



# Towards better lumpfish: Changes in size variation, cataract development, behaviour and sea lice grazing through selective breeding

Albert Kjartan Dagbjartarson Imsland<sup>a,b,\*</sup>, Patrick Reynolds<sup>c,1</sup>, Lauri Kapari<sup>d</sup>,  
Simo Njabulo Maduna<sup>e</sup>, Snorre B. Hagen<sup>e</sup>, Anna Hanssen<sup>f</sup>, Ólöf Dóra Bartels Jónsdóttir<sup>a</sup>

<sup>a</sup> Akvaplan-niva Iceland Office, Akralind 4, 201 Kópavogur, Iceland

<sup>b</sup> Department of Biosciences, University of Bergen, High Technology Centre, 5020 Bergen, Norway

<sup>c</sup> GIFAS AS, Gildeskål, 8140 Inndyr, Norway

<sup>d</sup> Akvaplan-niva, Framsenteret, 9296 Tromsø, Norway

<sup>e</sup> Norwegian Institute of Bioeconomy Research, Svanhovd, 9925 Svanvik, Norway

<sup>f</sup> Lerøy Aurora, Stortorget 1D, 9008 Tromsø, Norway

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## ABSTRACT

The aim of this study was to evaluate whether sea lice grazing efficiency, behaviour, size variation and cataract development can be improved through selective breeding of lumpfish. A series of studies was conducted over a four-year period where distinctive lumpfish families were established initially from wild caught mature fish and latterly from established breeding lines. Four subsequent trials (called: Phase I-IV) with ten families of lumpfish ( $N = 480$ ) with a mean ( $\pm$  SD) weight of  $46.4 \pm 9.4$  g (Phase I),  $54.8 \pm 9.2$  g (Phase II),  $42.0 \pm 7.4$  g (Phase III) and  $31.3 \pm 2.4$  g (Phase IV) were distributed among ten sea cages ( $5 \times 5 \times 5$  m) during autumn 2018 to spring 2022, each stocked with 400–404 Atlantic salmon with an average initial mean ( $\pm$  SD) of  $387 \pm 9$  g (Phase I),  $621 \pm 15$  g (Phase II),  $280 \pm 16$  g (Phase III) and  $480 \pm 66$  g (Phase IV). All the ten cages were stocked with 48 lumpfish (12% stocking density). In all phases there was a large inter-family variation of lice grazing of lumpfish of both *L. salmonis* and *C. elongatus*. When sea lice grazing was scaled in relation to sea lice infestation numbers on the salmon the highest sea lice grazing activity was found in Phase IV and in particular in families sired from farmed parents. There was a general trend for mean start weights and standard deviations to decrease as the phases continued. A significant increase was found in frequency of behaviour associated with feeding on natural food sources and grazing sea lice from salmon during each subsequent phase. The increase in incidence of cataracts from start to end of each trial phase was significantly reduced from Phase I (16%) to Phase IV (2%). Overall, present findings showed that sea lice grazing of both *L. salmonis* and *C. elongatus*, size variation, cataract prevalence and behaviour types can be enhanced through selection and targeted breeding programs.

## 1. Introduction

Members of two of the parasitic copepod Caligidae genera – *Lepeophtheirus* and *Caligus* - have achieved notoriety by having the greatest economic impact of any group of parasites in salmonid fish mariculture (Costello, 2006; Igboeli et al., 2012, 2014; Hemmingsen et al., 2020) and have become collectively known as “sea lice”. The sea lice *Lepeophtheirus salmonis* L. and *Caligus elongatus* (von Nordmann 1832) have a major impact on salmonid aquaculture in Norway causing losses of over €440 million annually (Abolofia et al., 2017). The problems of adverse welfare outcomes associated with infestations and increased infestation

pressure have escalated with commercial production of Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum) in sea cages (Igboeli et al., 2012, 2014; Torrissen et al., 2013). Biological control using cleaner fish that pick the sea lice from salmonids (Skiftesvik et al., 2013; Imsland et al., 2014a; Powell et al., 2018; Treasurer, 2018) has been effective in reducing lice numbers and is being adopted widely by the salmon farming industry. As a cold-water cleaner-fish alternative, the common lumpfish *Cyclopterus lumpus* L. is currently used successfully to control sea lice infestations (Imsland et al., 2014a, 2018a, 2020a; Boissonnot et al., 2022; Imsland and Reynolds, 2022).

However, high mortality and loss of cleaner fish in salmon cages is

\* Corresponding author at: Akvaplan-niva Iceland Office, Akralind 6, 201 Kópavogur, Iceland.

E-mail address: [albert.imsland@akvaplan.niva.no](mailto:albert.imsland@akvaplan.niva.no) (A.K.D. Imsland).

<sup>1</sup> Equal authorship between: Imsland and Reynolds.

one of the most serious problems the aquaculture industry in Norway faces at present (Reynolds et al., 2022; Boissonnot et al., 2023). Recently, operational welfare indicators for lumpfish (Noble et al., 2019; Imsland et al., 2020b; Gutierrez Rabadan et al., 2021) have been published which can be used to define best practice guides for better welfare and reduced mortality. Recently Reynolds et al. (2022) investigated causes of mortality and loss of lumpfish from both small- and large-scale studies in Northern Norway. Results showed that causes of mortality varied within and between research sites. For lumpfish in hatcheries as well as for those deployed at small-scale sea pens, the primary cause of mortality was identified as pathogenic, while for lumpfish deployed at large-scale sea pens, transporting, grading and mechanical delousing were the primary causes of mortality. The results indicated that more research is required to clarify best practices both in commercial hatcheries and salmon cages and further understanding on lumpfish biological requirements and stress physiology is necessary to develop better methods that safeguard lumpfish welfare and meet their needs. One possible way of improving the welfare of lumpfish in sea pens is through the use of selective breeding as recent findings (Imsland et al., 2021) have indicated that welfare can vary between lumpfish families.

Intensive Atlantic salmon aquaculture is a relatively new innovation, compared to other livestock production. The process of domestication has only just begun in salmonids, and considerable effort is devoted to selecting for and estimating the heritability of economically important traits in these fish, such as growth rate, size at maturity, muscle composition, disease resistance and stress responsiveness (see e.g., Gjerde, 1993; Gjedrem, 2000; Midtlyng et al., 2002; Øverli et al., 2006). A number of aquaculture 'common garden' experiments, in which rearing effects, nutritional status and other confounding variables are removed (Huntingford, 2004), have demonstrated differences in risk taking between fish from lines that have been farmed for several generations and fish from the wild stock from which the farmed stock was originally derived, reared in identical conditions in the laboratory (Huntingford and Adams, 2005). This may suggest that a number of behavioural traits are under genetic control and that behaviour can be modified by selection. In other organisms it is known that behavioural traits respond to both natural and sexual selection, show geographic adaptation, and that such traits may be heritable (reviewed in Roff and Mousseau, 1987; Meffert et al., 2002; Stirling et al., 2002). With this information, it may be possible to predict inclination for sea lice grazing in individual fish, and families, with clear applications for the aquaculture industry. These includes the ability to select fish for breeding purposes, which have not been in contact with salmon, and to introduce a breeding programme for continuous improvements of the cleaning efficiency of lumpfish (Imsland et al., 2016a, 2021).

For lumpfish a significant individual differences in feed intake and preference for sea lice has been seen (Imsland et al., 2014a, 2014c, 2015), and genetic influence has been suggested to be a possible factor (Imsland et al., 2016a, 2021). A series of studies (called Phase I, II, III and IV) has now been conducted over a four-year period where distinctive lumpfish families have been established initially from wild caught mature fish and latterly from established breeding lines. For each phase, ten distinct lumpfish families were assessed and published results from some of these studies showed that growth, feeding behaviour and lice grazing efficacy varied between families (Imsland et al., 2021) and these observed differences may have a genetic basis which allows for these favourable traits to be maintained in future lumpfish populations.

The aim of this study was to evaluate whether sea lice grazing efficiency, behaviour, size variation and cataract development can be improved through selective breeding of lumpfish.

## 2. Materials and methods

### 2.1. Ethical statement

The use of lumpfish for experimental purposes was accepted by the

Norwegian Food Safety Authority (FOTS ID: 20736). All fish were carefully handled based on the Norwegian law on Regulation of Animal Experimentation (FOR-1996-01-15-23). All personnel involved in the study have previously completed the FELASA-C course, developed by the Federation of European Laboratory Animal Science Association. The experiment was planned and conducted using the ARRIVE guidelines (Kilkenny et al., 2010). All four phase experiments were approved by the local responsible laboratory animal science specialist under the surveillance of the Norwegian Animal Research Authority (NARA) and registered by the Authority.

### 2.2. Experimental layout

Data were sourced from the four inputs (hereafter called: Phase I, II, III and IV) of lumpfish assessed at GIFAS small-scale facility Langholmen, Nordland, Norway between September 2018 and April 2022. Each input comprised of ten distinct families derived from either wild or farmed paternal stock (Table 1). Each family comprised of 48 individuals ( $N = 480$  per phase;  $N_{\text{total}} = 1920$ ) selected and the differences

**Table 1**

Mating information, generation status and % fertilisation of each of the 40 families evaluated during the project period.

Phase I (H18): September – November 2018, 73 days					
Family	Father ID	Generation	Mother ID	Generation	% Fertilisation
H18 F1	79	Wild	789	Wild	95
H18 F2	79	Wild	790	Wild	77
H18 F3	77	Wild	791	Wild	86
H18 F4	77	Wild	792	Wild	90
H18 F5	75	Wild	795	Wild	67
H18 F6	75	Wild	796	Wild	70
H18 F7	32	Wild	798	Wild	88
H18 F8	32	Wild	799	Wild	94
H18 F9	38	Wild	800	Wild	87
H18 F10	38	Wild	801	Wild	86
Phase II (H19): October – December 2019, 68 days					
H19 F1	117	Wild	968	Wild	80
H19 F2	117	Wild	969	Wild	84
H19 F3	119	Wild	971	Wild	76
H19 F4	119	Wild	973	Wild	87
H19 F5	118	Wild	974	Wild	100
H19 F6	118	Wild	976	Wild	92
H19 F7	120	Wild	977	Wild	81
H19 F8	120	Wild	978	Wild	88
H19 F9	121	Wild	980	Wild	90
H19 F10	121	Wild	981	Wild	86
Phase III: July – October 2020, 77 days					
H19 F1	1541	H18 F6	872	Wild	97
H19 F2	1541	H18 F6	873	Wild	95
H19 F3	1427	H18 F5	876	Wild	89
H19 F4	1427	H18 F5	877	Wild	96
H19 F5	1505	H18 F6	883	Wild	96
H19 F6	1505	H18 F6	1226	H18 F3	84
H19 F7	1576	H18 F6	887	Wild	88
H19 F8	1576	H18 F6	1126	H18 F3	86
H19 F9	1589	H18 F6	911	Wild	99
H19 F10	1589	H18 F6	913	Wild	96
Phase IV: April – June 2022, 73 days					
H21F1	3560	H19 F6	3115	H19 F2	95
H21F2	6536	H19 F6	3167	H19 F2	97
H21F3	3943	H19 F1	3116	H19 F2	93
H21F4	6975	H19 F10	3116	H19 F2	96
H21F5	6883	H19 F9	3153	H19 F2	87
H21F6	6930	H19 F10	3122	H19 F2	99
H21F7	3599	H19 F6	346	Wild	97
H21F8	6326	H19 F4	347	Wild	95
H21F9	3940	H19 F1	348	Wild	93
H21F10	6469	H19 F5	349	Wild	89

in mean weights between families reflect the mean weights for each family found by individual weighing all fish upon arrival at GIFAS.

### 2.3. Atlantic salmon

The Atlantic salmon ( $N_{\text{total}} = 4000\text{--}4040$  in each Phase) 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. The fish were transferred to small-scale sea pens ( $5 \times 5 \times 5$  m,  $125 \text{ m}^3$ ) in August 2018 (Phase I), September 2019 (Phase II), June 2020 (Phase III) and March 2022 (Phase IV), and remained in those sea pens during the trial period. The salmon had an average initial mean ( $\pm$  SD) of  $387 \pm 9$  g (Phase I),  $621 \pm 15$  g (Phase II),  $280 \pm 16$  g (Phase III) and  $480 \pm 66$  g (Phase IV). All salmon 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.

### 2.4. Lumpfish

Sexually mature wild lumpfish were caught by Akvaplan-niva staff in gill nets at Hekkingen outside Kvaløya, Senja, Troms County, Norway. Eggs were stripped, fertilized and incubated at natural sea water temperature between 5 and 8 °C at Akvaplan-niva research station at Kraknes, Troms County, Norway where they hatched in January 2018 (Phase I), January 2019 (Phase II), January–February 2020 (Phase III) and September–October 2021 (Phase IV). For Phase I–III five paternal half-sibling families and ten maternal sibling families were used in the study obtained by crossing the different males and females (Table 1). For Phase IV ten sibling families were used.

All fish in Phase I and II were wild progenies (wild ♂  $\times$  wild ♀, Table 1). For Phase III 5 males derived from Phase I were used along with 8 wild caught females and 2 females from Phase I. Hence, for Phase III 2 families (F6, F8) were 1G (farmed ♂  $\times$  farmed ♀) and 8 families were 0.5G (farmed ♂  $\times$  wild ♀) progenies. For Phase IV 10 males from Phase II were used along with 6 females from Phase II and 4 wild caught females. Hence, for Phase IV 6 families (F1–6) were 1G and 4 families were 0.5G (farmed ♂  $\times$  wild ♀).

The juveniles from each family were started in 100 l conical incubators, after that reared in raceways  $40 \times 280 \times 5\text{--}10$  cm, with volume of 60–120 l depending on the fish size. The juveniles were initially fed with Otohime B1 and B2 250–650  $\mu\text{m}$ , 56% protein, 16% lipid, 14% ash (PT Aqua, Dublin, Ireland). After approximately 30 days, the juveniles were fed with 800–1800  $\mu\text{m}$  dry feed pellets (Clean Assist, Skretting, Norway; 57% protein, 15% lipid, 10% ash). Once the fish had attained a mean weight of 8.0–10.0 g all fish were anaesthetized (benzoak 80  $\text{mg l}^{-1}$ ) 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) prior to transfer to GIFAS. After transfer to GIFAS the lumpfish were maintained in a  $5 \times 5 \times 5$  m cage fitted with a specially designed net at Gifas small-scale research facility Langholmen. The fish were fed at a feeding rate of 1.5–2.0%  $\text{BW}^{-1}$  with feed blocks (World Feeds, UK, Imsland et al., 2018b, 2019a, 2020b) during the acclimation period and during all trial periods. The feed blocks were suspended in the water column. Each individual feed block was an average of  $26 \times 100$  mm with a 10 mm hole through the centre and had grooves created on their surface during the extrusion process (Imsland et al., 2018b; Imsland et al., 2019a, 2019b). 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 2.0%  $\text{BW}^{-1}$ .

### 2.5. Experimental set-up

At the start of each phase experiment (Phase I – 6 September 2018;

Phase II – 11 October 2019; Phase III – 19 July 2020; Phase IV – 11 April 2022), 4000–4040 Atlantic salmon were bulk weighed, counted and randomly distributed between ten cages of  $125 \text{ m}^3$  ( $5 \times 5 \times 5$  m), with 400 (Phase I, III and IV) to 404 (Phase II) 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 (Phase I – 18 November 2018; Phase II – 11 December 2019; Phase III – 11 October 2020; Phase IV – 24 June 2022).

All ten cages were stocked with 48 lumpfish (12% stocking density) during all four trials. The average initial mean ( $\pm$  SD) of the lumpfish was  $46.4 \pm 9.4$  g (Phase I),  $54.8 \pm 9.2$  g (Phase II),  $42.0 \pm 7.4$  g (Phase III) and  $31.3 \pm 2.4$  g (Phase IV). The stocking of cages was such that each cage consisted of two random families where families were randomly allocated to the different cages. All lumpfish from each family were anaesthetized (Metacaine, 200  $\text{mg l}^{-1}$ ) 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 four studies lasted for 68–77 days. Daily mean temperature in the sea pens was as follows:

- Phase I. Decreased from 10.5 °C on the 6 September to 7.2 °C on 18 November 2018.
- Phase II. Decreased from 10.5 °C on the 11 October to 6.0 °C on 11 December 2019.
- Phase III. Increased from 11.5 °C (19 July) to 14.8 °C (22 August), and then decreased to 10.9 °C on 11 October 2020.
- Phase IV. Increased from 3.8 °C (11 April) to 9.9 °C (24 June 2022).

Salinity in all four studies ranged from 29.6 ppt. to 32.8 ppt., while dissolved oxygen ranged between 8.6  $\text{mg l}^{-1}$  and 13.3  $\text{mg l}^{-1}$  during the four trial periods. Secchi depth in the sea pens was between 8 and 10 m in the four studies.

### 2.6. Gastric lavage of lumpfish

During the trial period gastric lavage (Imsland et al., 2014a, 2015, 2016a, 2021) was performed every two weeks to assess the feeding preferences of individual lumpfish. Individual weight (g) and total length (cm) of all the lumpfish were measured on the same dates that gastric lavage was performed. 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  $\text{mg l}^{-1}$  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. In addition, infestation numbers for each cage were recorded by inspecting 30 sedated individual Atlantic salmon.

### 2.7. Behavioural observations

During all four phase trials intra and interspecific behaviour was recorded by direct observations in all cages. The behaviour of lumpfish in the cages was assessed 2–4 times per week throughout the trial period. Behavioural observations commenced one week after the cages had been established to allow for an acclimation period. Behaviour was classified by recording for 30 s intervals the principal activity of individual fish. Ten Floy tagged fish from fish from each family were observed (to prevent pseudo replication) to identify family members, giving a total of 20 activity records per duplicate group, on each observation time point for each cage. Each Floy tag had a unique number on the shaft and this was used to identify each tagged fish. In addition, eight different colours of tag were used to assist in individual identification (white, white with

black strip, blue, green, yellow, red, black and orange) (five of each for eight fish). There was no algae growth on the tags so they were always visible. The tags were sited on the highest point of the back just beside the dorsal array. As each fish was identified directly (if on or near the surface), the number was normally seen. If the number was not visible, then another fish would be observed until all 20 recordings were achieved by different fish. Behavioural observations were classified using behavioural indices as used by Imsland et al. (2014b, Imsland et al., 2016a, b) (Table 2). These behaviours were classified and grouped into “positive/cleaning”; “negative” and “normal” types (Table 2) for the propose of this summary study. Positive behaviours are associated with feeding on natural food sources withing the cage environment, feeding on feed blocks and grazing sea lice from salmon. Negative behaviour types are mostly associated with consuming salmon feed pellets either obtained from the edge of the salmon feeding zone or actively competing for access to this feed source. Normal behaviour is shoaling with conspecifics, resting and hovering within the sea pen.

## 2.8. Cataract scoring

During weighing and counting of lumpfish throughout all four study periods, 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 <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.

**Table 2**

Classification of behaviour types for lumpfish used in this study. See Imsland et al. (2014 b, 2016) for details of the different behaviour types.

Behaviour type	Description
<i>Positive behaviour</i>	
Feeding on net fouling	Observed feeding on side of nets
Feeding on free-swimming organisms	Observed feeding on organisms entering the sea pen
Eating from feed blocks	Observed eating from feed blocks
Swimming along net side	Swim up or down at the sea pen net
Inspecting Atlantic salmon	Swimming along individual Atlantic salmon
Cleaning Atlantic salmon	Displaying cleaning of <i>L. salmonis</i> from Atlantic salmon
<i>Negative behaviour</i>	
Actively competing for salmon pellets	Observed competing with Atlantic salmon for pellets
Eating salmon pellets	Observed eating pellets on the periphery of feeding Atlantic salmon
Swimming in between Atlantic salmon	Observed swimming among Atlantic salmon
Swimming at observer	Observing the observer
Aggression salmon	Observed showing aggression towards Atlantic salmon
Aggression lumpfish	Observed showing aggression towards conspecifics
<i>Normal behaviour</i>	
Shoaling	Groups of lumpfish swimming together
Resting on substrates	Observed resting in folds or substrates of the sea pen net
Hovering	Near motionless in sheltered areas of the sea pen

## 2.9. Statistics

All statistical analyses were conducted using Statistica™ 13.3 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). A two-way nested analysis of variance (ANOVA, Searle et al., 1992) where replicates are nested within family groups was applied to calculate the effect of different families within and between each experimental Phase on growth performance, behaviour, cataract scores and sea lice counts. The model equation of the nested ANOVA had the form:

$$X_{ijk} = \mu + \alpha_i + C_{ij} + \varepsilon_{ijk}$$

where  $\mu$  is the general level;  $\alpha_i$  is the family group effect;  $C_{ij}$  is the contribution caused by replicate (sea cage)  $j$  in feeding frequency  $i$  and  $\varepsilon_{ijk}$  is the error term. We assume that  $\varepsilon_{ijk} \sim \text{Normal distributed } (0, \sigma^2)$ .

Significant differences revealed in ANOVA were followed by Student-Newman-Keuls (SNK) post hoc test to determine differences among experimental groups. Significance level ( $\alpha$ ) of 0.05 was used if not stated otherwise.

## 3. Results

### 3.1. Growth

There was a general trend for mean start weights and standard deviations to decrease subsequently from Phase I to Phase IV (Fig. 1) from an initial mean ( $\pm$  SD) start weight of  $46.4 \pm 9.4$  g for lumpfish in Phase I to  $31.3 \pm 1.7$  g in Phase IV. Mean end weights varied from  $90.1 \pm 10.8$  g for Phase IV to  $117.8 \pm 13.1$  for Phase III (Fig. 1). In addition, the variation in mean start weights within each phase population decreased significantly between Phase I and Phase IV (two-way ANOVA,  $P < 0.01$ , Fig. 2). For Phase I, the variation in mean weights for all ten families was 27.1 g while for Phase IV lumpfish was 4.6 g (Fig. 2).

### 3.2. Behaviour

There was a significant increase in frequency of positive behaviour during each subsequent phase which accounts for a 45% increase in those behaviour types between Phase I and Phase IV (two-way ANOVA,  $P < 0.05$ , Fig. 3A). The frequency of negative behaviour types calculated for each phase (Fig. 3B) showed a sharp decline from Phase I onwards. The frequency of negative behaviours for Phase I was calculated to be 35% of all observed lumpfish for all ten families while for Phase IV 10% of all observations were linked to these behaviour types. The frequency of normal behaviours calculated for each phase (Fig. 3C) showed little variation in these behaviour types for Phase I, II and IV with percent frequencies ranging between 24% for Phase IV and 34% for Phase III. There was a higher frequency of these behaviours recorded during Phase II (two-way ANOVA,  $P < 0.05$ , 42% of all lumpfish observed).

### 3.3. Cataracts

Cataract prevalence was calculated to be 19% for Phase I while for Phase IV it was 9% and was significantly different between those two phases (two-way ANOVA,  $P < 0.05$ , Fig. 4). There were no significant differences (two-way ANOVA,  $P > 0.45$ ) in cataract prevalence at the start of each phase. There were persistent variations in prevalence between families within each phase with some families scoring low (Student-Newman-Keuls post hoc tests,  $P < 0.05$ ) levels to families scoring much higher levels of cataracts. Further there were indications that lumpfish families with higher prevalence had the lowest levels of ingested sea lice. Generally, most of the families which were tested arrived at GIFAS with varying levels of cataracts and only two families were scored as having no cataracts (families 7 and 10 from Phase II).



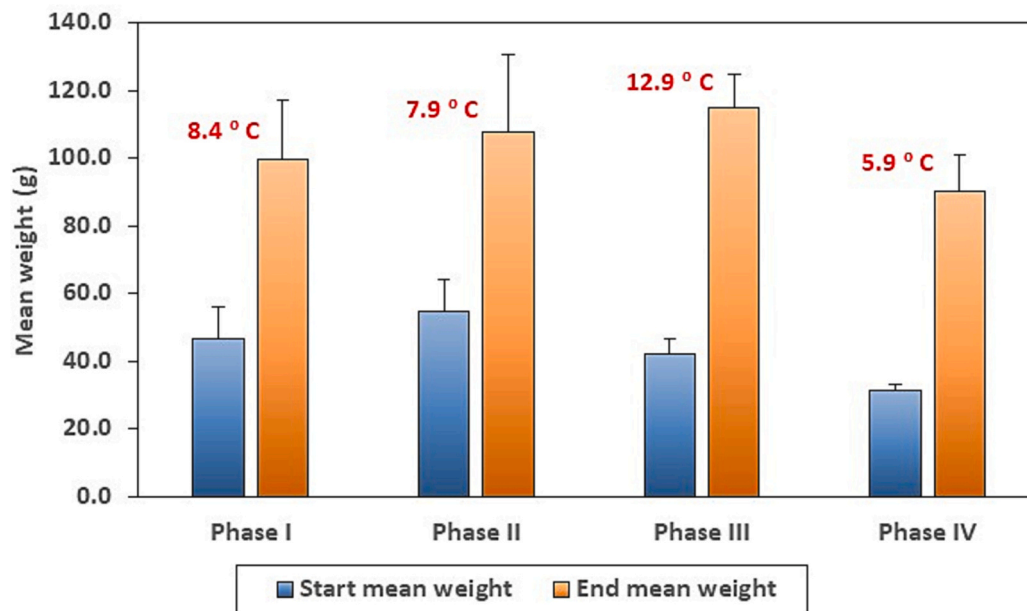


Fig. 1. Mean start, and final weights calculated for each phase (combining individual lumpfish weight for all ten families,  $N = 470\text{--}480$  for each bar). Values represent means  $\pm$  S.D. Average temperature through each phase is indicated in the above the bars.

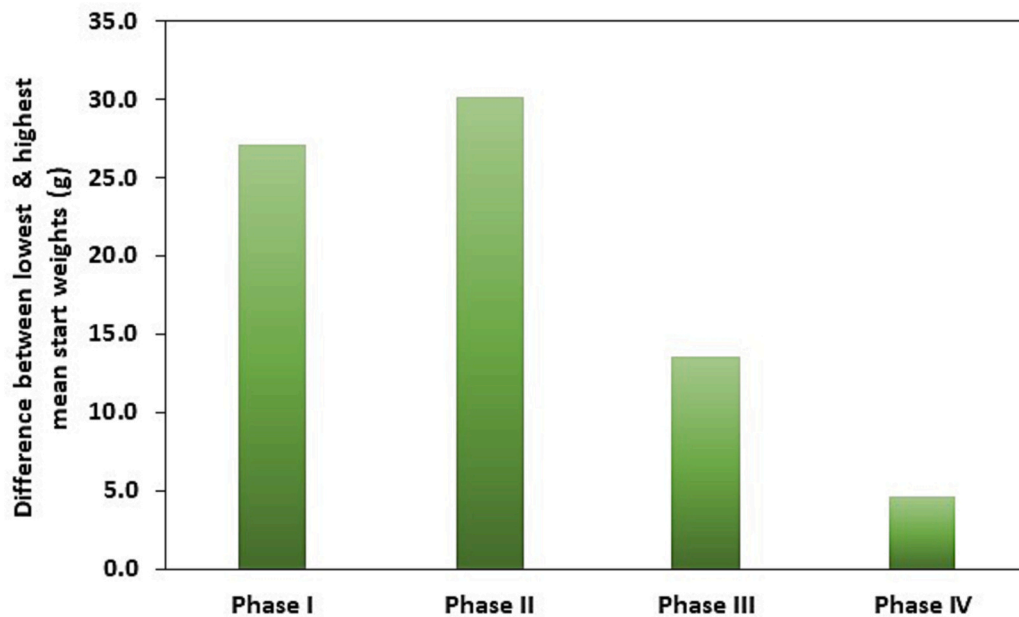


Fig. 2. Difference between lowest and highest mean start weights between the families within each of the experimental phases.

Further, cataract prevalence increase during each experimental phase declined from Phase I to Phase IV.

### 3.4. Sea lice grazing

Infestation numbers of *L. salmonis* were generally very low during all phases particularly for Phases III and IV (Fig. 5). Only during phase II were the Norwegian treatment threshold (0.5) for mature female stages of *L. salmonis* reached. The mean number of mature female *L. salmonis* (Fig. 5A) and all stages of *L. salmonis* (Fig. 5B) indicate that at higher infestation numbers then there is a higher incidence of ingested sea lice (see Fig. 6). Phase II had the highest infestation numbers as well as the higher numbers of lumpfish found with ingested lice (Fig. 6). The average infestation numbers of female *L. salmonis* varied between the

different phases and were found to be: Phase I – 0.14; Phase II – 0.30; Phase III – 0.10; Phase IV – 0.04. In order to visualise sea lice grazing in relation to sea lice infestation numbers we scaled the values of Fig. 6 with the observed infestation numbers (using the highest numbers (Phase II) as the base number). First, we averaged the values shown in Fig. 5A for each phase (i.e., Phase I: 0.14; Phase II: 0.30; Phase III: 0.10; Phase IV: 0.04). Secondly, the relative scaling was then found by using the highest infestation number observed (i.e., Phase II: 0.3) as base number and dividing the base into each of the Phases e.g., for Phase I =  $0.3/0.14 = 2.1$ , for Phase II =  $0.3/0.3 = 1$ , for Phase III =  $0.3/0.1 = 3$  and for Phase IV =  $0.3/0.04 = 7.5$ . This gives us the relative scaling of each phase depicted in Fig. 7. This relative scaling of each experimental phase (Fig. 7) indicates the highest sea lice grazing activity in Phase IV. Similar sea lice grazing activity was found in Phase I-III,

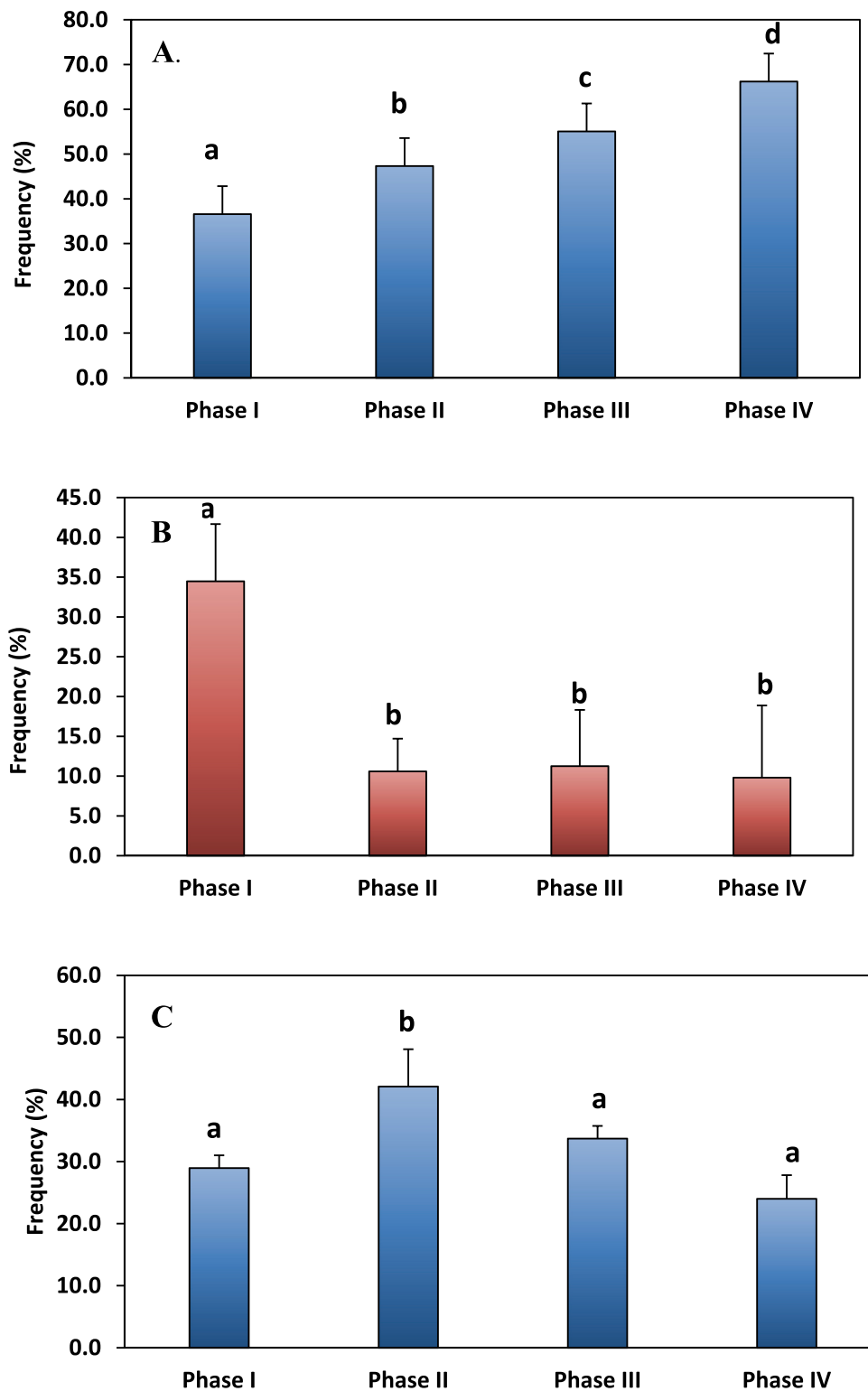


Fig. 3. Mean percentage frequency of observed positive behaviour (A), negative (B) and normal (C) types for each of the four phases. Values represent means  $\pm$  S.D. Different letters indicate statistical differences (two-way nested ANOVA,  $P < 0.05$ ) in behavioural type between the different experimental phases.

whereas the inter-family variation is highest in Phases I-II (wild parents).

#### 4. Discussion

##### 4.1. Sea lice grazing in different families

Efficient delousing capability is an extremely important trait for ecological control with sea lice in salmon farming and the results from these studies indicate grazing activity even though infestation numbers

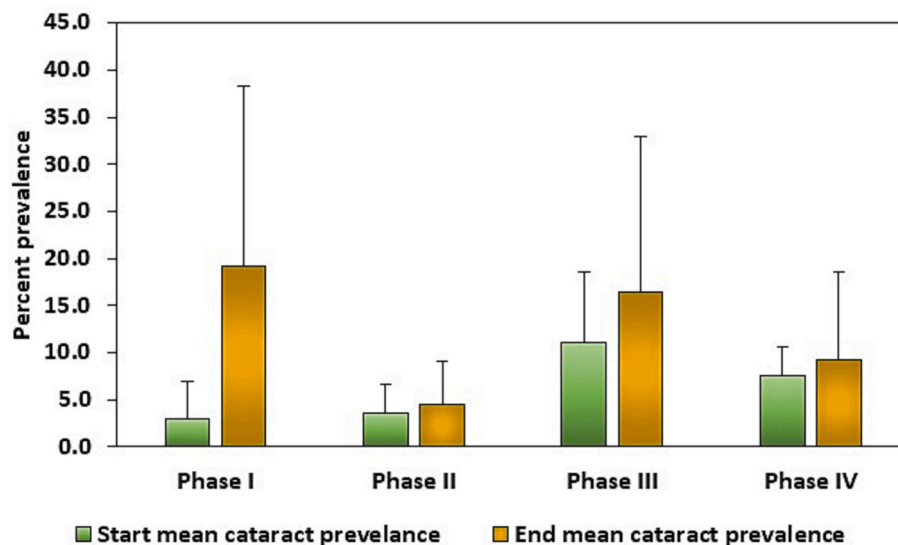


Fig. 4. Percentage cataract prevalence at the start and end of each phase. Values represent means  $\pm$  S.D.

of *L. salmonis* were low, but typical to what is observed in the industry (Imsland et al., 2018a; Boissonnot et al., 2022; Imsland and Reynolds, 2022), during most of the phases. Further, there was clear differences between families during each phase. When families for each phase were ranked in descending order of frequency, there was a clear trend for some families to higher incidences of sea lice grazing than others. Further, when sea lice grazing was scaled in relation to sea lice infestation numbers in each experimental phase a trend of increased sea lice grazing in the 1G breed fish (Families 1–6) used in Phase IV. The top 4 performing families of Phase IV were sired from farmed parents. Highest inter-family variation was seen in Phases I–II (wild offspring). Further, there was direct evidence of grazing of *C. elongatus* when gastric lavage sampling was undertaken during all experimental phases. This further supports the suggestion that the lumpfish grazed on *L. salmonis*. Previous studies (Imsland et al., 2014a, 2016a, 2021) have shown that both species combined are normally exploited as a food source. This means that increasing grazing of *L. salmonis* through selective breeding will also lead to increased grazing of *C. elongatus* which is in line with present findings. Grazing of *C. elongatus* was more pronounced in Phase I, III and IV (data not shown) compared to grazing of *L. salmonis*. Infestation numbers of this species were particularly high during the Phase II project period and was not considered the norm for the site where the study was undertaken. Infestation numbers ranged between an average of 1.3 per fish and 1.7 per fish during the Phase II. 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 numbers.

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 a later study (Imsland et al., 2021) supported this. In this study initial size of the lumpfish was similar (31–46 g) in all four experimental phases which makes size-related differences in sea lice grazing between the experimental phases highly unlikely. More likely, the lice grazing activity recorded during this study suggests a likely genetic effect in line with previous findings (Imsland et al., 2016a, 2021). Earlier studies have further indicated that this influence may be from both male and female broodstock rather than an individual gender (Imsland et al., 2021). Given that, this genetic effect has successfully been used in this selection programme to enhance grazing behaviour of lumpfish.

Juvenile lumpfish displays omnivorous feeding behaviour both in

the wild (Ingólfsson and Kristjánsson, 2002; Vandendriessche et al., 2007) and in culture (Imsland et al., 2015, 2016b, 2021). Although the nutritious and energy dense feed blocks are equally available for all families in all experimental phases of this study, and the energy demand is well covered, there was a clear family-based 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. Work is underway in our research group to establish a genotype-phenotype association of sea-lice delousing-related traits (S. Maduna, pers. comm.).

The frequency of individuals to graze *L. salmonis* and *C. elongatus* on repeated occasions was significantly different between families (data not shown). In Phase IV fish sired from farmed parents were dominant among the repeated grazers. This indicates a strong preference to select sea lice as a food source by certain individuals within these 1G 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.

Sea lice grazing observed in this study is similar to that seen in earlier small-scale studies (Imsland et al., 2014a, 2014c, 2015, Imsland et al., 2016a, b; Imsland and Reynolds, 2022). However, it must be noted that all the data in present study are from small-scale studies allowing for a much more controlled sampling of sea lice grazing and lower sea lice grazing has been reported from large-scale observations. For example, Engebretsen et al. (2023) found only 3.1% of the lumpfish stomachs containing salmon lice whereas this varied from 0 to 80% in the different families in this study. It should be noted that large-scale observations (Imsland et al., 2018a; Engebretsen et al., 2023) and small-scale studies (Imsland et al., 2014a, 2014c, 2015, Imsland et al., 2016a, b; Imsland and Reynolds, 2022) often give different results in sea lice grazing although high sea lice grazing numbers have also been noted in large scale trials (e.g., Eliassen et al., 2018).

#### 4.2. Lumpfish growth and size variation

Variation in mean start weights within each phase population decreased significantly between Phase I and Phase IV. It has been observed that variation in individual weights exists within lumpfish populations reared from the same male and female broodstock (Imsland et al., 2016a, 2021). For future establishment of lumpfish families, a percentage of outliers (smallest and largest individuals) should be

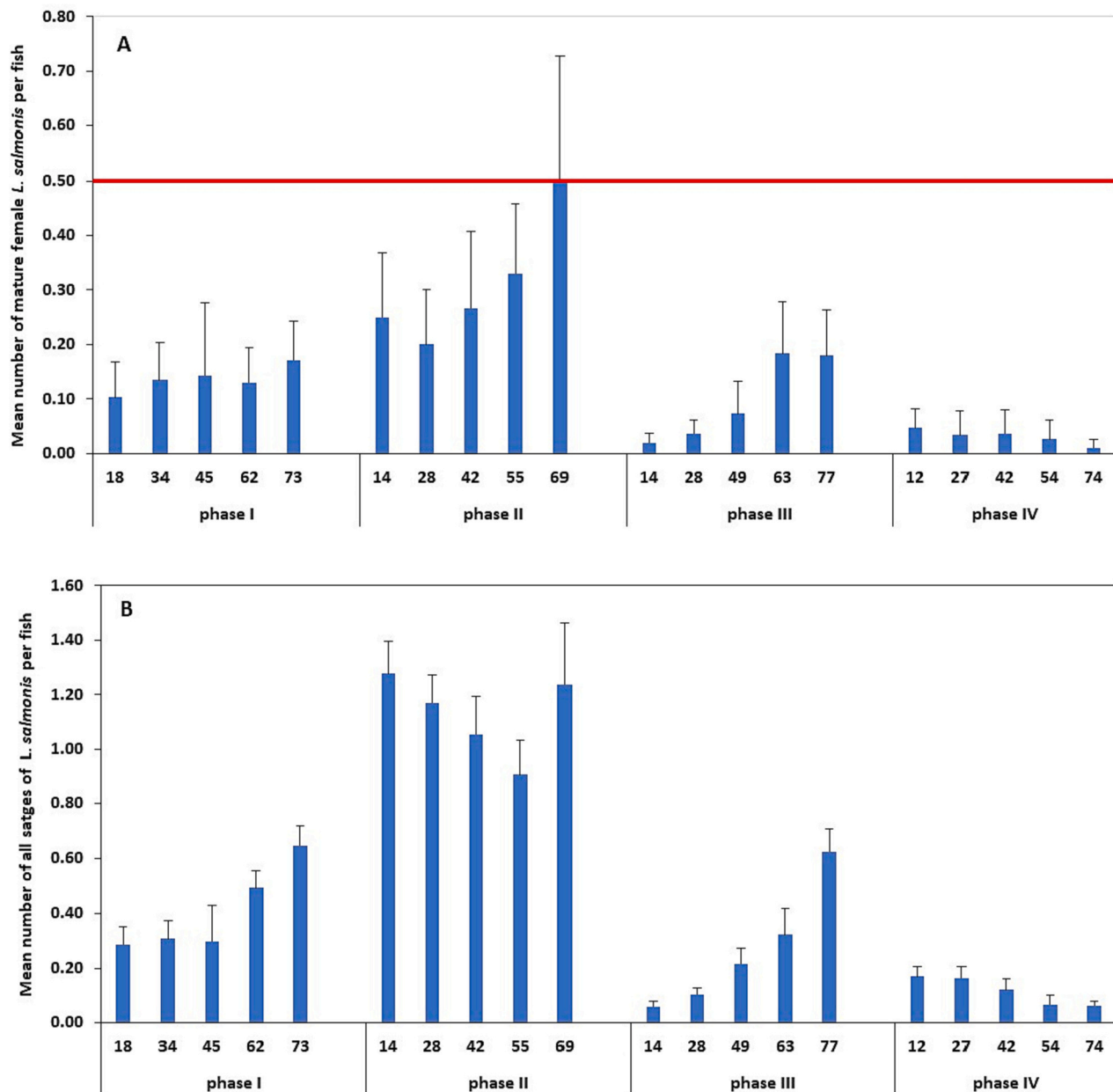


Fig. 5. Mean sea lice counts for A) mature adult female *L. salmonis* and B) pre-adult and mature male and female stages of *L. salmonis*. Values represent means  $\pm$  S.D. Red line denotes treatment threshold number for mature female *L. salmonis*. Numbers on X-axis denote day number during each experimental Phase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

removed from each family during the hatchery phase to maintain as close as possible the true mean/medial range of each family. This criterion, in part has been used during the later phases and may have contributed to the reductions in mean start and end weights observed. In addition, the establishment of more farmed families as the study progressed has reduced reliance on wild mature fish and as a result may have reduced the historical observed variations in individual weights within populations.

It has been proposed from previous studies (Imsland et al., 2016a, 2021) that differences in mean start weights between families cannot be attributed to time of hatching of the eggs or differences in water quality during incubation as all families were reared at the same location and at the same time of year (apart from the fish in Phase IV) and 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, unpublished data) as

the two smallest lumpfish families shared the same male. It may also be linked to female phenotype which results in production of smaller eggs compared to the normal egg size of the species. 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 (i.e., this function varies in relation to maternal phenotype). Further research is required to determine parental influence on early juvenile growth of lumpfish.

It should be noted that high growth is not an aim for lumpfish used as cleaner fish. Imsland et al. (2021) found that small lumpfish (initial size approx. 39 g) have a higher overall preference for natural food items, including sea lice, compared to larger conspecifics (initial size 67 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



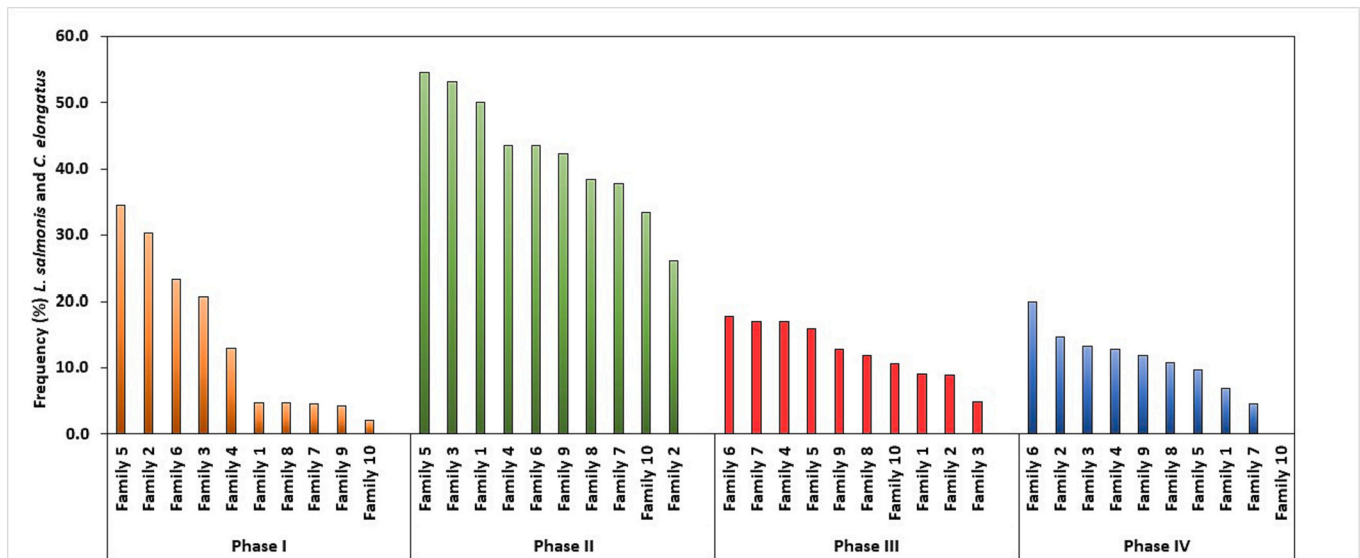


Fig. 6. Percentage of each lumpfish from each family found with ingested *L. salmonis* and *C. elongatus*. Values represent means  $\pm$  S.D.

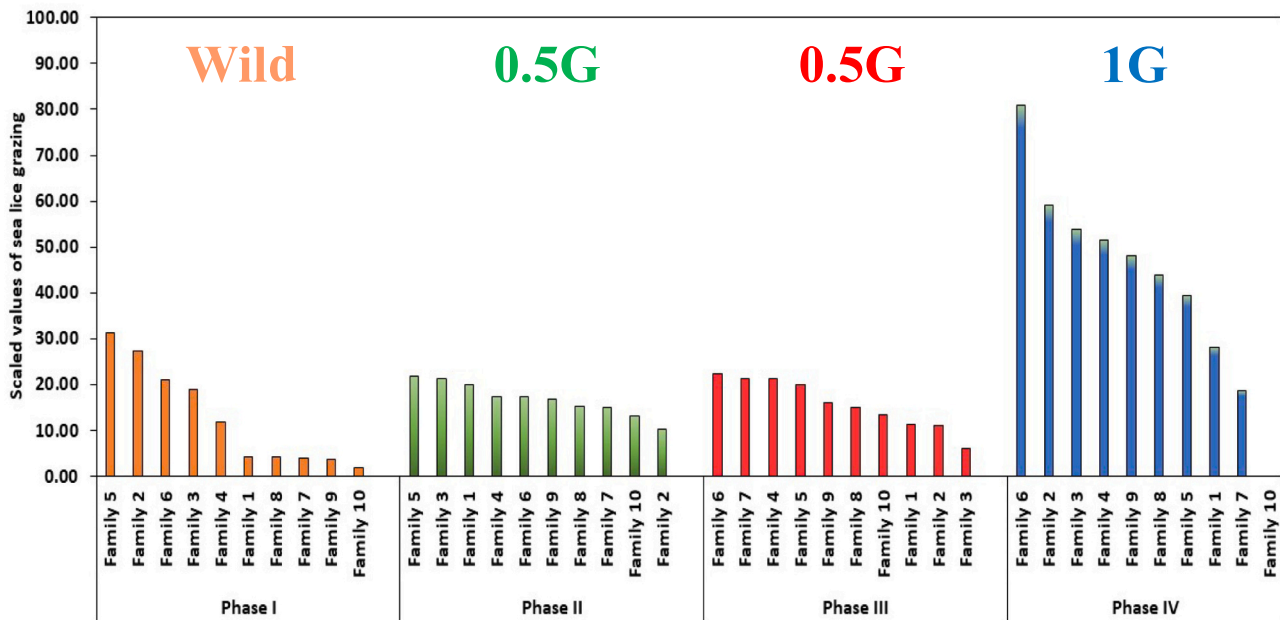


Fig. 7. Comparison of how much lice found in lumpfish stomachs related to the amount available.

cages may allow for the prolongation of sea lice grazing behaviour and allow salmon farmers to alter their stocking strategies and potentially reduce the number of times restocking of lumpfish occurs as well as perhaps enhancing sea lice grazing potential. Reducing initial size variation, as seen in present study, is an important step towards optimal use of lumpfish in sea pens.

#### 4.3. Lumpfish behaviour

Juvenile lumpfish exhibit a limited palette of behaviour types (12–14 types) with the majority based on food location and/or feeding (Imslund et al., 2014b, 2016a). These behaviour types were classified and grouped into “positive/cleaning”; “negative” and “normal” types for the purpose of this study (Table 2). Positive behaviours are associated with feeding on natural food sources within the cage environment, feeding on feed blocks and grazing sea lice from salmon. The natural feeding

behaviours such as feeding on net fouling and free-swimming organisms have been shown in previous studies to be linked with grazing sea lice from Atlantic salmon (Imslund et al., 2014b, 2016a). Negative behaviour types have been shown in previous studies to be linked to low or none-existent sea lice grazing for both *L. salmonis* and *C. elongatus* (Imslund et al., 2014b, 2016a). These behaviour types are mostly associated with consuming salmon feed pellets either obtained from the edge of the salmon feeding zone or actively competing for access to this feed source. Previous studies have also shown that lumpfish which are exhibiting this behaviour strongly, persist in this behaviour to the extent of most others (Imslund et al., 2014b, 2016a).

Previous studies have shown that wild juvenile lumpfish forage using one of two modes: they can actively search for prey while swimming (here: positive) or they can ‘sit and- wait’ for prey (here: normal) while clinging to the substrate using a ventral adhesive disk (Killen et al., 2007). The study suggested that juvenile lumpfish forage in a manner

that reduces activity and conserves space in their limited aerobic scope. The authors noted that this behavioural flexibility is of great benefit to this species, as it allows young individuals to divert energy towards growth as opposed to activity. Present data are in line with these observations as lumpfish displayed large behavioural flexibility with significant increase in positive behavioural mode in subsequent experimental phases of this study. Phase II had the highest sea lice infestation numbers of all the phases, and this may, in part, have reinforced normal behaviour types as indicated in previous studies performed by our research group (Imsland et al., 2014b, 2016a). Based on the data from the present study we suggest that this natural feeding dimorphism might be genetic due to the observations recorded during the study period as the first mode (search for prey while swimming) increased significantly from Phase I to Phases IV concurrently with the increased use of breed fish. However, it must be noted that changes between Phase I and II cannot be attributed to selective effect as both as all families in those two Phases were from wild populations.

#### 4.4. Cataracts

The increase in incidence of cataracts from start to end of each trial phase was reduced from Phase I (16% increase) to Phase IV (2% increase). However, prevalence was lower than observed in previous studies using pelleted feeds (Imsland et al., 2019b), but comparable, or lower, 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 of wild origin (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). Comparing fish from Phase I (wild) with Phase IV (1G breed fish) we suggest that the difference in development of cataract (48% lower in Phase IV) is linked to the hatchery origin of the broodfish. Development of cataract means that less light passes to the retina and vision becomes impaired or disappears (Bjerkås and Sveier, 2004). Especially the more severe degrees of cataract may then reduce feed intake (Savino et al., 1993) and competition for food (Barber et al., 2000), thus reducing growth.

Importantly, if lumpfish are stocked in commercial salmon and have some degree of cataracts (Jonassen et al., 2017), there is the issue that if the fish have reduced vision, then they lose their ability to graze sea lice from salmon. It has been suggested that cataracts can affect how effectively fish catch natural feed, such as in Arctic charr (*Salvelinus alpinus* L.) where fish with no cataracts caught zooplankton more effectively than fish with cataracts (Voutilainen et al., 2008). Fish that were assessed as having cataracts which covered >75% in both eyes would be visually impaired to a point where locating food items would be challenging and as a result growth performance would be limited to a point where the fish would starve and suffer weight loss. A previous study (Imsland et al., 2016a) showed that lumpfish with a low degree of cataracts does not affect their ability to detect and consume sea lice nor affect their overall feed intake and growth negatively. However, in a previous study (P. Reynolds, unpublished data) over 80% of lumpfish developed severe cataracts and of these, 50% were found to have weight loss after 50 days. Upon closer examination, cataracts scored as 4 (covering over 75% of the eye surface) varied in their level of opacity and it may be the degree of opacity which is the limiting factor in fish being able to find food items.

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. 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 and that this can be used in selection for lowering the risk of developing cataracts as has been seen in the present study.

#### 4.5. Gastric lavage sampling

Sea lice grazing was studied by gastric lavage of all lumpfish every two weeks during all four trial periods. The method is a nonlethal and harmless method where the stomach contents of the lumpfish are flushed out by a stream of water. The method was used with 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 considered 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 (Imsland et al., 2014a, 2015, 2016a, 2021). This method has been used on other fish species (Stehlik et al., 2015; Braga et al., 2017) with no detrimental effects.

### 5. Conclusions

There were clear differences in sea lice grazing efficacy, size variation, behaviour and cataract prevalence between the families tested in the different phases of the study. Present findings may suggest that at higher infestation numbers on the salmon then there may be found a higher incidence of ingested sea lice in lumpfish. In all phases there was a large inter-family variation of lice grazing of both *L. salmonis* and *C. elongatus*. When sea lice grazing was scaled in relation to sea lice infestation numbers on the salmon the highest sea lice grazing activity was indicated in Phase IV and in particular in families sired from farmed parents. Mean start weights and standard deviations decreased in subsequent phases. Behaviour associated with feeding on natural food sources within the cage environment, feeding on feed blocks and grazing sea lice from salmon increased during each subsequent phase. Further, the frequency of behaviour linked to low or non-existent sea lice grazing showed a sharp decrease from Phase I onwards. A general decrease in mean cataract prevalence at the end of each phase was observed. The increase in incidence of cataracts from start to end of each trial phase was reduced from Phase I (16% increase) to Phase IV (2% increase). Overall, present findings showed that sea lice grazing of both *L. salmonis* and *C. elongatus*, size variation, cataract prevalence and behaviour types can be enhanced through selection and targeted breeding programs.

#### CRedit authorship contribution statement

**Albert Kjartan Dagbjartarson Imsland:** Project administration, Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Patrick Reynolds:** Conceptualization, Investigation, Project administration, Writing – review & editing. **Lauri Kapari:** Investigation, Writing – review & editing. **Simo Njabulo Maduna:** Conceptualization, Writing – review & editing. **Snorre B. Hagen:** Conceptualization, Writing – review & editing. **Anna Hanssen:** Funding acquisition, Writing – review & editing. **Ólöf Dóra Bartels Jónsdóttir:** Conceptualization, Writing – original draft, Writing – review & editing.

#### Declaration of Competing Interest

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

#### Data availability

Data will be made available on request.

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