Quantification of grazing efficacy, growth and health score of different lumpfish (*Cyclopterus lumpus* L.) families: possible size and gender effects

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Abstract

To investigate the possible family influence on sea lice grazing of lumpfish on Atlantic salmon, ten families of lumpfish (N = 480) with a mean (± SD) weight of 54.8 ± 9.2 g were distributed among ten sea cages (5 × 5 × 5 m) each stocked with 400 Atlantic salmon with a mean (± SD) weight of 621.4 ± 9.2 g. All the ten cages were stocked with 48 lumpfish (12% stocking density). The stocking of cages was such that each cage consisted of two random families where full- and paternal half-sib families were randomly allocated to the different cages. There were clear differences in sea lice grazing efficacy, growth and cataract prevalence between the ten families assessed in this study. Lumpfish from families 2, 6 and 10 had the lowest mean weights but showed comparable growth rates compared to the other families throughout the study and this may be as a direct result of genetic influence. In addition, fish from these families had a significantly higher incidence of lice grazing of both *L. salmonis* and *C. elongatus* compared to the other families. Using mixed linear model to analyse the data revealed significant family and paternal effect on sea lice grazing. There was a trend for a reduction in sea lice grazing with increased size within each family. The results indicated that it was the smallest size classes of lumpfish (40-140 g) which exhibited higher sea lice grazing potential compared to the larger size classes within families. There were no clear differences in the lice grazing potential between male and female lumpfish within and between families. Overall, present findings showed that sea lice grazing of both *L. salmonis* and *C. elongatus* can be enhanced using targeted family production and if this behaviour has a genetic basis it may further enhanced through selection and targeted breeding programs.

1. Introduction

The biological control of sea lice using cleaner fish has become a feasible option due to the increased occurrence of resistance towards medical treatments in salmon lice, *Lepeophtheirus salmonis*. Previous studies have shown up to 93–97% less sea lice infestation (adult female lice) in sea cages with lumpfish compared to salmon in sea cages without lumpfish present (Imsland et al., 2014a). Significant individual differences in feed intake and preference for sea lice has been seen (Imsland et al., 2014a, c; 2015), and genetic influence has been suggested to be a possible factor (Imsland et al., 2016a). If these differences are genetically influenced, certain genotypes may be better suited than others for stocking in open cages with Atlantic salmon. Maintenance of genetic diversity within an aquaculture species is critical to its long-term sustainability and to avoid future consequences of inbreeding, such as increased disease susceptibility, reduced growth and fecundity. Genetic markers have been used in the management of farmed species (Koljonen et al., 2002; Jackson et al., 2003; Borrell et al., 2004) and breeding selection for lice grazing families in lumpfish could further enhance this species potential to be used commercially.

In the central and northern parts of Norway, high abundance of the sea lice *Caligus elongatus* Nordmann on farmed fish frequently occurs in autumn (Øines et al., 2006). Infections have been assumed to be connected to passing schools of pollock *(Pollachius pollachius* L.), saithe (*Pollachius virens* L.) or herring (*Clupea harengus* L.) (á Norði et al., 2015). Mature stages of *C. elongatus* are smaller than mature *L. salmonis* (Piasecki, 1996) with both sexes of equal sizes (around 6 mm). *C. elongatus* is a much better swimmer than *L. salmonis* and can re-infect other fish species if removed from the original host (Øines et al., 2006; Hemmingsen et al., 2020). Lumpfish are now extensively used as cleaner fish in Northern Norway (Imsland et al., 2018a), Ireland (Bolton-Warberg, 2018), Scotland (Treasurer et al., 2018), Iceland (Steinarson and Árnason, 2018) and the Faroese Island (Eliasen et al., 2018). Recent data indicate that lumpfish graze on *C. elongatus* (Imsland et al., 2020a) but to this date there exists little knowledge if there are differences in *C. elongatus* grazing related to the genetic background of the lumpfish deployed in commercial sea pens. Earlier research has clearly indicated that lumpfish prefer the adult female *L. salmonis* (Imsland et al., 2014a, c; 2016a-b; 2018a), but lumpfish in sea pens can be classified as strongly opportunistic (Imsland et al., 2014c, 2015) and the fish do not restrict themselves or rely on a single food source if others are present. They may, therefore, readily graze on *C. elongatus* but whether this may depend on the parental background is at present unclear.

The present study aims to investigate the observed variation in sea lice (*L. salmonis* and *C. elongatus*) foraging behaviour of lumpfish (Imsland et al., 2014a-b) to reveal potential correlations between inclination to graze sea lice and genetic composition. With this information, it may be possible to introduce a breeding programme for continuous improvements of the cleaning efficiency of lumpfish. Previous research has indicated a size-related (Imsland et al., 2016a) and sex-related (P. Reynolds, Gifas, unpublished data) sea lice grazing of lumpfish but whether this can differ in different families of lumpfish has not been studied before.

The principal aim of this study is to quantify the grazing of different lumpfish families on sea lice found on Atlantic salmon in cages and investigate possible differences in growth, cataract prevalence and health of lumpfish with different parental background.

2. Materials and methods

2.1. Atlantic salmon

The Atlantic salmon (N_{total}=4040) used in the study were under yearling (0+) 11G (eleventh generation of the Norwegian breeding program for Atlantic salmon) produced at Sundsfjord Smolt AS (Nordland, Norway) and delivered to Gildeskål Research Station (GIFAS), Nordland, Norway in April 2019. The fish were transferred to small-scale sea pens (5 × 5 × 5 m, 125 m^3) in September 2019 and remained in those sea pens during the trial period. The salmon had an average initial mean (± SD) weight of 621.4 ± 15.4 g on 11 October 2019. All fish originated from the same group of fish and shared the same genetic and environmental background. These fish had not been used in any previous trials. The health status of the fish (N = 40) was assessed immediately prior to the start of the trial. Sample fish were obtained and delivered to BioVivo AS, Bodø. Health status was assessed by qPCR screening for known pathogens in lumpfish and salmonids, including *Aeromonas salmonicida*, *Pasteurella spp.*, *Moritella viscosa*, salmnoid alphavirus (SAV), IPN-virus, VHS-virus, Nodavirus and *Paramoeba perurans*. During the study period the salmon were fed a standard commercial diet (Energy Range, Biomar, Århus, Denmark) from automatic feeders once daily.

2.2. Lumpfish

Sexually mature wild lumpfish (5 males and 10 females) were caught by Akvaplan-niva staff in gill nets in Sandnessundet outside Kraknes, Troms County, Norway during November 2018. Eggs were stripped, fertilized and incubated at 9–10°C at Akvaplan-niva research station at Kraknes, Troms County, Norway where they hatched between 17-25 January 2019 (Table 1). Five paternal half-sibling families and ten maternal full-sibling families were used in the study obtained by crossing the different males and females (Table 1). The juveniles from each family were reared in replicate tanks (230 L), from hatching to tagging. The juveniles were initially fed with Gemma Micro (150–500 µm, Skretting, Norway; 62% protein, 14% lipid, 9% ash). After 30 days, the juveniles were fed with 500–800 µm dry feed pellets (Gemma Wean Diamond, Skretting, Norway; 57% protein, 15% lipid, 10% ash). Once the fish had attained a mean weight of 8.0 g all fish were anaesthetized (benzoak 80 mg l^{-1}) and tagged at the dorsal array with a Trovan® Passive Integrated Transponder (PIT). All lumpfish were vaccinated with AMARINE micro 3-1 (Pharmaq AS, Oslo, Norway) on July 2019. The fish were transferred to Gifas, Inndyr, Nordland on 23 September 2019 and maintained in a 5x5x5 m cage fitted with a specially designed net at Gifas small-scale research facility Langholmen. The fish were fed at a feeding rate of 1.5% BW^{-1} with feed blocks (World Feeds, UK, Imsland et al., 2018a, c, 2019) during the acclimation period and during the trial period. The feed blocks were suspended in the water column. Each individual feed block was an average of 26 x 100 mm with a 10 mm hole through the centre and had grooves created on their surface during the extrusion process (Imsland et al., 2018a. 2019a). Feed blocks were placed in each of the cages three days per week (Monday, Wednesday and Friday) and were weighed prior to placement to ensure enough feed was available to maintain a feeding rate of 1.5% BW^{-}

^{1}.

The following experiment was approved by the local responsible laboratory animal science specialist under the surveillance of the Norwegian Animal Research Authority (NARA) and registered by the Authority.

2.3. Experimental set-up

At the start of the trial (11 October 2019), 4040 Atlantic salmon were bulk weighed, counted and randomly distributed between ten cages of 125 m^3 (5 × 5 × 5m), with 404 fish in each cage. To minimize the effects of water quality and current, experimental groups were assigned randomly among predetermined duplicate distributions of the cages. There was one final weighing for Atlantic salmon in all ten cages at the end of the study period (17 December). Without prior starvation, all fish in all cages were counted and bulk weighed. Feed conversion ratio (FCR) was calculated as:

$$FCR = FI\,(B_2 - B_1 + B_{dead})^{-1}$$

where FI is feed consumed, B_1 and B_2 are the biomass at the start and end respectively for the period and B_{dead} is the biomass of dead fish during the period.

All ten cages were stocked with 48 lumpfish (12% stocking density). The stocking of cages was such that each cage consisted of two random families where full- and paternal half-sib families were randomly allocated to the different cages (Table 1). All lumpfish from each family were anaesthetized (Metacaine, 200 mg l^{-1}) and tagged with a separate colour external short fine fabric anchor tag (Floy Tag Inc. Seattle, Washington, USA) at the highest ventral point of the dorsal array. All lumpfish were identified by scanning each fish for their PIT-tag ID prior to placement. The study lasted for 68 days and was terminated on the 15 December 2019. Daily mean temperature in the sea pens decreased from 10.5°C on the 11 October to 6.0°C on 11 December. Salinity ranged from 29.6 ppt. to 32.8 ppt., while dissolved oxygen ranged between 8.6 mg l^{-1} and 11.7 mg l^{-1} during the trial period. Secchi depth in the sea pens was constant at 10 m throughout the study. Individual weight (g) and total length (cm) of all the lumpfish were measured on the same dates that 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. Condition factor (K) was defined as:

$$K = 100 * W / L^3$$

2.4. Gastric lavage of lumpfish

During the trial period gastric lavage (Imsland et al., 2014a, 2016a) was performed every two weeks to assess the feeding preferences of individual lumpfish. All samplings started at the same time in the morning. After each lavage, the stomach contents were transferred to a clean Petri dish and the amount of sea lice i.e. all stages of *L. salmonis* and *C. elongatus* identified under a dissecting scope. All lumpfish were individually anaesthetised with Metacaine 200 mg L^{-1} before gastric lavage and after sampling, the fish were placed into a recovery tank containing aerated seawater and allowed to recover before being placed back into their specific cages.

2.5. Size of lumpfish and effect on sea lice grazing

At the end of the study, all fish from each family which were found with ingested sea lice (*L. salmonis* and *C. elongatus*) during gastric lavage were arranged into the following 9 size classes: 40-59; 60-79; 80-99; 100-119;120-139; 140-159; 160-179; 180-199 and 200-229 from individual weights (g) at the end of the study period. For each family, the percentage of each size class consuming sea lice during the project period was calculated separately for each species of sea lice. This applied to cases where either one or both species of sea lice were found. The percentage of each size found to have consumed both species was also calculated.

2.6. Lumpfish sex determination and comparison of lice grazing by gender

At the end of the study period all remaining lumpfish in the cages were humanely dispatched with an anaesthetic overdose (metacaine 600 mg l^{-1}). For each fish, PIT-tag number was recorded to identify its specific family. The fish were then dissected to determine whether the fish was male, or female based on the presence of gonads. Sex distribution was determined for each family and the data used to assess frequency of lice grazing by individuals and potential differences of efficacy by gender.

2.7. Cataract scoring

During weighing and counting of lumpfish throughout the study period, the cataract score of all sampled fish was recorded. After weighing, each fish was transferred to a darkened room and a hand-held Heine HSL 150, C-002,14,602 (HEINE Optotechnik, Herrschingunder, Germany) slit lamp with a magnifying glass at 10 x magnification used to examine both eyes. After scoring, the fish were transferred to a holding tank containing well-aerated seawater until fully recovered before being placed back in its respective cage. Each eye was scored on a scale from 0 to 4 in accordance with Wall and Bjerkås (1999) where 0 = no cataract, 1 = cataract covers less than 10% of the lens, 2 = cataract covers 10-50 % of the lens, 3 = cataract covers 50-75% of the lens and 4 = cataract covers 75-100% of the lens. Mean scores (cataract index) of all examined individuals within the experimental groups was calculated. Both affected and non-affected individuals were included in calculated average group scores. There was no cataract scoring at day 55 due to equipment malfunction.

2.8. Health assessment of lumpfish

Assessment of the health status of all the lumpfish for each family group was undertaken during routine sampling points. At each sampling point, all lumpfish from each cage was weighed and length recorded. The status of fins was scored along with assessment of body condition, caudal, dorsal and pectoral fin damage, deformities, cataract/eye ulceration status and condition factor (Table 2). Any obvious wounds to the body or fins of the fish was recorded and digitally photographed on each occasion. In addition to the external condition status of the lumpfish, evidence of any continual individual loss of growth and/or mortality rates was assessed.

At each sampling time point, scores obtained for each fish was summated and the average score per cage calculated and evaluated according to Imsland et al. (2020b). In short if scores were between 0 and 11, health status are deemed satisfactory and no action was required. A score of 11 to 16 indicated health status has deteriorated and action is required. A score of over 16 indicates extensive health deterioration and immediate action required to alleviate suffering (Imsland et al., 2020b).

2.9. Statistics

All statistical analyses were conducted using Statistica™ 12.0 software. A Kolmogorov-Smirnov test (Zar, 1984) was used to assess for normality of distributions. The homogeneity of variances was tested using the Levene’s F test (Zar, 1984). Possible differences in mean weights, growth rates, cataract and health scores and sea lice counts were tested with two-way nested analysis of variance (ANOVA) where replicates were nested within families. Significant differences revealed in ANOVA were followed by Student-Newman-Keuls (SNK) post hoc test to determine differences among experimental groups. Data on mortality was tested with a χ^2 test with the mean overall mortality set as expected value. Possible gender and size specific sea lice grazing was tested with a χ^2 test. Significance level (α) of 0.05 was used if not stated otherwise.

The contribution of the different variables (i.e. different families, maternal ID, paternal

ID) for sea lice grazing was estimated using the Variance Estimation and Precision program in Statistica™. In this model the variance components for both the random (maternal and paternal effect) and fixed (family) effects are estimated with a Restricted Maximum Likelihood Estimate (REML) procedure (Searle et al., 1992; Demidenko, 2004).

3. Results

3.1. Growth and mortality of lumpfish

Initial mean (±SD) weight ranged from 39.2 ± 6.8 g, 44.4 ± 6.5 g and 46.9 ± 7.4 g for family 2, 6 and 10, respectively to 69.3 ± 8.2 g for family 4 and was significantly different (two-way nested ANOVA, $F_{9, 468} = 51.4$, $P < 0.001$, Fig. 1A) throughout the trial. At the end of the study, fish from family 2, 6 and 10 were still smallest and had a mean weight of 71.8 ± 27.9 g, 82.2 ± 28.9 g and 93.3 8 ± 32.0 g, respectively whereas, fish from family 7 had the highest mean weight of 144.7 ± 52.3 g. No differences were found in the specific growth rate (SGR) of the lumpfish between the families in the two first periods (two-way nested ANOVA, $P > 0.15$, Fig. 1B). From day 42 onwards SGR varied between the families with significantly higher growth seen in family 5 compared to all other families (SNK post hoc test, $P < 0.05$). Fish from the three smallest families (families 2, 6 and 10) exhibited similar growth rates as seen in the other families and no significant differences were seen (SNK post hoc test, $P > 0.15$).

Mortality rates were low throughout the study period for all families. Families 1, 7 and 9 recorded the highest mortality at 8.3% (4 fish) whereas fish from families 3, 4 and 8 had the lowest mortality rate of 2.1% (1 fish). All mortalities were fish found dead in cages apart for 1 during sampling. Overall mortality did not vary between the families ($\chi^2 = 1.1$, $P > 0.35$).

3.2. Growth, feeding and mortality of salmon

Overall mean weight (± SD) of the Atlantic salmon increased from 621.4 ± 89.2 g to 1150.9 ± 21.6 g at termination of the trial. No differences in mean weights between the salmon in the experimental cages were seen (two-way nested ANOVA, $F_{9, 391} = 1.7$, $P > 0.45$). Specific growth rate of the salmon in the ten sea cages varied between 0.97 to 1.07 and no significant differences were found (two-way nested ANOVA, $P > 0.55$). No difference was seen in feed consumption of the salmon in the ten experimental cages ranging from 210 to 225 kg nor in the feed conversion rate of the salmon in the ten sea cages which varied between 1.0 to 1.1. Sea lice levels on the salmon were similar across all cages at the onset of the trial.

3.3. Gastric lavage of lumpfish – consumed sea lice levels

The percentage of lumpfish found with ingested *L. salmonis* varied between families on each of the sampling days (two-way nested ANOVA, $F_{9, 468} > 1.5$, $P < 0.05$, Fig. 2A). The incidence of consumed sea lice increased for family 10 on the first four sampling time points and was significantly higher at day 42 (SNK post hoc test, $P < 0.05$, Fig. 2A). The incidence of consumed sea lice also increased for family 2 and 6. A higher incidence of consumed *C. elongatus* compared to *L. salmonis* was seen in all families (Fig. 2B) at all sampling dates. Families 10, 2 and 6 displayed the highest consumption throughout the experimental period (SNK post hoc test, $P < 0.05$). The REML based variance component analysis (VEPAC) of sea lice grazing showed significant paternal ($F_{4, 39} = 2.9$, $P < 0.05$) and family ($F_{9, 39} = 3.5$, $P < 0.05$) effect on *L. salmonis* grazing. For *C. elongatus* grazing the VEPAC analyses found significant family ($F_{9, 39}$ = 3.2, P < 0.05) effect whereas the paternal effect tended towards significance ($F_{4, 39}$ = 2.5, P = 0.06).

3.4. Gender effect on lice grazing

The percentage females (Fig. 3A) varied between 44% (family 1) to 59% (family 6) and similarly the percentage males varied from 37% (family 6) to 52% (family 1). Seven of the families had small numbers of lumpfish with undetermined gender with percentage values between 2% and 7%.

The total percentage of lumpfish by gender for each family found with ingested *L. salmonis* differed between families (χ^2 test, P < 0.05, Fig. 3B). More females were found to have consumed *L. salmonis* in families 3 and 5 (62% and 55%) compared to males (39% and 36%). The opposite finding was seen for families 1 and 9 where a higher percentage of males (60 and 64%, respectively) were found to have consumed *L. salmonis* compared to males (40 and 27%, respectively). No differences were seen for the other six families.

There was a higher percentage of female lumpfish found with ingested *C. elongatus* for families 2, 6, and 8 (χ^2 test, P < 0.05, Fig. 3C). Values ranged between 60% for family 2 and 71% for family 8 whilst a higher percentage of males were found for family 1 (55%) and family 9 (53%). There were small numbers of lumpfish with undetermined gender found with ingested *C. elongatus* in six of the families.

3.5. Size effect on sea lice grazing in different families

The size class distribution of lumpfish found to have ingested sea lice in their stomachs at each gastric lavage sampling varied between families (Fig. 4). There was a general trend for sea lice grazing to decrease as the lumpfish grew, but this trend varied between the families. For family 2 and 6 fish between 40 and 79 g had higher levels (χ^2 test, $P < 0.05$) of ingested sea lice compared to the other size classes with very few or no lumpfish grazing sea lice at sizes over 120 g. For families 1, 3 and 8 the highest percentage incidence of sea lice grazing occurred for fish between 60 and 119 g (χ^2 test, $P < 0.05$). A wider, more equal size class distribution of sea lice grazing of fish between 40-179 g was seen in the other families (i.e. families 4, 5, 7, 9 and 10). Consumption of both sea lice species were seen in all size categories (Fig. 4) and in all families.

3.6. Cataracts

There was a general trend for the prevalence of cataracts to increase for all ten families as the study progressed. At the start of the study period two families had no cataracts present (families 7 and 10). Of the eight families with cataracts, prevalence ranged from 2.1% for fish from families 2, 5 and 8 to 10.4% for fish from family 3. These differences were significantly different at each of the subsequent sampling time points (two-way nested ANOVA, $F_{9, 468} > 3.30$, $P < 0.001$, Fig. 5). At the end of the study period (day 69), fish from family 3 had the highest prevalence of all families (21.2%) whereas fish from families 2, 6, 7 and 10 had the lowest prevalence of cataracts (between 9.8% and 10.3%).

3.7. Health assessment of lumpfish

There was a small increase in health scores for all ten families indicating minimal deterioration in health status with time with significant differences between all families throughout the study (two-way ANOVA, $F_{9, 468} > 2.7$, $P < 0.01$, Fig. 6). Lumpfish from family 5 had the highest score at the start and end of the study (SNK post hoc test, $P < 0.05$) period with the mean score increasing from 2.1 to 2.9 whilst lumpfish from family 10 had the lowest mean health score at the end of the study period (2.0).

4. Discussion

4.1. Sea lice grazing in different families

Efficient delousing capability is an extremely important trait for ecological control on sea lice in salmon farming (Imsland et al., 2014a, 2016a, 2018b) and delousing efficacy varied between the families as observed in a previous similar study (Imsland et al. 2016a), whereas there was a clearer paternal than maternal effect. Lumpfish from family 2, 6 and 10 had the highest persistent percentage prevalence of consuming *L. salmonis* and *C. elongatus*. The lice grazing activity recorded during this study suggests a likely genetic effect, but influence may be from both male and female broodstock rather than an individual gender. Given that, the likely genetic effect can be used for future selection programmes for lumpfish grazing behaviour. Low sea lice grazing was observed in all families during the last experimental period. During the last two weeks of the project period, adverse weather conditions prevailed for most of the time. It may well be that the fish became stressed during this period and sought easier food items to ingest whilst being unable to locate smaller prey items such as sea lice due to lack of daylight.

Grazing of *C. elongatus* was more pronounced compared to grazing of *L. salmonis* with high incidences of ingestion recorded particularly for lumpfish from family 10 where 57% of all fish were found with this species of sea lice in their stomachs. Consumption of this species was evident for all families until at day 69 very few fish were found to have ingested them probably linked to adverse weather conditions. Infestation levels of this species were particularly high during the project period and was not considered the norm for the site were the study was undertaken. Infestation levels ranged between an average of 1.3 per fish and 1.7 per fish during the project. Lumpfish clearly exploited this species as a viable additional food source when available and shows potential for lumpfish to be used in areas where this species can cause significant damage to the salmon due to high infestation levels.

The present data shows temporal changes in feed choice throughout the period seemingly linked with food availability and previous studies have shown that lumpfish seem to switch between natural food choice to whatever becomes available to them within their environment (Imsland et al., 2015). This omnivorous feeding behaviour has been reported in wild juvenile lumpfish (Ingólfsson and Kristjánsson, 2002; Vandendriessche et al., 2007). Although the nutritious and energy dense feed blocks are equally available for all families, and the energy demand is well covered, lumpfish particularly from families 2, 6 and 10 showed a preference for sea lice which are much less energy dense. These fish may be more predisposed to actively seeking out natural food sources as compared to feed pellets and this behaviour may well have a genetic basis. If so, the genetic composition for these families requires further elucidation.

4.2. Lumpfish growth and mortality

There were significant differences in mean weights between the ten families at the start of the study period. The differences in mean start weights between the families was not as a result of differences in time of egg hatching as all families were hatched over a period of 7 days under similar rearing conditions. In addition, the differences in mean weights between families reflect the mean weights for each family found by individual weighing all fish prior to the start of the study. Lumpfish were only selected to reflect the actual mean weights for each family based on the existing deviations present. The differences in mean weight for fish from the smallest families may be as a direct result of genetic influence, but whether it be paternal or maternal influence remains unclear. A previous study had shown that paternal influence may have been involved (P. Reynolds, Gifas, unpublished data) as the two smallest lumpfish families shared the same male, but this was not the case in the present study. It may also be linked to female phenotype which results in production of smaller eggs compared to the normal egg size of the species. The fish used in this study were obtained from wild mature fish caught by gill nets outside Tromsø thus there was no prior selection criteria when harvesting the eggs. It has been suggested that variation in mean egg size is commonly correlated with female phenotype (e.g., body size, age, Einum and Fleming, 2002) in that there is a maternal effect on the egg size–offspring fitness function.

The growth rates for all ten families observed in this study were lower at each sampling time point compared to previous studies (Imsland et al., 2014a-b, 2018a). The lower growth was attributed to the use of feed blocks to maintain the lumpfish during the study period. Previous studies have shown that using feed blocks controls growth in lumpfish without apparently compromising the health status of the fish (Imsland et al., 2018a, c, 2019a). It is important that lumpfish populations have access to a regular food source particularly in wintertime when naturally occurring food items become scarce. This food source is vital to maintain healthy and robust populations. It should be noted that high growth is not an aim for lumpfish used as cleaner fish. Imsland et al. (2016b) found that small lumpfish (initial size approx. 20 g) have a higher overall preference for natural food items, including sea lice, compared to larger conspecifics (initial size 77 and 113 g). This makes slow to moderate and uniform growth of lumpfish more desirable than fast growth for its optimal use as cleaner fish in salmon aquaculture. Controlling growth rates of lumpfish in commercial sea cages may allow for the prolongation of sea lice grazing behaviour.

There was no significant mortality of lumpfish during the study. The highest mortality recorded was 8.3% for both families 1, 7 and 9. The mortality recorded may be attributed to repeated handling during sampling and/or individual fish unable to acclimate to the cage environment during wintertime as there were no obvious signs of bacterial or viral infections of the dead fish.

4.3. Effects of gender and size on lice grazing

There was a higher percentage of females present compared to males for seven of the ten families tested in this study and very small numbers of lumpfish found to have no gonadal development. At present no published information exists regarding whether wild and/or farmed lumpfish favour a strong male or female bias in population structure. Previous research by the present research group (unpublished data) on gender effect on lice grazing efficacy has indicated some potential for female lumpfish to consume more sea lice compared to males. However, present results do not support this as it varied between the families which gender was dominant in sea lice grazing. Further, there was no consistent trend in gender related grazing of the two sea lice species as only two families

(1 and 9) displayed the same trend for both sea lice species. More research is required to determine whether male or female fish perform better as lice grazers or whether fish with little/no gonadal development would be preferred. It has been shown that as male lumpfish mature, they tend to be increasingly aggressive and territorial (Davenport, 1985) and if maturation in males occurs in commercial salmon cages then sea lice grazing efficacy may be compromised dependent on the percentage of males present in the cage. In a previous study (Imsland et al., 2016a), male lumpfish showed signs of sexual maturation at 450 g and exhibited weak lice grazing efficacy. However, in the present study there were no obvious signs of maturation (colour changing in males and/or eggs deposited on submerged substrates or on the side of nets) in any of the ten cages throughout the trial period. Overall present data indicate only weak gender related sea lice grazing in lumpfish.

Previously it has been indicated that smaller lumpfish (initial size approx. 20 g) have a higher overall preference for natural food items, including sea lice, compared to larger conspecifics (Imsland et al., 2016b) and results from this study seem to support this. At the end of the present study, the lumpfish from each family were arranged into size classes and the level of sea lice grazing for each was recorded. Generally, it was found that it was the smallest size classes which exhibited higher sea lice grazing potential compared to the larger size classes. Lumpfish between 40 and 140 g had the greatest grazing effect overall, however, variations between families existed. These results suggest that smaller lumpfish are more desirable than larger conspecifics and as such smaller fish are potentially more desirable to produce commercially. It is important to consider that when selecting smaller lumpfish that these fish must reflect the true mean weight of any given population and are not selected through a grading process which could result in using lumpfish that may be not as healthy compared to their larger conspecifics. The smallest families assessed in this study were a true representation of the population size as they displayed similar growth trends compared to larger fish from other families. The frequency of individuals to graze *L. salmonis* and *C. elongatus* on repeated occasions was significantly different between families (data not shown). The highest percentage of repeat grazers were from families 2, 6 and 10 with the same individuals found to have consumed both species of sea lice in their stomachs in three or more of the five gastric lavage sampling points during the study period. These three families were also the smallest populations throughout the study period. This indicates a strong preference to select sea lice as a food source by certain individuals within these three families. As sea lice grazing efficacy is one trait that is strongly desirable in future breeding programmes then also the frequency of lice grazing by individuals within families should also be used as a selection criterion for such programmes.

4.4. Lumpfish cataract and health

The incidence of cataracts increased as the study progressed for all ten families. However, prevalence was lower than observed in previous studies using pelleted feeds (Imsland et al., 2019b), but comparable to studies when lumpfish were fed with feed blocks (Imsland et al., 2019a). A previous study has shown that the prevalence of cataracts can vary between 20% and 100% in lumpfish populations (Jonassen et al., 2017). Such high prevalence of severe cataract is only comparable with the highest incidences previously found in farmed Atlantic salmon caused by a histidine-deficient diet. In farmed salmon, it has been shown that even moderate degrees of cataract can result in reduced growth (Breck and Sveier, 2001). Development of cataract means that less light passes to the retina and vision becomes impaired or disappears (Bjerkås and Sveier, 2004). A previous study comparing lumpfish fed with feed blocks or pelleted feed (Imsland et al., 2019a) found that cataract prevalence for fish fed with feed blocks only increased from 3% to 9% over the whole study period whilst prevalence for fish fed with pelleted feed increased from 4% to 87% over the same period. These differences may be attributed to dietary effects as both groups shared the same husbandry and environmental conditions throughout the project period.

Rapid growth can increase the risk of cataracts in Atlantic salmon (Ersdal et al., 2001). Further, previous studies on lumpfish (Jonassen et al., 2017; Imsland et al., 2018c) found that high growth increased risk of developing cataracts as has been observed in salmon. In contrast, the results from this study show that the lumpfish had similar specific growth rates, but differences in cataract prevalence existed between the families. Although, some of these effects may be partially attributed to differences in food sources consumed, there may well be an additional genetic factor which manifests as certain lumpfish families being less predisposed developing cataracts.

Sea lice grazing was studied by gastric lavage of all lumpfish every two weeks during the trial period. The method is a nonlethal and harmless method where the stomach contents of the lumpfish are flushed out by a stream of water. The limitations of this technique are that people carrying out gastric lavage need to be trained to ensure that the technique does not lead to excessive stress or death of the fish. In addition, it is time and labour intensive and only about, 100–200 fish can be processed per day. Further, the

Norwegian Food Safety Authority recently stated that the flushing technique is in violation of the Norwegian Animal Welfare Act and recommended the use of dissection instead (Mattilsynet, 2016). In this experiment we applied for, and got, allowance from the Norwegian Animal Research Authority to perform the gastric lavage as it was done by experienced, and trained, personnel with several years of experience of conducting such samplings. Possible negative effects on sea lice grazing was consider minimal and if any it would be a systematic effect in all groups analysed. Previous studies undertaken by our research group have shown no negative effects on growth or incidences of mortality or reduced health status for fish that have routinely been assessed with gastric lavage (P. Reynolds, GIFAS, unpublished data). This method has been used on other fish species (Stehlik et al., 2015; Braga et al., 2017) with no detrimental effects.

The welfare of cleaner fish in cages is a prime concern and the focus of some of the fish welfare schemes (RSPCA, 2015). Lumpfish can lose condition within six weeks of transfer to sea cages. This can be alleviated by the supply of robust fish and by providing supplementary feed. Cleaner fish should be regularly checked for weight, condition, and by applying operational welfare indicators (OWIs) on the farm. Treasurer and Feledi (2014) developed a categorisation scale of fin erosion (FEI) and fin splitting (FSI) for five wrasse species stocked on salmon farms and gave a good working representation of condition of the fins. This was allied to other external indicators such as examination for incidence of cataracts, jaw erosion, external lesions and scale loss. A similar scale of OWIs was assessed with lumpfish juveniles in the hatchery and for broodstock (Treasurer, 2018). For the fish in this study, OWIs were developed to assess health and condition for all ten families. There was a slight deterioration in condition manifested by increasing health scores for all families. Lumpfish from family 2 had the lowest scores throughout the study. The deterioration in condition observed in this study was assessed as non-critical and no action was required. The scoring system used in this study was shown to be robust and reliable and gave an accurate indication of the health status of the fish in this study. It is proposed that this assessment criteria should be used in future studies to evaluate the fish welfare of lumpfish.

5. Conclusions

There were clear differences in sea lice grazing efficacy, growth and cataract prevalence, between the ten families assessed in this study. Lumpfish from families 2, 6 and 10 had the lowest mean weights but showed comparable growth rates compared to the other families throughout the study and this may be as a direct result of genetic influence. In addition, fish from these families had a significantly higher incidence of lice grazing of both *L. salmonis* and *C. elongatus* compared to the other families. Cataract prevalence for these three families was also significantly lower compared to the other families. There were no clear differences in the lice grazing potential between male, females and unsexed lumpfish. These results indicate that further research may be required to fully elucidate potential differences. Overall there was a trend for a reduction in sea lice grazing with increased size within each family. The results indicate that it was the smallest size classes of lumpfish (40-140 g) which exhibited higher sea lice grazing potential compared to the larger size classes within families.

Acknowledgements

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Conflict of interest

There is no conflict of interest in relation to this study.

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Figure legends

Fig. 1. (A) Mean weight (g) and (B) Specific growth rates (% day $^{-1}$) for ten families of lumpfish throughout the experimental periods. Values represent means ± SE. * indicates significant differences between the families (two-way nested ANOVA, $P < 0.05$); n.s., not significant.

Fig. 2. Percentage values of food choices of lumpfish of the ten lumpfish families sampled at each sampling time point. Values are presented as means ± S.E. (A) *L. salmonis* sea lice; (B) *C. elongatus* sea lice. * indicates significant differences between the families (two-way nested ANOVA, $P < 0.05$).

Fig. 3 (A) Percentage of males, females and lumpfish with unknown gender for each of the ten families; (B) Total percentage of lumpfish by gender for each family found with ingested *L. salmonis*; (C) Total percentage of lumpfish by gender for each family found with ingested *C. elongatus*.

Fig. 4. Total percentage of individual lumpfish per family arranged in size classes from individual weights (g) at the end of the study period ingesting sea lice (1. Only *L. salmonis*, 2. Only *C. elongatus* or; 3. ate both species) during gastric lavage sampling in the experimental period.

Fig. 5. Occurrence of lumpfish with cataracts (% prevalence) calculated for each of the ten families at day 1, 14, 28, 42 and 69. Values represent means ± S.E. * indicates significant differences between the families (two-way nested ANOVA, $P < 0.05$).

Fig. 6. Mean heath scores for each of the ten lumpfish families at sampling days 1, 14, 28, 42, 55 and 69. Values are presented as means ± S.E.

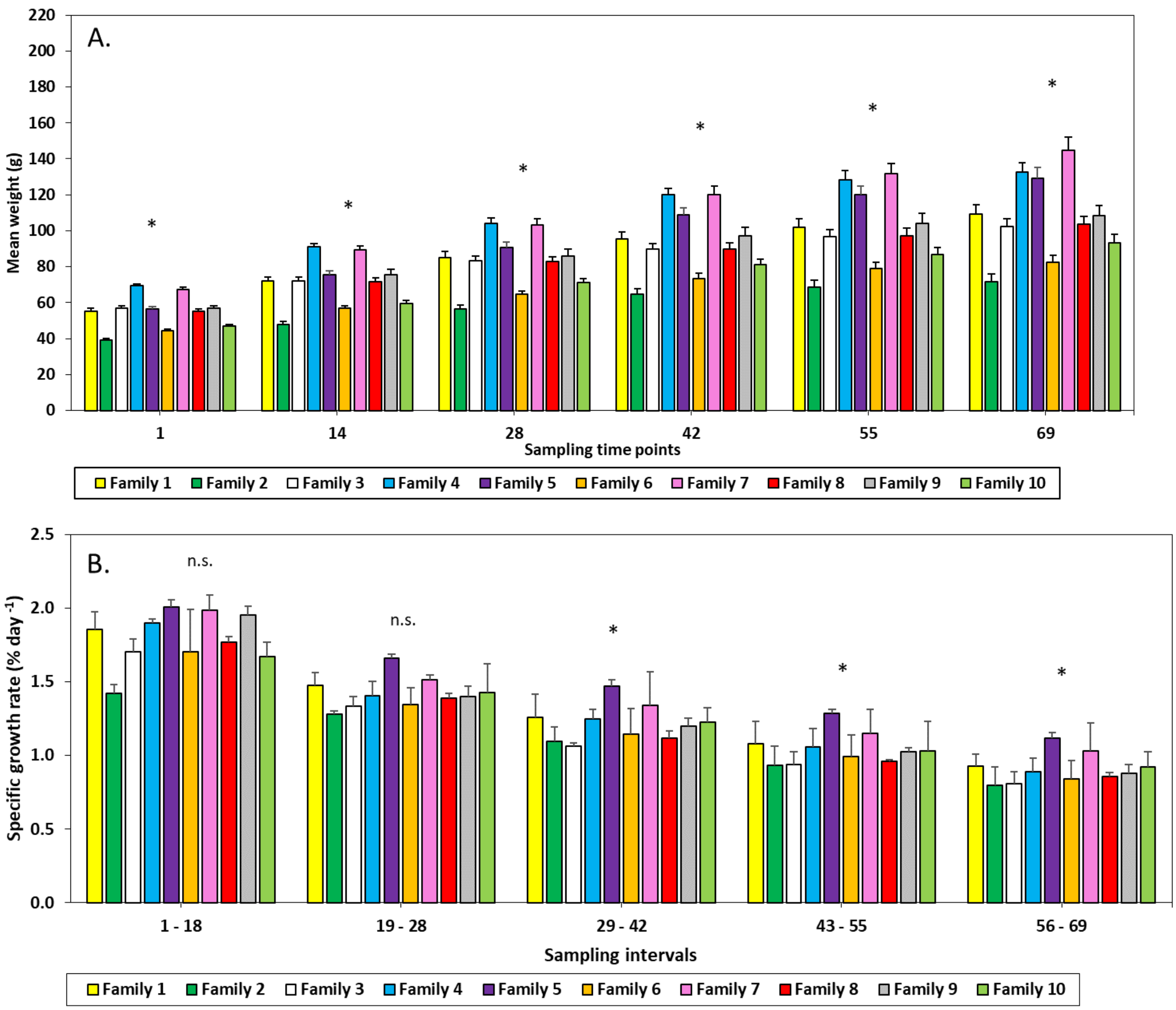


Fig. 1. Imsland, Reynolds at al.

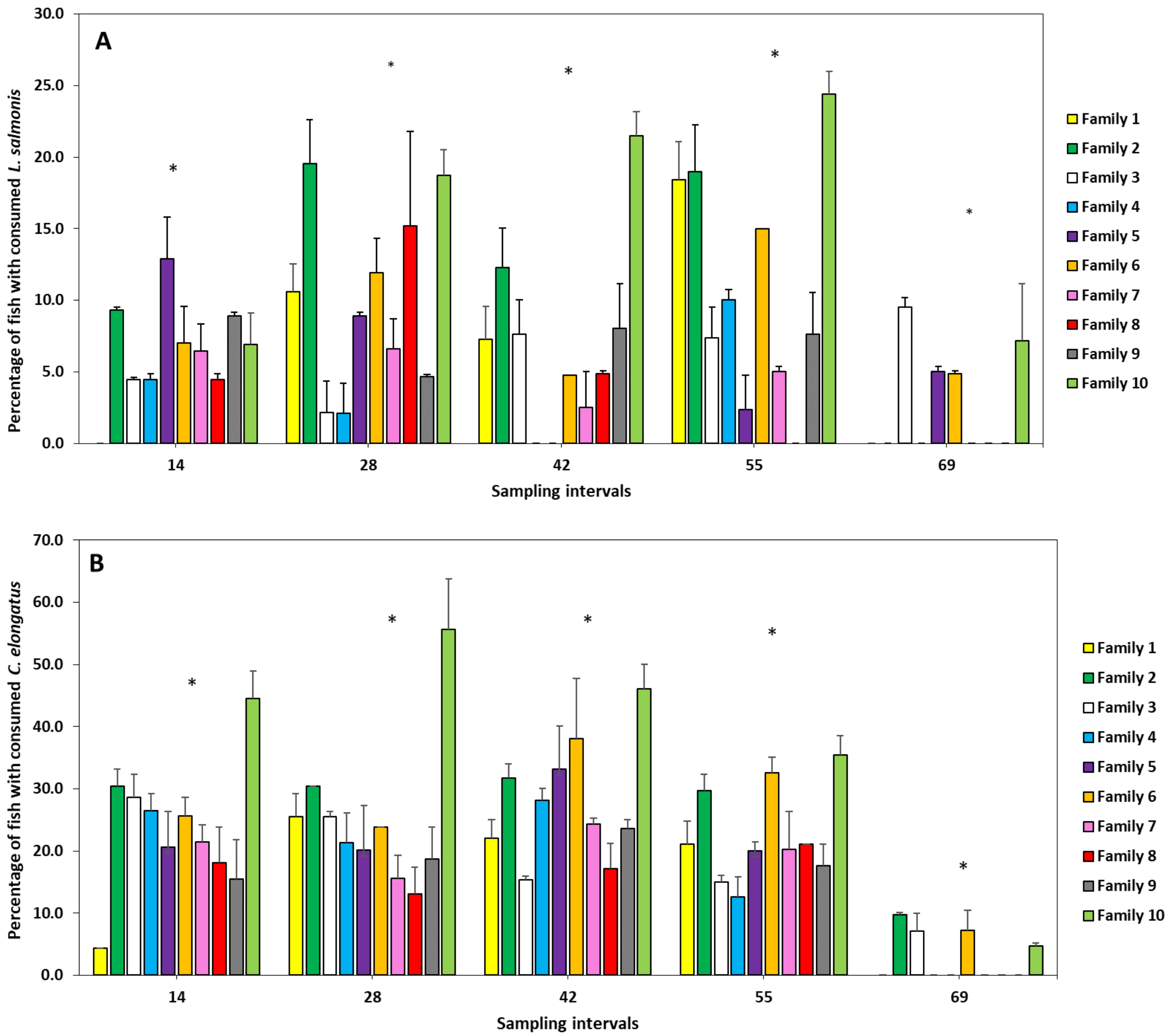


Fig. 2. Imsland, Reynolds et al.

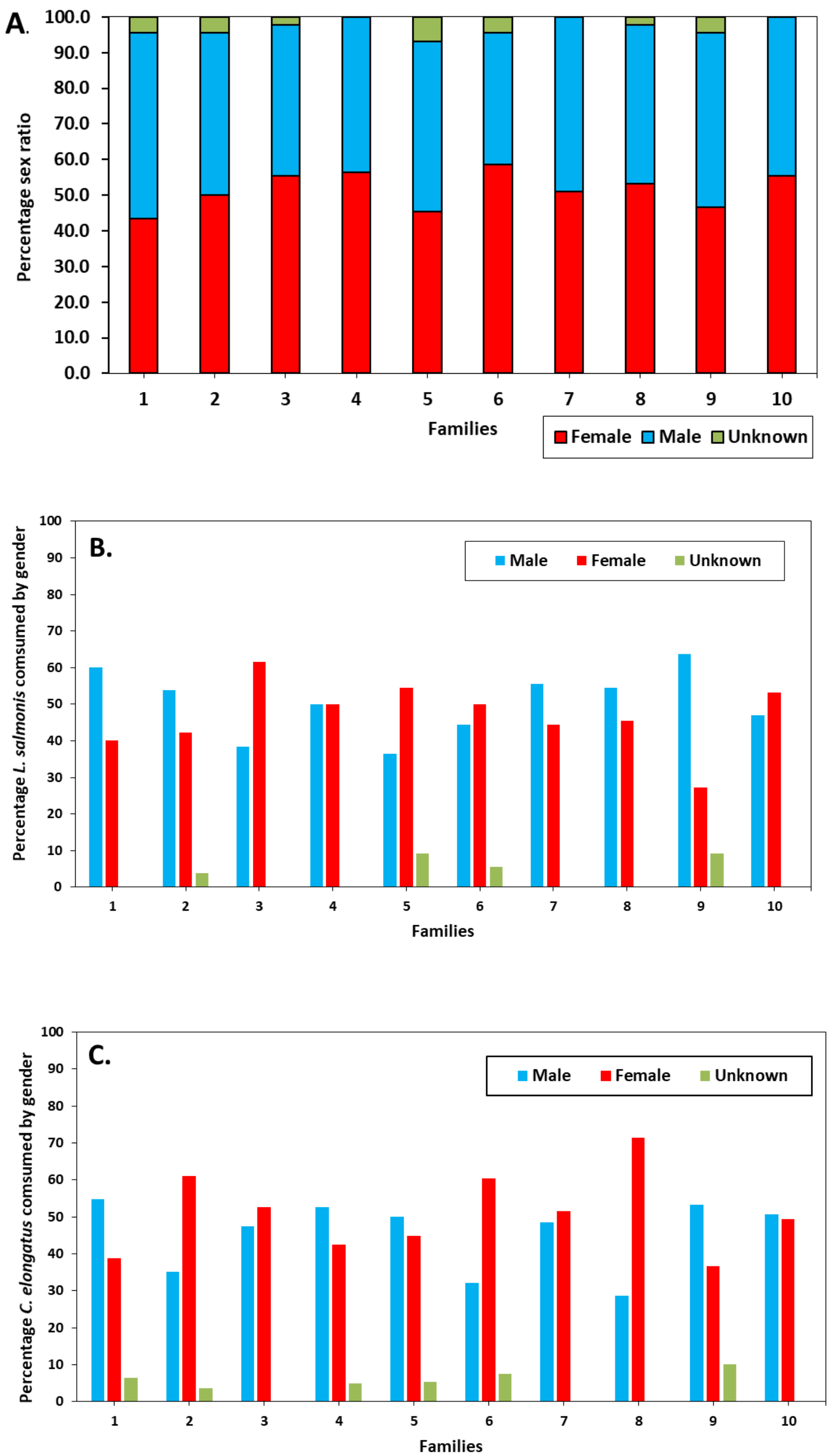
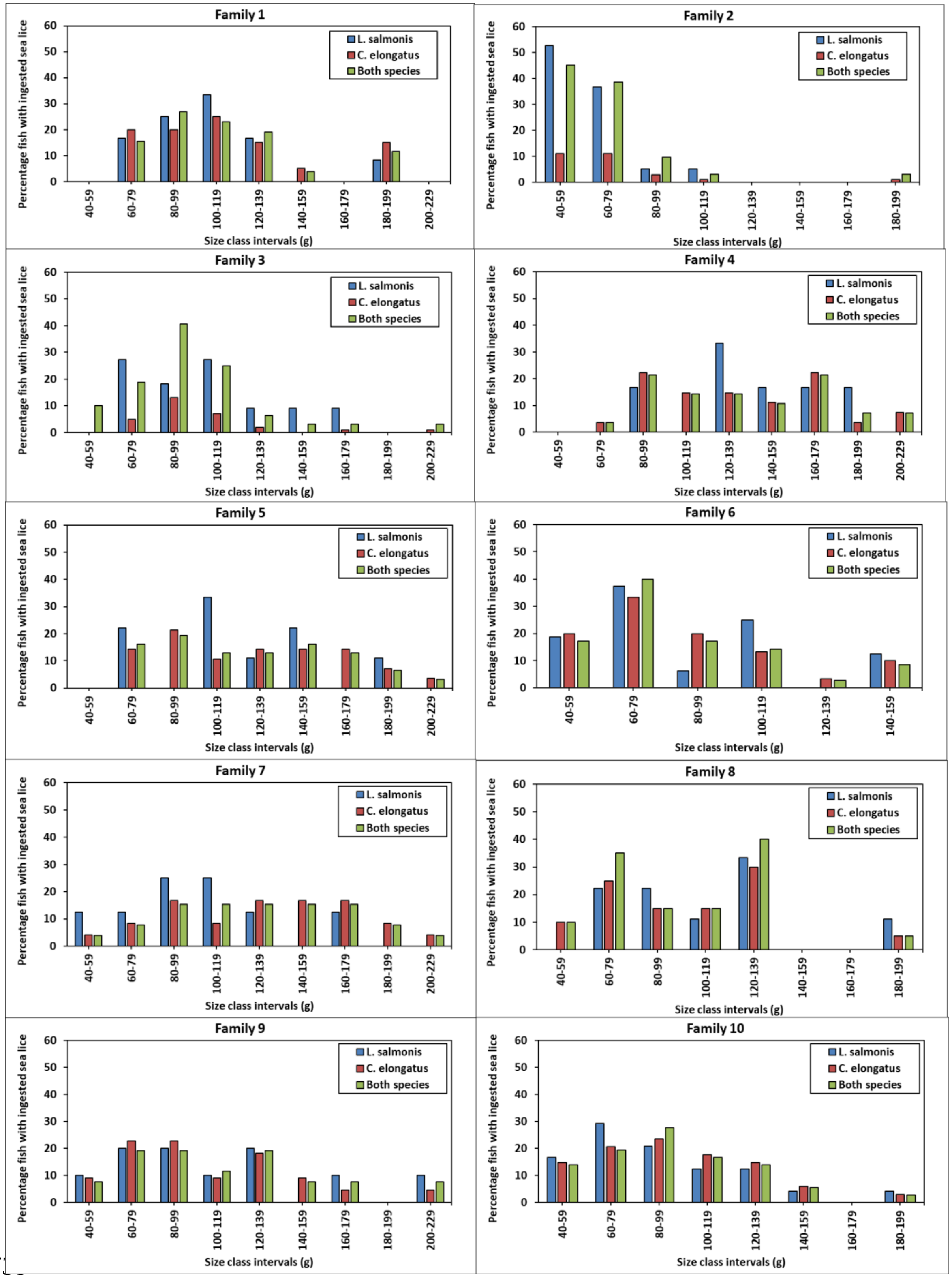


Fig. 3. Imsland, Reynolds et al.



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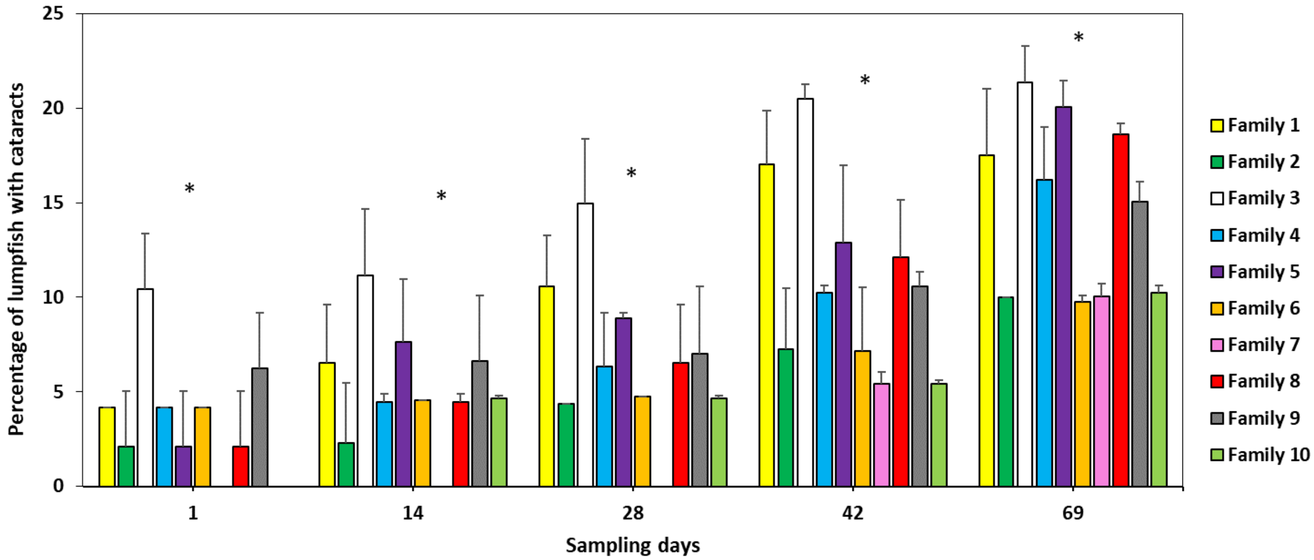

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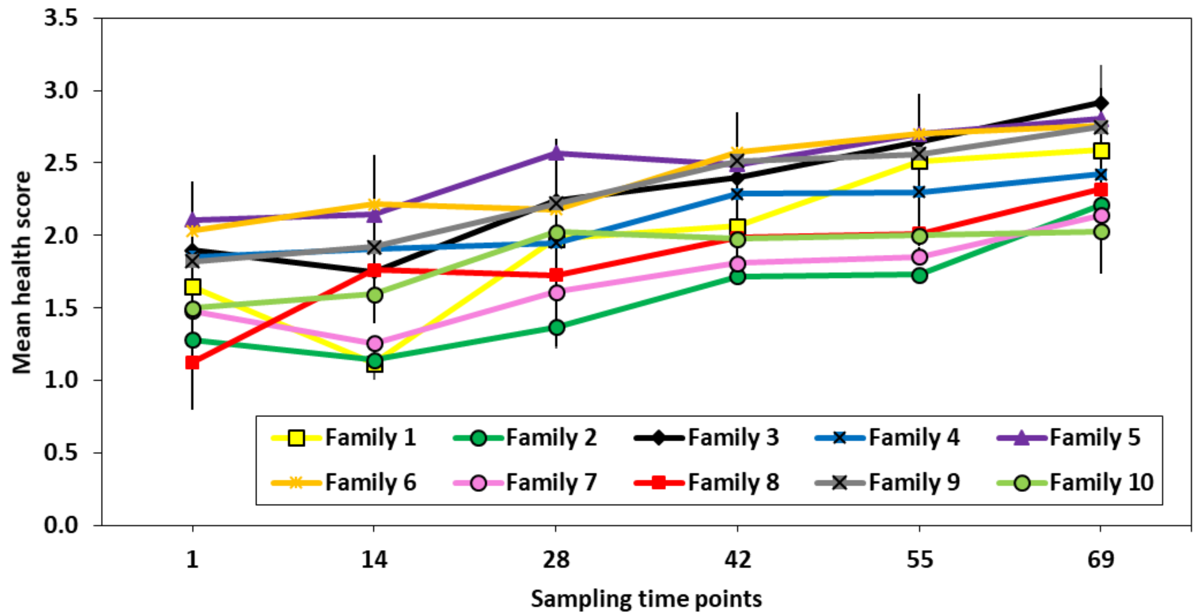

Fig. 6. Imsland, Reynolds et al.

Table 1. Mating information, fertilization and hatching date, mean weight (g) after the start-feeding period and sea pen numbers where each family was mixed with salmon for each of the ten families used in the study.

Family number	Male no.	Female no.	Fertilized	Hatched	Mean weight (± SD) on 17 March 2019	Sea pen no.
1	M1	F1	7 December	17 January	0.14 ± 0.04	301, 307
2	M1	F2	7 December	19 January	0.14 ± 0.04	304, 310
3	M2	F3	10 December	22 January	0.14 ± 0.05	308, 309
4	M2	F4	10 December	23 January	0.15 ± 0.05	306, 309
5	M3	F5	10 December	22 January	0.11 ± 0.03	301, 305
6	M3	F6	11 December	24 January	0.11 ± 0.04	304, 308
7	M4	F7	11 December	23 January	0.11 ± 0.03	303, 310
8	M4	F8	11 December	25 January	0.11 ± 0.03	302, 306
9	M5	F9	12 December	25 January	0.11 ± 0.04	303, 305
10	M5	F10	12 December	24 January	0.10 ± 0.03	302, 307

Table 2. Health assessment criteria for lumpfish used in this study.

Score		Body condition: Wounds/sores/damage
0		Intact. No evidence of any injuries.
		Minimal localised damage to body, tubercles and/or head. Injuries confined to one
1		/two locations
2		More widespread injuries. Not to be considered at high risk
3		Damaged areas more pronounced. Assessment of potential recovery.
		Recovery unlikely. Health status compromised. Fish removed
Score		Tail fin: Damage/erosion
0		No visible damage.
1		Marginal biting or fin spliting
2		Major fin ray loss
3		Complete removal of fin and tissue damage evident
		Complete erosion & tissue damage: Fish removed
Score		Other fins (2nd dorsal & pectoral): Damage/erosion
0		No visible damage.
1		Marginal biting or fin spliting
2		Major fin ray loss
3		Complete removal of fin and tissue damage evident
		Complete erosion & tissue damage: Fish removed
Score		Deformities: Suction disc; Spine; other
0		No deformities evident
1		Minimal deformation. Fish able to attach and function normally

2		More obvious deformation. Fish observed to be functioning normally
3		Extensive malformation. Fish unable to attach or function normally.
		Fish removed if unable to attach and/or feed.
Score		Cataracts
0		No cataracts
1		Less than 10 %
2		Between 10 and 50%
3		Between 50 and 75 %
4		Over 75 %, fish shall be removed if growth is negative
Score		Eye ulceration/swelling
0		None
1		light localised swelling (< 10%)
2		Swelling between 10 - 50%
3		Swelling between 50 - 75%
4		Eye completely affected and over > 75%. Fish shall be removed if growth is negative
Score		Condition factor
0		4.5 to 5.5: Good condition
1		3.5 to 4.5: Moderate condition.
2		3.0 to 3.5: Poor condition.
3		Under 3.0: Fish emaciated. Potential recovery will be assessed by evaluation of the other scores.