Aquaculture 531 (2021) 735777

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Development, validation and testing of an Operational Welfare Score Index for farmed lumpfish *Cyclopterus lumpus* L

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ARTICLE INFO

Keywords:
OWI
Relative weight
Salmon farming
Cleaner fish
Welfare indicators

ABSTRACT

Lumpfish (*Cyclopterus lumpus* L.) are widely used for controlling sea lice in salmon farming, but their welfare is often challenged by poor husbandry, stress, and disease outbreaks, which compromise their ability to delouse salmon and cause public concern. It is hence important to identify when the welfare of lumpfish is being compromised in a simple and effective manner so that remedial actions can be taken. We developed, validated and tested a Lumpfish Operational Welfare Score Index (LOWSI) based on a visual assessment of skin and fin damage, eye condition, sucker deformities and relative weight, operational welfare indicators that fish farmers considered to be the most informative and were validated against cortisol measurements. We also present percentile length-weight charts to enable fish farmers to detect underweight and emaciated lumpfish at different stages of development. The lumpish welfare score index was quick and easy to score and was highly repeatable (intra class correlation coefficient = 0.83 ± 0.05). Most lumpfish (71%) displayed good welfare, but significant differences were found between six commercial sites and 28% of lumpfish had lower than normal weights for their length, and 10% were emaciated. The most common welfare problems were sucker deformities and fin damage in hatcheries, and poor eye condition and body damage in sea cages, conditions that may increase the risk of emaciation. Being able to score the welfare of lumpfish quickly and accurately will help improve their welfare, reduce stress-related mortalities, and improve the sustainability of the salmon farming industry.

1. Introduction

Growing consumer demand for ethically-produced food has led to the development of specific welfare standards for a few farmed fish such as Atlantic salmon (Pettersen et al., 2014; RSPCA, 2018), but only generic guidelines exist for most farmed species (Cooke, 2016). Given the large diversity of fish, and their very different habitat and social requirements, welfare criteria that may work well for some species may not be applicable to others (Toni et al., 2019; Treasurer and Feledi, 2014).

The lumpfish (*Cyclopterus lumpus* L.) is a novel species to aquaculture that is increasingly being used to control sea lice (*Lepeophtheirus salmonis*), an ectoparasite that represents one of the major threats to salmon farming (Torrissen et al., 2013). Sea lice cause substantial economic losses to industry (Costello, 2009b), impact the survival and welfare of wild and farmed salmon alike (Costello, 2006; Costello, 2009a), and tarnish public's perception of salmon farming (Hersoug, 2015; Jackson et al., 2018). Increasing resistance to chemotherapeutants traditionally used to combat sea lice (Aaen et al., 2015) has prompted an interest on the use of cleaner fish as an environmentally-friendly 'green' alternative to medicines (Powell et al., 2018b). Commercial production of lumpfish has grown exponentially over the past few years, and reached 4.8 million in 2017 in the UK alone (Treasurer et al., 2018b). However, their survival is often poor, and there is increasing concern regarding their welfare (Brooker et al., 2018; Treasurer and Feledi, 2014). Studies suggest that between 33% and 50% of lumpfish may die following deployment in salmon cages (Imsland et al., 2016; Noble et al., 2019; Stien et al., 2020), reaching 100% in some cases (European Union Reference Laboratory for Fish Diseases, 2016; OneKind, 2018). Infectious diseases are a common cause of mortality in lumpfish (Brooker et al., 2018; Powell et al., 2018b), but they are not the only ones. Starvation, poor husbandry, high water temperatures, strong currents, low oxygen, and traumatic injuries caused by rough handling have also been cited as sources of mortality (Anon, 2020; Grefsrud et al., 2019; Stien et al., 2020) and compromise the welfare of lumpfish (Hjeltnes et al., 2018; Treasurer et al., 2018a). The public and retailers generally support the use of lumpfish for controlling sea lice because of the environmental and efficacy benefits that they provide (Anon, 2013), but only as long as the welfare of cleaner fish is not compromised (Treasurer et al., 2018a).

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https://doi.org/10.1016/j.aquaculture.2020.735777
Received 4 April 2020; Received in revised form 3 July 2020; Accepted 25 July 2020
Available online 04 August 2020

Thus, the development of suitable metrics of lumpfish welfare is important, not only for identifying those activities that compromise welfare, but also for quality assurance (Brooker et al., 2018; Treasurer et al., 2018a) and for restoring public confidence on the salmon farming industry and its ability to tackle the threat posed by sea lice (Hersoug, 2015; Jackson et al., 2018).

To be effective, operational welfare indicators need to be practical and easy to use, or they will not be used by fish farmers (North et al., 2008; van de Vis et al., 2012). The use of Operational Welfare Indicators (OWIs) represents a practical approach to achieve this, as these indicators are designed to be easily scored at the farm (Folkedal et al., 2016; Noble et al., 2018). Welfare indicators need to be fit for purpose and be tailored to particular species and uses (Gismervik et al., 2018; Kolarevic et al., 2018), although some OWIs are more generic than others and may be used across species and contexts. For example, a high prevalence of deformities, external injuries, and fin damage may signal low welfare in many species, particularly those reared at high densities (Hoyle et al., 2007; Noble et al., 2012). Although genetic factors should not be excluded, fin damage can result from aggression, but also from stress (Turnbull et al., 1996) and may cause detrimental effects on growth and survival by increasing the susceptibility to infection, potentially impacting swimming ability (Noble et al., 2012). In contrast, some welfare indicators, like eye and body darkening, can be indicative of social stress in territorial or aggressive species like Nile tilapia (Champneys et al., 2018; Freitas et al., 2014) or Atlantic salmon (O'Connor et al., 1999; Suter and Huntingford, 2002), but may be totally unsuitable for shoaling fish. Other individual based indicators, such as plasma cortisol (Pavlidis et al., 2013), expression of stress-related genes (Rodriguez-Barreto et al., 2019; Uren Webster et al., 2018), or the presence of bacterial biomarkers (Uren Webster et al., 2020), require analytical equipment and training that are not typically available within an aquaculture setting; they are laboratory-based welfare indicators, not operational ones (Noble et al., 2018).

Assessing the welfare of lumpfish under farm conditions poses particular challenges. Lumpfish are weak swimmers (Hvas et al., 2018) and lack a swim bladder, which makes them particularly vulnerable to exhaustion and barotrauma if they cannot attach to a suitable substrate (Powell et al., 2018a). Juveniles tend to aggregate in clumps, display a low cortisol response (Treasurer et al., 2018a) and lack Mauthner neurons (Hale, 2000), which makes it difficult to assess their stress response and determine optimal rearing densities (Powell et al., 2018b). In addition, lumpfish can easily suffer from malnutrition, as they cannot survive grazing on sea lice alone and need supplemental feeding (Imsland et al., 2018b; Treasurer et al., 2018a). Thus, some measure of body condition should be included in an operational welfare indicator for this species (Johannesen et al., 2018). A recent report has reviewed some potential welfare indicators for lumpfish (Noble et al., 2019), but there is no validated index that can be used under farm conditions. Here we used an aggregated welfare indicator approach (Rousing et al., 2001) to develop, validate, refine and test a Lumpfish Operational Welfare Score Index (LOWSI) based on the visual assessment of several operational individual-based welfare indicators that can be easily scored in hatcheries and sea cages.

2. Materials and methods

To develop a practical index of welfare for lumpfish (LOWSI) we adopted a workflow that consisted of four steps (Fig. 1): (1) selection and screening of individual-based OWIs in collaboration with lumpfish farmers; (2) validation of OWIs against measures of cortisol and body condition; (3) refinement and simplification of OWIs, and (4) testing of an aggregated operational welfare score index at six commercial sites.

2.1. Selection and screening of individual-based OWIs

To select potential welfare indicators for testing, a questionnaire was given to 53 lumpfish farmers and other participants in the Lumpfish Welfare Workshop at the First Welfare in Aquaculture Symposium (Swansea, 14th May 2019). During the focus group, respondents were asked to assess the potential utility of 12 welfare indicators for lumpfish (Table S1). We excluded from further testing those indicators that were group based (mortality, growth), laboratory based (blood analysis), or required specialized training (parasite/disease screening), or had proved unreliable (body darkening) or shown limited or no variation in pilot trials (operculum erosion, body deformities). We focussed on 6 potential OWIs (external body damage, fin damage, eye condition, eye darkening, suction cup deformities, and relative weight) as described below. For these, we scored high resolution photographs (Canon EOS 800D; EFS 18-55 mm, TAMRON 90 mm lens) of the body, eyes, fins and suction cup of 95 freshly euthanized lumpfish (overdose of anaesthesia, UK HO schedule 1) sampled at two hatcheries (n = 60, 5-152 g) and one salmon cage (n = 35, 22-100 g) in the UK. A tripod and a standard black background fitted with a scale and a reference colour chart (Colour Checker Passport, X-rite) were used to ensure consistency between photographs.

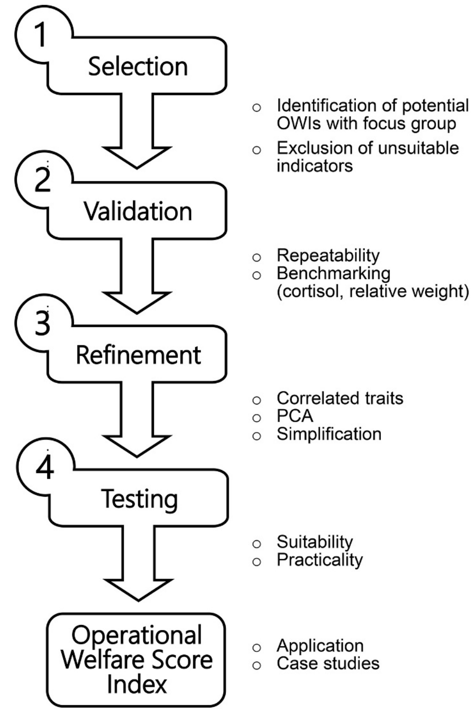


Fig. 1. Work-flow used to develop an operational welfare score index for farmed lumpfish.

2.2. External body damage

External body damage was assessed on a 2-point scale (absence: 0, presence: 1) depending on the presence of skin lesions, including erosion, reddening, abrasion and body ulcers (Fig. S1).

2.3. Fin damage

We scored damage of the 4-rayed fins (dorsal, caudal, anal and pectoral) on a 5-point Likert scale according to the extension of the tissue area affected (Fig. S2). Left and right pectoral fins were averaged, and an aggregated total fin damage score ranging between 0 and 20 was obtained by summing the erosion of the four fins.

2.4. Eye condition

Three eye conditions (eye damage, exophthalmia and cataracts) were scored on a 3-point scale, depending on the extension of the condition (0: absence; 1: one eye affected; 2: both eyes affected; Fig. S3). An aggregated eye condition score was obtained by adding the three scores, with values ranging from 0 to 6.

2.5. Eye darkening

To quantify eye darkening, we divided photographs of the eye sclera into eight equal sections and assessed the percentage of darkening in each octant (Champneys et al., 2018). The average of the left and right eyes was converted to a 5-point Likert scale depending on the extent of the darkened area (Fig. S4).

2.6. Suction cup deformities

Deformity of the ventral suction cup was assessed according to five parameters that were found to vary on a pilot screening: (1) symmetry of the suction cup, (2) indentations, (3) depressions, (4) papillae development, and (5) deformity or curling of the ventral section of the pectoral fins. Each condition was assessed on a 5-point Likert scale depending on severity and extent (Fig. S5), and all scores were summed to provide a suction cup deformity score, with values ranging from 0 to 20, that classified the fish into five suction cup deformity classes: Class A – Perfect suction cup (total score = 0). Symmetrical, without indentations, flat, with well-developed papillae and with pectoral fins that do not obliterate the suction cup. Class B – Mild deformity (total score 1–5). Slight asymmetry, with some depression and/or indentations and minor under-development of the papillae or slight curling of the pectoral fins. Class C – Moderate deformity (total score 6–10). Moderate asymmetry, depressions and indentations, and clear under-development of the papillae or curling of the pectoral fins that hide parts of the suction cup. Class D – Substantial deformity (total score 11–15). Substantial asymmetry, with deep depressions and indentations, substantial under-development of the papillae, and significant curling of the pectoral fins that hide most of the suction cup. Class E – Severe deformity (total score > 15), non-functional suction cup. Severe asymmetry, with severe depressions, indentations and under-development of the papillae and totally deformed or curled pectoral fins that cover all the suction cup.

2.7. Relative weight

Relative weight (W_r) was used as an index of body condition, rather than Fulton's condition factor (Blackwell et al., 2000), because it is more appropriate for fish like lumpfish that have an unusual body shape (Al Nahdi et al., 2016). We collected data on total length (TL, mm) and body mass (W, wet weight, g) of 2658 farmed lumpfish sampled during 2015–2019 at four stages of development: (S1) Larvae (0-1 g), (S2) Pre-deployment juveniles (1–10 g), (S3) Pre-deployment juveniles (+10 g) and (S4) Post-deployment. From these, expected standard weights (W_s) were computed for each stage of development using the parameters of the fitted regressions $\log10\ W_s = a + (b * \log10\ \mathrm{TL})$, where a is the intercept, b is the slope and TL is the total length. Relative weight was then calculated as $W_r = 100*(W/W_s)$, where W is the observed weight and W_s is the standard (i.e. expected) weight for fish of that length and that stage of development (Blackwell et al., 2000). We considered that fish were underweight if they were 10–25% below their expected weight (i.e. Wr = 90–75%) and severely underweight or emaciated if they were 25% or more below their expected weight (i.e. $Wr < 75\%$), when their head typically becomes the widest part of the body (Noble et al., 2019). To aid farmers to quickly identify underweight fish we constructed percentile length-weight charts for each stage of development (Fig. S6).

2.8. Validation of individual-based OWIs

We used two criteria for OWI validation: (1) reliability, and (2) construct validity. Reliability measures the magnitude of the measurement error in relation to the inherent variability between subjects (Bartlett and Frost, 2008) and was calculated by scoring the same fish twice by the same and different raters. Construct validity is the degree to which scores are consistent with a priori hypothesised differences between relevant groups, based on the assumption that the scale validly accurately captures the construct it purports to measure (Mokkink et al., 2010).

2.9. Reliability

Two raters (A and B) working independently scored the images of the 95 lumpfish used for the OWI screening above. Images were allocated at random. Observer A also scored the same images after 8 months, to provide a measure of intra-rater reliability. For each OWI, we calculated the intraclass correlation coefficient (ICC) using the *irr* R package (Gamer et al., 2019) and the single-rating, absolute-agreement, 2-way random-effects model. The ICC is a suitable tool to measure reliability, as it considers both the strength of the correlation and the agreement between measurements (Koo and Li, 2016).

2.10. Construct validity

In the absence of an agreed standard for measuring lumpfish welfare, construct validity was evaluated via two surrogate welfare measures previously tested on other species (Noble et al., 2018): relative weight (as an indication of poor growth) as described above, and plasma cortisol (involved in the stress response). An aggregated welfare score was calculated for each fish by adding the scores of each OWI (range: 0 to 51), these were standardized and centred by subtracting the mean and dividing by the standard deviation before being analysed by principal component analysis (PCA) using the *factoextra* R package (Kassambara and Mundt, 2019).

To measure plasma cortisol, we collected blood through a puncture of the caudal vein (lithium-heparinised Vacutainer Blood Collection System) in a sample of recently euthanized lumpfish used in the OWI scoring above ($n = 55$, range = 22–152 g). Blood was collected mainly in the morning (0900–1300 h), by the same person, using the same equipment, and within 30 s from cessation of opercular movement to reduce unwanted variation. We employed the plasma preparation protocol from ThermoFisher Scientific to separate plasma from whole blood (Thavasu et al., 1992) consisting of centrifugation at 1500 rpm for 10 min at 15 °C and storage at −80 °C until analysis. For cortisol quantification we used a competitive ELISA test (DetectX Cortisol Enzyme Immunoassay Kit, Arbor Assays, Michigan, USA), that has been widely used before to measure plasma cortisol in fish (Huyben et al., 2019; Uren Webster et al., 2020).

Each plasma sample was treated with a dissociation reagent to increase its yield (mean recovery rate 96.7%) and diluted with buffer (1:50) before cortisol determination. Standards, blanks and test samples were loaded in duplicate and absorbance values (OD) were read with a SpectroStar Nano Plate Reader at 450 nm wave-length. The average of two duplicates was used to create a standard curve ($R^2 = 0.994$), and concentrations were multiplied by the dilution factor (1:100) to obtain plasma cortisol values (ng/ml). Assay sensitivity was 24.7 pg/ml, while detection limit was 18.01 pg/ml. Intra-assay and inter-assay precision (CV%) for duplicate samples was 15.4% and 6.8%, respectively.

2.11. Refinement & simplification

As fish that had one eroded fin typically showed erosion in others, we examined the pairwise matrix of Spearman's rank correlation coefficients between fin erosion scores to identify the most sensitive fins for

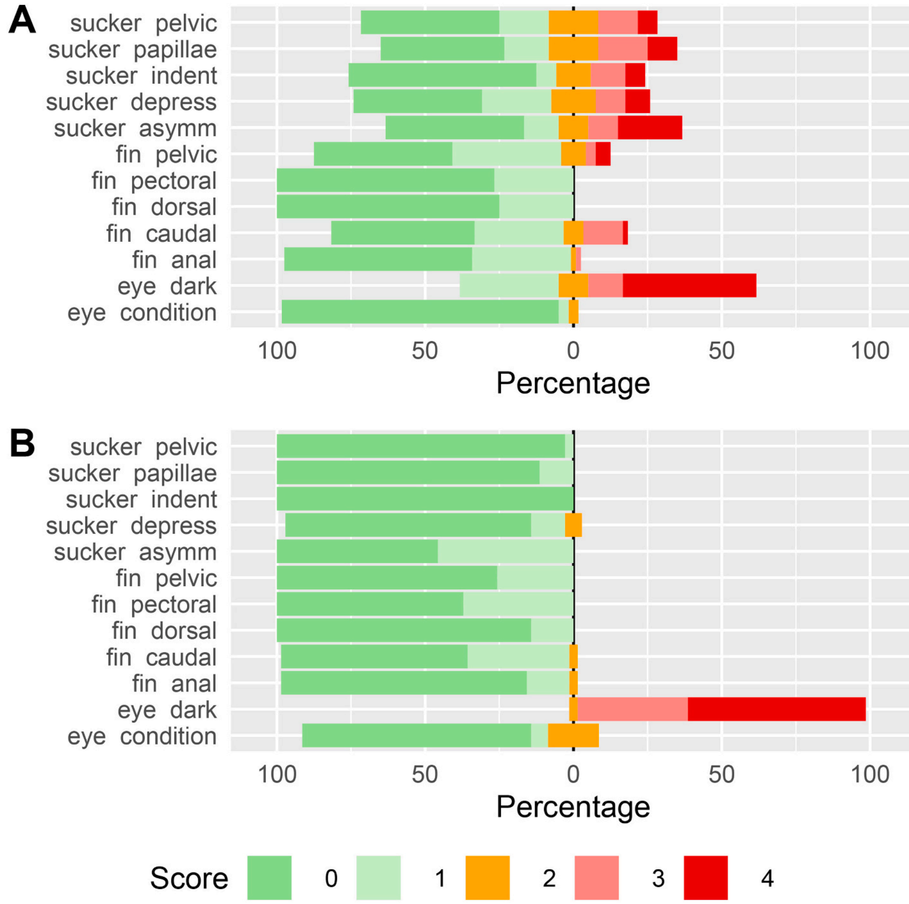


Fig. 2. Variation in operational welfare indicators (OWIs) in lumpfish sampled from (A) hatcheries (pre-deployment, n = 60) and (B) in sea cages (post-deployment, n = 35). Shown are the percentage of fish scored on a 5-point Likert scale (score 0–4) for each indicator, depending on the extent and severity of each condition.

damage scoring. We then simplified the original OWI scores into a 10-point Lumpfish Operational Welfare Score Index (LOWSI) that included relative weight and the four most reliable OWIs, all scored on a 3-point scale to ensure equal weighting.

2.12. Testing

To test the application of the LOWSI, 245 lumpfish from three hatcheries (H1-H3, n = 120) and three sea farms (F1-F3, n = 125) were scored by one or two raters during 2018–2019. Rearing temperature ranged between 8 and 12 °C, density between 4.5 and 24 kg/m^3 in hatcheries and between 4 and 15% of salmon numbers in sea cages, and photoperiod between 12 and 24 h light at hatcheries, depending on stage of development. Lumpfish were classified into three welfare classes depending on the values of the LOWSI: (A) Good welfare (< 3 points), (B) Moderately compromised welfare (3–5 points), and (C) Severely compromised welfare (> 5 points). ICC estimates of reliability were computed as above, using the scores of the lead author and 8 fish farmers on a subsample of 150 fish that were scored twice. To assess the practical implementation of the LOWSI, a questionnaire (Table S2) consisting of four questions and five possible responses (1-Strongly disagree, 2-Disagree, 3-Neutral, 4-Agree, 5-Strongly agree) was given to 8 fish farmers who had previously scored the welfare of lumpfish.

2.13. Statistical analysis

All data were analysed using R version 3.6.1 (R Core Team, 2019). We used the Wilcoxon test to compute pseudo-medians and 95% CI on the Likert scale to estimate the perceived utility of each welfare indicator (Mangiafico, 2016), and a cumulative link mixed model with the *clmm2* function in the R package *ordinal* to assess the degree of consensus among participants, having tested the proportional odds assumption via the *nominal_test* and the *scale_test* (Christensen, 2019). Consensus among lumpfish farmers on the practical application of the aggregated welfare score index was analysed with a cumulative link mixed model, as above.

To asses construct validity, we used changes in the Akaike Information Criterion (AIC) and the *MuMIn* package (Barton, 2019) to investigate how the first two principal components of the aggregated welfare score (PC1, PC2) varied in relation to plasma cortisol and relative weight while statistically controlling for variation in body size. We tested model assumptions by examining the distribution of residuals with respect to linearity, normality, homogeneity of variances, and leverage using the *plot* command and the *gvlma* package in R (Pena and Slate, 2019). Two observations were identified as overly influential outliers by the *olsrr* R package (Hebbali, 2020) and were excluded from the validation of relative weight (obs. #19, Cook's distance = 0.142, Studentized residual = 2.77) or cortisol (obs #17, Cook's distance = 2.31, Studentized residual = 2.84).

2.14. Ethical statement

The study was conducted following approval by Swansea University Ethics Committee (Permits SU-Ethics-Student-130718/692, SU-Ethics-Student-110618/713).

3. Results

3.1. Selection of operational welfare indicator (OWIs)

3.1.1. Perceived utility

The 12 OWIs considered by participants at the Lumpfish Welfare Workshop group differed significantly in perceived utility (type III analysis of deviance, $\chi 2 = 68.041$, df = 11, $P < .001$); model assumptions were met (tests of nominal effects, background LRT = 10.2, $P = .12$; tests of scale effects, trait LRT = 17.03, $P = .11$; background LRT = 1.13, $P = .57$). Fin erosion and body damage were considered to be the most useful, while body/eye darkening and blood parameters were considered to be the least useful (Table S1). Although participants differed in opinion about the utility of different OWIs for lumpfish, depending on their background ($\chi 2 = 11.504$, df = 2, $P = .003$), consensus was high, and 87% of them did not deviate significantly from the responses of the average rater.

3.1.2. Variation in OWIs

The prevalence of different welfare conditions varied significantly between stages of development (Fig. 2). For example, the prevalence of fish with external body damage was rare in hatcheries (2%), but common in sea cages (46%; z-test with continuity correction, $\chi 2 = 26.27$, df = 1, $P < .001$). In general, lumpfish in hatcheries were mostly affected by fin erosion, particularly of the caudal (52%) and pelvic fins (53%), and by suction cup deformities (37–58%), whereas lumpfish in the sea cages were more affected by eye damage (23%), which was significantly less common in hatcheries (7%, $\chi 2 = 3.89$, df = 1, $P = .048$). Such separation was confirmed by Principal Component Analysis (Fig. 3). PC1 accounted for 39% of variation and was mainly associated with sucker deformity (−0.78), eye darkening (0.67) and external body damage (0.64), while PC2 (21%) mostly captured variation in eye condition (−0.81) and fin damage (−0.46).

3.1.3. Incidence of underweight and emaciated fish

The length-weight relationships of farmed lumpfish differed significantly between life stages (Table 1; life stage x total length interaction, $F_{3,2650} = 61.346$, $P < .001$). Growth was positively allometric (i.e. b > 3.0) in hatcheries, i.e. fish became progressively fatter as they grew, and negatively allometric (i.e. b < 3.0) in the sea, i.e. fish became progressively thinner over time. Using a common length-weight regression for all stages of development (instead or four) would introduce a mean absolute error of 5.2% in the estimation of relative weight, but this varied between 1.1% for stage S3 (just prior to deployment) to 9.5% for stage 1 (larvae). In general, a single length-weight regression would overestimate relative weight in hatcheries (i.e. lumpfish would appear to be fatter than they really are) and underestimate it in sea cages (i.e. lumpfish would appear to be thinner).

The frequency of underweight fish (i.e. those with weights between 10% and 25% below their expected value) varied significantly between life stages ($\chi 2 = 8.235$, df = 3, $P = .041$), being highest prior to deployment (stage S3, 20.3%). In contrast, the incidence of emaciated fish (i.e. fish weighing 25% or less below the expected value) was significantly higher during the larval S1 stage (18.4%) than at any other stage ($\chi 2 = 121.51$, df = 3, $P < .001$; Fig. 4). Overall, 28% of the 2658 farmed lumpfish we sampled had lower than normal weights for their length, and 10% were emaciated.

3.1.4. Variation in plasma cortisol

Mean plasma cortisol differed significantly between life stages (Welch two sample *t*-test for unequal variances, $t = 6.56$, df = 35.975, $P < .001$), being approximately 7 times higher among post-deployment lumpfish sampled in salmon sea cages (mean = 84.70 ng/ml ± 10.99 SE) than among pre-deployment juveniles sampled in hatcheries (mean = 11.61 ng/ml ± 1.88 SE; Fig. 5). Cortisol values were also significantly more variable post-deployment (CV = 76.7%) than pre-deployment (CV = 72.6%; Fligner-Killeen test, $\chi 2 = 21.84$, df = 1, $P < .001$).

3.2. Validation of OWIs

3.2.1. Reliability

All welfare indicators showed good (ICC = 0.75–0.90) or excellent reliability (ICC > 0.9; Table 2) except for eye condition, which was

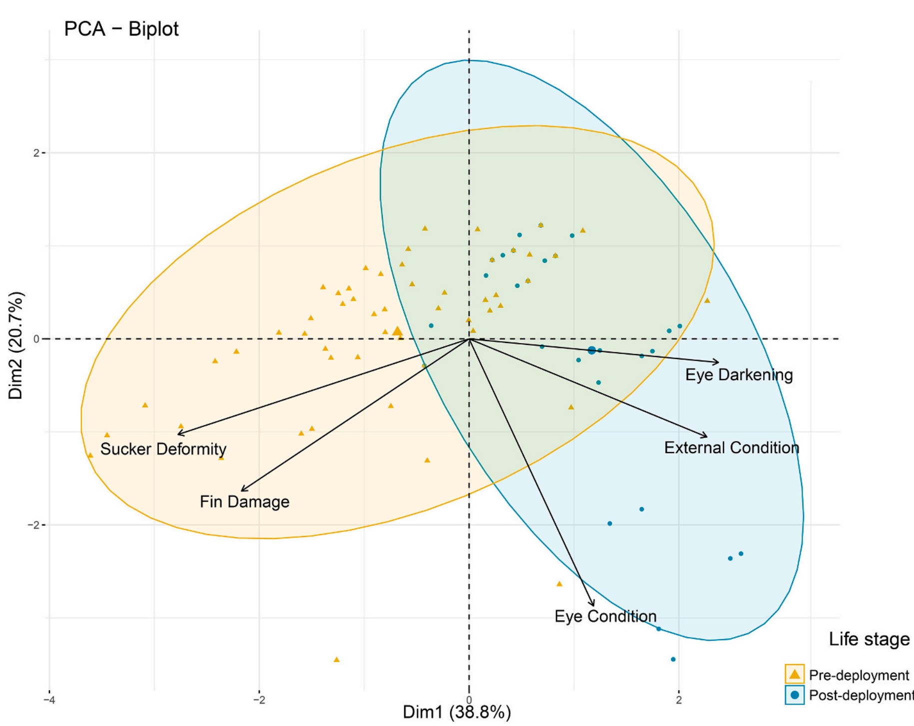


Fig. 3. Principal Components Analysis biplot of lumpfish welfare showing separation of individuals depending on their stage of development (pre-deployment vs. post-deployment) and relative influence of different welfare indicators.

Table 1
Length-weight regression coefficients (± SE) for farmed lumpfish at different stages of development (log10 Ws = a + b·(log10 TL), where Ws = standard weight (g) and TL = total length (mm).

Stage	Weight range (g)	*N*	*a*	*b*	R^2	*P*
S1. Larvae	0–1	948	− 5.023 ± 0.035	3.532 ± 0.038	0.903	< 0.001
S2. Pre-deployment	1–10	126	− 4.301 ± 0.101	2.926 ± 0.060	0.950	< 0.001
S3. Pre-deployment	+ 10	1229	− 4.737 ± 0.034	3.181 ± 0.016	0.970	< 0.001
S4. Post-deployment	+ 10	355	− 3.516 ± 0.117	2.559 ± 0.058	0.847	< 0.001
All Stages	0–1380	2658	− 4.692 ± 0.007	3.157 ± 0.004	0.996	< 0.001

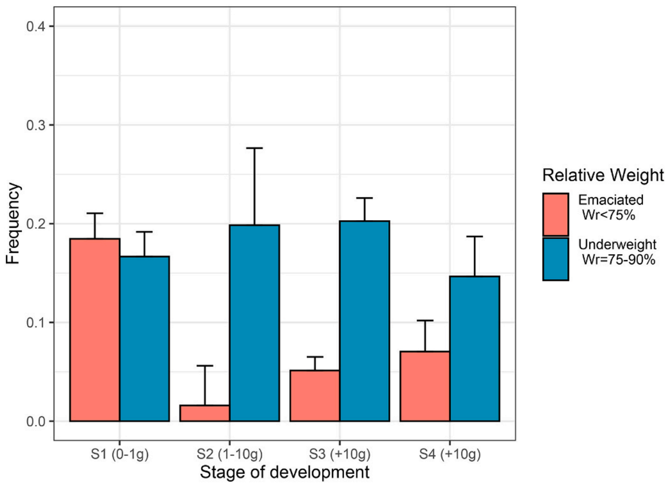


Fig. 4. Variation in the proportion (± binomial 95% CI) of underweight (Wr = 90–75%) and emaciated (Wr < 75%) lumpfish (*n* = 2658) at different stages of development sampled from three hatcheries (stages S1-S3) and three sea farms (stage S4).

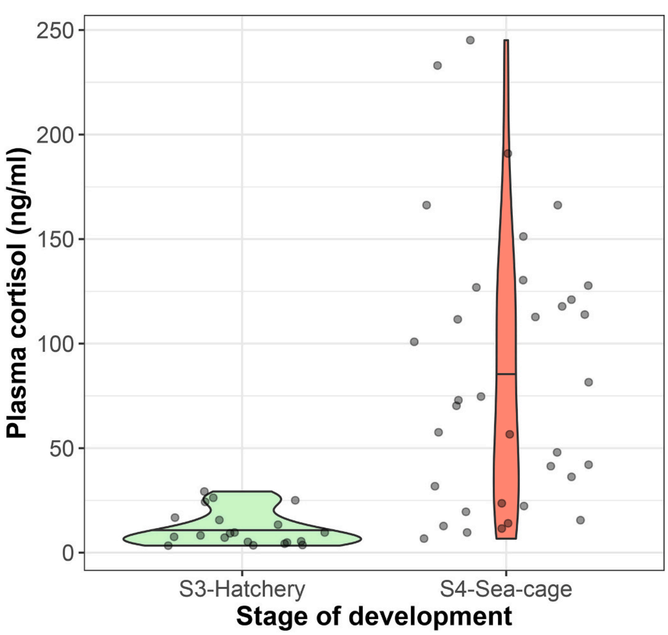


Fig. 5. Variation in blood plasma cortisol (ng/ml) of lumpfish (*n* = 55) sampled in two hatcheries (stage S3 - pre-deployment) and one sea farm (stage S4 - post-deployment).

highly repeatable when measured by different raters (ICC = 1.0), but had a modest repeatability when it was assessed at two different times by the same rater (ICC = 0.60, 95% CI = 0.46–0.72).

3.2.2. Construct validity against relative weight and plasma cortisol

As both relative weight and cortisol differed significantly between stages of development ($P < .001$), construct validity was assessed separately for the pre- and post-deployment stages. Relative weight was found to be dependent on PC1 and PC2 and their interactions with total length during the pre-deployment stages ($F_{5,54} = 6.6$, $R^2_{adj} = 0.32$, $P < .001$), but was only dependent on total length during post-deployment ($F_{1,32} = 6.19$, $R^2_{adj} = 0.14$, $P = .02$). PC1 was a significant predictor of plasma cortisol during pre-deployment ($F_{1,17} = 8.98$, $R^2_{adj} = 0.31$, $P < .01$), while PC2 and the interaction between PC2 and total length were significant cortisol predictors at post-deployment ($F_{3,31} = 4.58$, $R^2_{adj} = 0.24$, $P < .01$).

3.3. Refinement and simplification

The caudal was the fin most commonly damaged (47%), the easiest to score, and also the one that showed the highest variation among individuals, with scores ranging from 0 to 4. The ventral section of the pectoral fins was also highly variable, but it was less affected by erosion (43%) than the caudal fin. Damage of the dorsal and anal fins was positively correlated (Spearman's $\rho = 0.302$, $P < .01$), as was damage on the anal fin and the ventral section of the pectoral fins (Spearman's $\rho = 0.222$, $P < .05$), both positioned in the ventral part of the longitudinal axis of the lumpfish. Scores for the ventral section of the pectoral fin and deformities of the suction disc were also positively correlated ($\rho = 0.531$, $P < .001$). Inspection of the data indicated that scoring of the caudal fin would identify 74% of individuals that had also damage in other fins, and that scoring of the suction papillae was positively correlated with other suction cup conditions ($\rho = 0.786$, $P < .001$), indicating redundancy and lending support to the use of a simplified Welfare Score Index (Table 3).

3.4. Testing and application

The simplified Lumpfish Operational Welfare Score Index was tested on 245 farmed lumpfish from six commercial sites (three hatcheries and three sea farms), showing high repeatability (ICC = 0.826, 0.767–0.871, $P < .001$). Consensus among farmers on the performance of the index was high, as 75% of them did not deviate significantly from the average response; model assumptions were met (tests of nominal effects, trait LRT = 3.12, $P = .37$). Farmers agreed that it was easy to score, lasting less than 2 min per fish, and that it was practical and easy to implement at the farm. Moreover, they were also willing to train their staff to implement it (Table S2).

Overall, 71% of lumpfish were classified as class "A" (good welfare, unlikely to be compromised), 27% as class "B" (moderately compromised welfare) and 2% as class "C" (severely compromised welfare) but this differed significantly among farms (analysis of deviance on binomial proportions, $\chi 2 = 47.397$, df = 5, $P < .001$; Fig. 6). Mean LOWSI values ranged from 0.82 to 3.37 among the six sites, suggesting there was a 4× difference in welfare conditions. Post-hoc Tukey contrasts indicated that the welfare at one of the sea farms (Farm 1) was significantly poorer than the rest with 57% of fish classified as class "B" and 11% classified as class "C"; poor welfare was typically associated with fin damage and suction cup deformities on hatcheries, and poor

Table 2
Repeatability of different operational welfare indicators (OWI) based on the inter- and intra-class correlation coefficients (ICC) of two raters (R1 and R2) independently scoring 95 farmed lumpfish.

OWI	Intra-rater repeatability (R1 at time 1 & time 2)			Inter-rater repeatability (R1 vs R2)		
	ICC	95 CI	*P*	ICC	95 CI	*P*
Body damage	0.82	0.74–0.87	< 0.001	0.83	0.76–0.88	< 0.001
Fin damage	0.79	0.68–0.86	< 0.001	0.93	0.90–0.96	< 0.001
Eye darkening	0.83	0.76–0.89	< 0.001	0.85	0.78–0.90	< 0.001
Eye condition	0.60	0.46–0.72	< 0.001	1.00	1.00–1.00	< 0.001
Sucker deformity	0.85	0.78–0.90	< 0.001	0.94	0.91–0.96	< 0.001

growth and eye damage in sea cages (Fig. 6).

4. Discussion

The salmon farming industry has been criticized for not doing enough to maintain the welfare of lumpfish (Compassion in World Farming, 2018; 2019; Stranden, 2020) and for causing unacceptably high mortalities in some cases (European Union Reference Laboratory for Fish Diseases, 2016; Imsland et al., 2016; OneKind, 2018). This has caused concern among consumers and prompted some pressure groups to discourage the use of cleaner fish until high mortalities are addressed and welfare standards can be guaranteed (Marine Conservation Society, 2018; OneKind, 2018), Compassion in World Farming, pers. comm. 27/02/2020). The Norwegian Food Safety Authority has recently warned salmon farms that they may have to stop using cleaner fish if welfare standards are not met (Anon, 2020). However, it is difficult to maintain good welfare if farmers do not know what to measure. The development of welfare standards has been flagged as an urgent priority for lumpfish (FAWC, 2014; Noble et al., 2019; OneKind, 2018), because without standards, mortality is often the only indicator of compromised welfare, which is of course too late to take remedial action (Stranden, 2020). Although several welfare indicators have recently been proposed for lumpfish (Imsland et al., 2020; Noble et al., 2019), these have not been validated and there is a need for a simple index that fish farmers can use under working conditions.

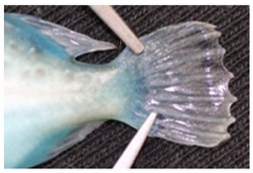
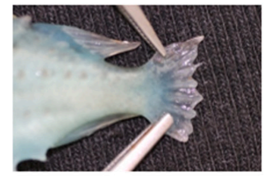
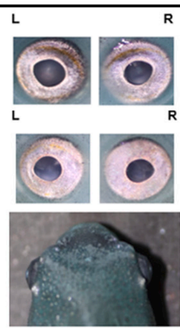
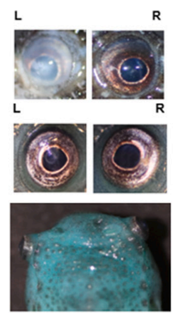
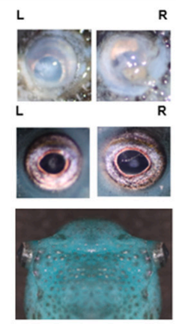
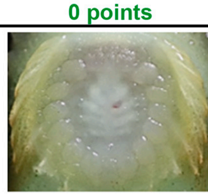
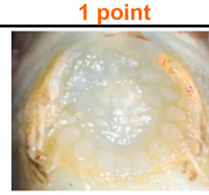
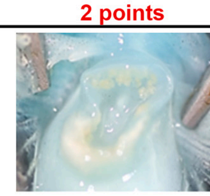
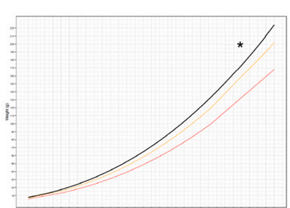
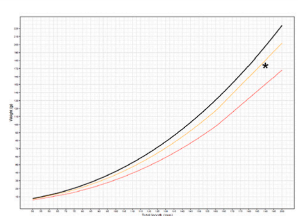
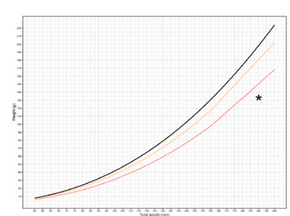
We developed and validated a repeatable lumpfish operational welfare score index (LOWSI) that is easy to score and can be used for routine welfare assessment under commercial conditions with minimal training (Table 3). Our welfare index is based on the same OWIs recently employed to assess variation in growth and mortality of lumpfish in sea cages in Norway (Imsland et al., 2020), indicating that the welfare metrics we used are meaningful across contexts. Our screening at six commercial sites indicates that although the welfare of most lumpfish (71%) was not compromised (class "A"), it was likely compromised in 27% of cases (class "B"), and was clearly poor in the remaining 2% (class "C"). However, welfare scores varied by a factor of 4× among sites, and the prevalence of fish with good welfare varied three-fold (from 31% to 97% class A). This indicates that while some farms are already achieving high welfare standards, others are not.

One key finding from our study was the relatively high incidence of under-weight lumpfish at all stages of development, which raises ethical concerns. Our results indicate that 28% of lumpfish had lower than normal weights for their length, and 10% were clearly emaciated. In previous studies, between 10% and 30% of lumpfish were found to have empty stomachs in sea cages (Eliasen et al., 2018), and only 13–38% were found to eat sea lice (Eliasen et al., 2018; Imsland et al., 2014; Imsland et al., 2015b; Imsland et al., 2016), although this can be as low as 0% during the summer (Eliasen et al., 2018). Clearly, reducing the risk of emaciation is a major challenge for the ethical use of lumpfish. This could be achieved by supplementary feeding, better diets, and novel feeding methods (Imsland et al., 2019a; Imsland et al., 2019d), but also by reducing stress and excessive energy expenditure. In this sense, our length-weight charts could be used by fish farmers to regularly monitor growth, and to take remedial actions before emaciation becomes a problem. They could also be used to select elite lines that are efficient sea lice eaters (Imsland et al., 2016; Imsland et al., 2018d; Powell et al., 2018b), as they provide a benchmark against which growth can be easily compared.

Four welfare conditions were identified in our study that affected lumpfish differently depending on the stage of development, and which may have also increased the risk of emaciation: suction cup deformities and fin erosion in hatcheries, and eye damage and external body injuries in sea cages. The prevalence of fish with suction disk deformities was relatively high (mean 37%, range 3–69%), and was higher in hatcheries than in sea cages, probably because juveniles are typically screened for deformities before they are deployed. Treasurer et al. (2018a) reported that 65% of juveniles had deformed suction disks at one rearing facility, but as deformed fish are more likely to die (Hustad, 2008), the true frequency of deformities at birth is probably higher. The causes of suction cup deformities are unclear but nutritional, environmental, and genetic factors have been implicated in deformities in other species (reviewed in Berillis (2015). Over one third of lumpfish larvae may show different types of malformations upon hatching (Hustad, 2008), which may be exacerbated by high temperatures during development (Imsland et al., 2019b). It has also been suggested that suction cup deformities may be associated with poor nutritional status (Kousoulaki et al., 2018), although it is more likely that deformities result in poor growth, rather than the other way around, as the suction cup is completely formed at hatching (Hanssen, 2018). Deformities may also result from inbreeding depression, as some lumpfish populations are very small and have gone through genetic bottlenecks (Whittaker et al., 2018). There is some evidence that deformities may vary among families (Danielsen, 2016), which might make it easier to select for deformity-free lines. Whatever the reasons, deformities are known to compromise the welfare of many farmed fish (Noble et al., 2012), and represent a particularly acute problem for lumpfish because they can affect the ability to rest (European Union Reference Laboratory for Fish Diseases, 2016; Imsland et al., 2018a; Imsland et al., 2015a; Johannesen et al., 2018; Leclercq et al., 2018), move (Davenport and Thorsteinsson, 1990), and perhaps also to cope with stress (Hvas et al., 2018). Unlike most other fish, lumpfish lack Mauthner neurons involved in the fast startle response, so their primary response to threat is to cling and hide, rather than to escape (Hale, 2000). A deformed, non-functional suction disc, therefore, will likely increase stress and energy expenditure.

Fin damage was another common welfare problem observed in our study. We found that 62% of juveniles had caudal fin damage in three hatcheries (range 50–93%), which is similar to the 69–87% prevalence reported by Johannesen et al. (2018). Many of the health conditions that affect lumpfish are stress related (Brooker et al., 2018; Powell et al., 2018b), and secondary bacterial and fungal infections will be exacerbated by fin and body damage, so any actions that reduce stress will likely improve welfare and survival. For example, manual feeding combined with automated pulse feeding may be used to reduce stress caused by competition (Johannesen et al., 2018), while regular grading may also reduce fin nipping and fin erosion (European Union Reference

Table 3
Rapid visual scoring of the Lumpfish Operational Welfare Score Index (LOWSI).

OWI	0 points	1 point	2 points
Skin damage • reddening • abrasion • wounds • ulcers	 No damage	 Moderate damage	 Severe damage
Caudal fin damage • ray splitting • fin erosion	 No damage	 Moderate damage	 Severe damage
Eye condition • cataracts • exophthalmia • injuries	 No damage	 One eye damaged	 Both eyes damaged
OWI	**0 points**	**1 point**	**2 points**
Suction disc • asymmetry • indentation • depression • papillae • curling	 No deformities, fully functional	 Moderate deformity, some impairment	 Severe deformity, non-functional
Relative weight	 Normal Normal weight for its size (Wr>90%)	 Underweight 10-25% below expected weight (Wr = 75-90%)	 Emaciated 25% or more below expected weight (Wr = <75%)
Average LOWSI	**Class A: <3 points** **Good welfare**	**Class B: 3-5 points** **Moderately compromised welfare**	**Class C: >5 points** **Severely compromised welfare**
ACTION PLAN	• No action needed • Continue monitoring	• Increase frequency of monitoring • Check mortality • Check diet and food delivery • Check use of shelters • Check diseases & parasites • Check sources of stress • Check environmental parameters	• Consider immediate corrective actions • Consult with Veterinary Services • Consider culling (under veterinary advice) • Continue monitoring & reassess

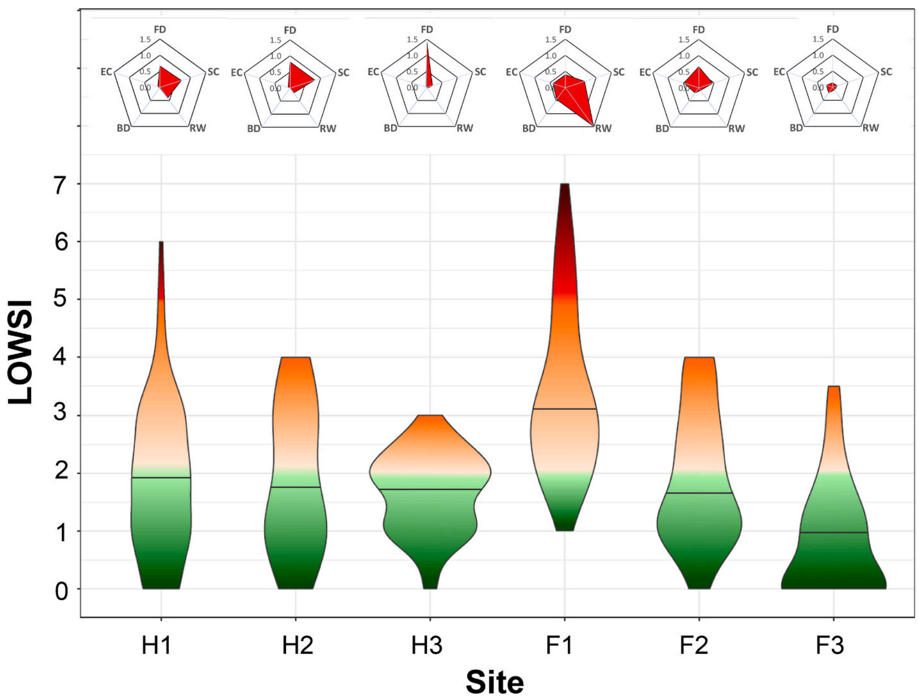


Fig. 6. Variation in the Lumpfish Operational Welfare Score Index (LOWSI) at six commercial sites (three hatcheries – H1-H3, and three sea farms, F1-F3; n = 245). The proportion of fish falling into each of the three welfare classes (A-C) is shown by the violin plots in different colours (class A, green: welfare unlikely to be compromised; class B, orange: moderately compromised welfare; class C, red: poor welfare). The radar plots at the top indicate the mean site scores for each of the five OWIs (scored from 0 to 2) that make up the LOWSI (FD – caudal fin damage; SC = suction cup deformity; RW – relative weight; BD – body damage; EC – eye condition).

Laboratory for Fish Diseases, 2016). Although lumpfish are relatively sedentary outside the spawning migrations (Powell et al., 2018a), delousing requires active swimming (Imsland et al., 2015b; Imsland et al., 2016; Leclercq et al., 2018), which may be compromised by damaged or eroded caudal fins. Salmon net pens are often thermally stratified which forces salmon to undertake vertical migrations (Oppedal et al., 2011), which cleaner fish must also follow to graze on sea lice. Some salmon pens may also be exposed to high current velocities (Johansson et al., 2014), which may exceed the 70–110 cm/s maximum current velocity lumpfish can withstand (Hvas et al., 2018). Damaged fins will likely make swimming less efficient and more energetically costly, leading to poor growth and increasing the risk of emaciation.

More than 15% of lumpfish displayed eye damage and poor eye condition in our study, particularly in sea cages, where this figure reached 26%. Maintaining healthy eyes is essential for sit-and-wait, visual feeders like the lumpfish (Powell et al., 2018a), which depend on having unimpaired vision to feed (Jonassen et al., 2017). Eye damage, cataracts and exophthalmia will likely affect feeding and may therefore also increase the risk of emaciation. While exophthalmia may be symptomatic of several underlying diseases (Austin et al., 2012), other conditions like cataracts may be improved by changes in diet (Imsland et al., 2018c) and feeding regimes (Imsland et al., 2019c). Eye cataracts are rare among wild lumpfish, but can affect 20–100% of lumpfish in captivity (Imsland et al., 2018c; Jonassen et al., 2017). Cataracts may be indicative of malnutrition (Jonassen et al., 2017), but also of overfeeding (Imsland et al., 2019c) and nutritional deficiencies (Imsland et al., 2018c). In our study, cataracts were detected in 17% of fish in sea cages, but only in 5% of juveniles in hatcheries (where nutrition is probably better controlled), which serves to highlight the importance of ensuring that lumpfish have access to suitable diets at all stages of development, and not just in hatcheries.

Seasonal changes in the welfare of lumpfish need to be monitored, and critical periods identified. For example, heavy mortalities have been reported during the summer (MOWI, 2019), when lumpfish tend to be more active (Leclercq et al., 2018), food is less abundant (Eliasen et al., 2018), and temperatures may exceed the species' optimum (Mortensen et al., 2020), making conditions more stressful. In this sense, our measurements of blood plasma cortisol provide some insights into the stress experienced by lumpfish in salmon net-pens. We found a mean cortisol value of 85 ng/ml ± 11 in sea cages, which is higher than that found for unstressed (5.6–16 ng/ml, or even stressed (36–63 ng/ml) lumpfish in other studies (Hvas and Oppedal, 2019; Hvas et al., 2018; Iversen et al., 2015; Jørgensen et al., 2017; Staven et al., 2019). Using a plasma cortisol cut-off of 63 ng/ml for stressed fish, our results suggest that 54% of the lumpfish we sampled in salmon net-pens might have been chronically stressed.

5. Conclusions

We developed and validated an operational welfare index for farmed lumpfish and tested its application across six commercial sites. The results indicate that the welfare of one third of the lumpfish we sampled was probably compromised, and in 2% of cases was undoubtedly poor.

6. Recommendations to improve the welfare of farmed lumpfish

Approximately one in four lumpfish was underweight, and one in ten was severely undernourished or emaciated. These figures appear unacceptably high, and highlight the need for a suitable feed management plan (lumpfish cannot be expected to rely on sea lice alone), as well as for the provision of suitable shelters where lumpfish can rest and be sheltered from strong currents. They also highlight the need for the artificial selection of elite lines that adapt well to captivity and are efficient at eating sea lice. Lumpfish are farmed to feed on sea lice, so underweight fish represent a system failure in every way, not just from a welfare and ethical angle, but also from an economic perspective.

Three of the four welfare conditions that affected lumpfish in our study may be expected to impact growth. Thus, loss of weight is a useful welfare metric for lumpfish because it results from multiple welfare insults. The percentile length-weight charts we developed should enable farmers to identify underweight and emaciated fish rapidly and easily at different stages of development.

A large proportion of lumpfish (37%) displayed suction cup deformities, both in hatcheries and in sea pens, and it is likely that this results in excessive energy expenditure, poor growth and compromised survival. A better understanding of the environmental and genetic basis of sucker deformities may help alleviate this problem, but rapid screening methods are also needed to identify larvae with deformed suckers and exclude them from commercial production.

Almost half of the lumpfish in sea pens were affected by eye or skin damage, which represent potential routes of infection and may be indicative of underlying pathologies, but also of physical injury. While the incidence of eye cataracts can be reduced by changes in diet, improvements are also needed in the way lumpfish are handled during farm operations in order to reduce the risk of physical injury.

Fin damage appears widespread in lumpfish hatcheries, in common with many other intensively farmed fish. Frequent grading, provision of shelters, improvements in diet, use of on-demand feeders, and in general husbandry practices that reduce stress and aggression, have proved beneficial in other species and may also reduce fin damage in lumpfish.

A 4× fold difference in welfare scores was found between the best and worst farms, indicating considerable scope for improvement. There are now more than 530 salmon farms using lumpfish in Europe, each facing slightly different welfare challenges, but most of which source their lumpfish from a small number of hatcheries. This provides unique opportunities for ensuring that juveniles sent for deployment are free of suction cup deformities and other conditions that compromise welfare. In this sense, it is recommended that a Code of Best Welfare Practices is drawn with farmers, regulators and NGOs to ensure that the welfare of lumpfish is properly monitored, that farmers are trained in the use of operational welfare indicators, and that best practices are agreed and shared. Improving the welfare standards of lumpfish will result in better survival, better delousing efficacy, and ultimately, in fewer cleaner fish and a more sustainable and ethically sound industry.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2020.735777.

Statement of relevance

The operational welfare index presented here will enable fish farmers to monitor and improve the welfare of farmed lumpfish, making the industry more sustainable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Funding for this research was provided by a Knowledge Economy Skills Scholarship (KESS-II) to CGR in collaboration with Ocean Matters Ltd., and the ERDF SMARTAQUA Operation. We are grateful to Dave Cockerill, Matt Tomlinson[†], Joel Ellis, Anita Nielsen, Ronnie Hawkins, Connie Patillo, Daniel Phillips, Luis Fonseca, Ben Jennings, Sara Barrento, Sofia Tretiak, Paul Howes and Rebecca Stringwell for help with the OWI development and scoring, and to Craig Pooley, Rob Smith, Ben Whittaker, Sergio Trevi, Alex McCurrach, Ben Overland, Dominic Jarvis, and Edward Miller for making their length-weight data available to us.

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