behaviour lumpfish retain to larger sizes. Biofouling, and the subsequent availability of alternative prey organisms, has been anticipated to have a negative effect on the cleaning efficacy of cleaner fish. However, this might be a more accurate assumption when using e.g. wrasse as a cleaner fish, since these are known to prey upon more sessile and/or benthic invertebrate species (Deady and Fives, 1996; Dipper et al., 1977; Ingólfsson and Kristjánsson, 2002; Figueiredo et al., 2005).

The omnivorous feeding behaviour of lumpfish described by Imsland et al., 2015 was also observed in the Faroe Islands. Here the lumpfish stomachs frequently contained numerous food types available at different locations within the salmon pen, e.g. sea lice, compound feed, organisms associated with biofouling and zooplankton organisms. In this study, approximately 35% of the lumpfish examined had at least two of the food categories in their stomach.

5. Conclusion

Lumpfish seems to favour zooplankton as prey when it is available. In the Faroes, this has a large influence on the cleaning efficacy of lumpfish, reducing the average effect by a factor of approximately five. This knowledge should be implemented in the sea lice strategies of farming sites using lumpfish as cleaner fish and where secondary production, i.e. naturally occurring food, can affect the cleaning efficacy negatively. To obtain the effect desired, the strategy could include restocking of lumpfish in periods with lowered grazing efficacy due to increased zooplankton abundance or co-production of e.g. bivalves to decrease the availability of zooplankton.

Our results further indicate that biofouling can have a positive influence on sea lice grazing by lumpfish. We can only speculate on the reason behind this phenomenon, but our suggestions would be that biofouling, and the organisms associated, stimulate a more active foraging behaviour than that of the artificial feed, and/or that biofouling on the nets provides a more natural and sheltered environment for the lumpfish, resulting in a better lumpfish welfare.

However, in this study we did not have data available on the abundance and species composition of the zooplankton, biofouling, and sea lice in the surroundings of the lumpfish. Hence, the selectivity for the different prey types is only indicative, and we cannot, in a broad sense, assure that the efficacy indices presented here are completely accurate.

Future studies should thus attempt to increase the knowledge of the influence on the grazing efficacy of lumpfish in regard to stimuli, lumpfish welfare and selection for specific food types and prey species. As well as other factors, which conceivably might influence the grazing efficacy of lumpfish seasonally, e.g. the sea lice burden of salmon, as well as abiotic factors such as temperature.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2018.01.026.

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