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Using model selection to choose a size-based condition index that is consistent with operational welfare indicators

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Abstract

Quantitative and qualitative measures of fish health and welfare are essential for management of both wild capture and aquaculture species. These measures include morphometric body condition indices, energetic condition and aquaculture operational welfare indicators (OWIs). Measures vary in ease of measurement (and may require destructive sampling), and it is critical to know how well they correlate with fish health and welfare so appropriate management decisions can be based on them. Lumpfish (Cyclopterus lumpus) is a new farming species that needs nondestructive OWIs to be developed and validated. In this study, we developed a C. lumpus fin damage score. Four different body condition indexes based on individual weight relative to either length-weight relationships or relative to other fish in its local environment were tested (using model selection) as predictors of individual fin damage. Results showed severity of fin damage was predicted by small size relative to the other individuals in the tank or cage. Body condition based on length-weight relationship was not found to predict fin damage, indicating that using established indices from fisheries or from other species would not predict welfare risks from fin damage. Implications are that especially in hatchery conditions grading will improve the condition index, and is expected to mitigate fin damage, but that low weight at length was not of use in predicting fin damage. Model selection to choose between a suite of possible indices proved powerful and should be considered in other applications where an easily measured index is needed to correlate with other health measures.

KEYWORDS

aquaculture, fin damage, grading, hatchery, length-weight relationship, welfare

1 | INTRODUCTION

Condition factors and operational welfare 1.1 indicators

It is essential in many contexts to be able to monitor the health, welfare and reproductive status of fish. Detailed evaluation of these is labour- and time-consuming, so practitioners rely on more easily measured proxy variables. Condition indices serve as a proxy for the energy reserves of a fish and may be used in fisheries assessment to indicate reproductive potential as well as general health and welfare status. Condition indices may be purely morphometric - ratios of lengths and weights - or physiological, such as using liver weights or lipid contents (e.g., Bolger & Connolly, 1989; McPherson et al., 2010). In some cases it can be impractical to measure physiological condition indices without destructive sampling, for example hepatosomatic

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index in gadoids or liver colour in Lumpfish (*Cyclopterus lumpus*) (Eliasen *et al.*, 2020). Operational welfare indicators (OWIs) are easily observed (and ideally nondestructive) measurements that indicate not only the welfare status of the fish but also other aspects of health, such as physical injury, that are used in aquaculture (Kiessling *et al.*, 2012; Noble *et al.*, 2018; Rey *et al.*, 2019). Both condition indices and OWIs can be applied to evaluate the status of individual fish or to evaluate the overall status of a stock of fish.

It is desirable that condition indices and OWIs are species-specific, and that there is evidence that they correlate to relevant health indicators. For novel species, this means collecting individual data of different aspects of health that can be used to evaluate which measures correlate with one another. In this study we evaluate a suite of potential morphometric condition indices to see which correlate with an injurybased OWI for a novel aquaculture species [lumpfish, Cyclopterus lumpus (Linnaeus, 1758)]. In fisheries science, morphometric condition indices are evaluated against standard (species-specific or more general) relationships between measurements: here we also evaluate indices that depend on the average size (and distribution of sizes) of fish in the same tank or cage. Our model selection method provides a general method for choosing condition indices to correlate with other measures of health, and could be applied to other indices in other contexts such as when using condition as a proxy for reproductive status. In addition, we validated the method against a different fish species using a similar data set derived from Atlantic salmon (Salmo salar).

1.2 | C. lumpus in aquaculture

C. lumpus is a native species to North Atlantic waters and has been adopted recently as a cleaner fish to control sea lice numbers, as an alternative or complement to the most commonly used cleaner fish by the salmon farming industry, wrasse (*Labrus* spp.) (Treasurer, 2002). *C. lumpus* has particular application in colder waters (Powell *et al.*, 2017; Treasurer, 2013), where most studies suggest it is an effective cleaner of the parasitic sea louse (*Lepeophtheirus salmonis*, Krøyer 1873 and *Caligus elongatus*, Burmeister, 1834) and more robust and active in winter conditions than wrasse (Barrett *et al.*, 2020; Eliasen *et al.*, 2018; Imsland, 2020; Imsland *et al.*, 2014, 2018, 2021; Overton *et al.*, 2020; Powell *et al.*, 2017).

C. lumpus is different in body form from other species used in aquaculture. As with the other 30 species in the family Cyclopteridae (Davenport & Thorsteinsson, 1989; Stevenson & Baird, 1988), the pelvic fins of *C. lumpus* form a pelvic sucker allowing attachment to different surfaces. The other major distinguishing feature of *C. lumpus* is the dorsal hump. The unusual morphology (large girth, concentration of muscle tissue around the pelvic sucker, mass of the dorsal hump, lack of streamlining and preference for median paired fin propulsion) mean that morphometric condition indices that work with other species may not be valid for *C. lumpus*.

Mature *C. lumpus* show pronounced sexual dimorphism (males are smaller and take on a red colouration), although in this study fish were all immature, so no sexual dimorphism was observed.

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Two main contrasting measures were developed and validated for this study: a fin damage index and a morphometric condition index.

1.3 | Fin damage

Good management practices and the development of reliable OWIs are key to ensuring high standards of welfare, good survival and that the use of cleaner fish, including *C. lumpus*, is sustainable (Rodgers, 2017).

The use of fin damage as an OWI has been described, validated and used in salmonids (Turnbull *et al.*, 1996, 2005). It is commonly used as an indicative of poor welfare but it is not always the case (MacLean *et al.*, 2000), *e.g.*, healthy dominant fish that fight for their hierarchical position versus small timid individuals that avoid interacting with conspecifics. In a previous study we described and validated *C. lumpus* fin damage by gross morphology and histology (Astier, 2016). Other authors have looked at multivariate OWIs for *C. lumpus* and found fin damage in addition to sucker deformities (in 37%–58% of fish) and poor eye condition (in 23% of fish) (Gutierrez Rabadan *et al.*, 2021).

C. lumpus fin damage in early stages (larvae and juveniles) is a result of agonistic interactions triggered by their innate aggressive behaviour in the wild and under captive conditions (Turner, 2016) or by stress-related events (Gutierrez Rabadan et al., 2021). Once animals move to sea cages, fin damage prevalence seems to decline (Brooker et al., 2018: Gutierrez Rabadan et al., 2021). As a common husbandry practice, the health and nutritional state of cleaner fish is assessed periodically in sea cages as well as OWIs such as fin damage and external appearance. This decrease in fin damage could be related to better health and nutritionally improved conditions than when they are in tanks (Eliasen et al., 2020; Treasurer et al., 2018). However, a significant number of animals were found emaciated, with empty stomachs (Eliasen et al., 2018; Gutierrez Rabadan et al., 2021) and with signs of disease (Eliasen et al., 2020) in salmon cages, pointing to aggression as the main cause of fin damage in cages. Because the lumpfish densities in cages is so low (8%-14% stocking densities in relation to salmon population in cages) the level of aggression due to resource competition is also low and this probably results in less fin damage being observed (Treasurer et al., 2018).

1.4 | Morphometric condition indices

In fisheries assessments, morphometric indices may be used to indicate the reproductive status of a stock. Indices may be generic (Fulton's K) or species-specific (variation from a species-specific length-weight relationship) and have been shown to correlate with both energetic condition indices and fecundity at an individual level, which can allow us to evaluate the habitat quality or the population fecundity level (Davidson & Marshall, 2010; Lloret & Planes, 2003; Marshall *et al.*, 1999; McPherson *et al.*, 2010; Rätz & Lloret, 2003).

It is current aquaculture husbandry practice to use whole-body condition indices to monitor the welfare and health of Atlantic salmon (*Salmo salar*) farmed fish stocks as well as for the cleaner fish JRNAL OF **FISH**BIOLOGY

(Bolger & Connolly, 1989). Due to the unusual morphology, anatomical features and limited knowledge of *C. lumpus* biological and physiological needs, it is currently difficult for fish farmers to identify specific indicators of health and condition that are relevant to *C. lumpus* and that allow the identification of problems to take remedial action (Brooker *et al.*, 2018; Powell *et al.*, 2017).

1.5 | Species-relative versus tank-relative condition

To find a condition index that predicts fin damage, we defined different indexes that can be calculated from the size measurements of the fish. We divided these into two classes: (1) the first class refers to the weight of the fish relative to species-specific length-weight relationship; (2) the second class refers to the weight of the fish relative to its peers in the same tank or sea cage. Comparing both classes of index allows us to distinguish whether individual condition relative to species-specific growth is more important in determining welfare or whether the status of the fish determined relative to its peers in the system or local population determines the welfare of individuals. Class (1) implies that maintaining good individual growth is important to husbandry, whereas class (2) implies that separating small fish from large is more likely to have positive welfare implications.

The main aim of this study was to establish the relationships between these distinct condition indices (see Table 1) and the fin damage score for the species to establish how they can be used as OWIs. This was done for *C. lumpus* in a hatchery and *C. lumpus* deployed at sea with *S. salar*. This methodology was also validated with a data set for fin damage of *S. salar* at a commercial farm.

These indices are intended to allow the detection of ill-health and poor welfare, to aid application of treatments and improve husbandry procedures like grading, both in tanks and sea cages. The model comparison methodology for selecting condition indices may also be applicable to other species, especially novel aquaculture species and species for which there are multiple candidates for proxies for reproductive potential.

TABLE 1 Descriptions of the two classes and the four different condition indices tested

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Index description	Illustration	Formula	Interpretation
1a: Weight relative to species- specific length-weight relationship	eight	$C_{1a} = 100 \times W/W_{predicted}$	Indicates whether a fish is above or below the expected weight for its length
Actual weight as percentage of predicted weight from length- weight relationship	≤ length	where $W_{\text{predicted}} = aL^b$ <i>a</i> and <i>b</i> fitted by log-log regression on species-specific data	A high value generally implies improved energy stores
1b: Fulton's K		$C_{1b} = 100 \times W/L^3$	As above, but without a species-
Conventional morphometric condition index. Equivalent to 1a, except with length ³ instead of predicted weight.		Equivalent to C_{1a} when $a = 1$ and $b = 3$	specific length-weight relationship
2a : Variation from mean weight within tank (or cage)	ta h	$C_{2a} = 100 \times W/W_{mean}$	Indicates whether a fish is larger or smaller than its peers
	eig A ⊢ +	where W _{mean} is the mean weight of fish with which this fish interacts (<i>i.</i> <i>e.</i> , those in the same cage or tank and taken on the same sampling date)	Measured as percentage of actual size
			A high value implies a large fish that is more likely to be dominant, and may have better health and growth
			Where a cage has been graded and there is small variation in weights, values of this will always be close to 100
2b : Variation from mean weight within tank (or cage), measured	Tank Tank Two tanks with same mean size Tank B has less variation in weight than tank A The same size of fish is given a higher value for the index in tank B	$C_{2b} = 100 \times (W - W_{mean})/sd_{weight}$	Indicates whether a fish is larger or smaller than its peers
in terms of tank standard deviations		where <i>sd</i> _{weight} is the standard deviation of weights of fish with which this fish interacts (<i>i.e.</i> , those in the same cage or tank and taken on the same sampling date)	Measured relative to the variation in weights in the tank or cage
			Where a cage has been graded and there is small variation, this index will still have a wide variation in values
			This index will be significant if dominance hierarchies exist even when variation in size is small

Note. Illustrations are not to scale. All indices have a multiplier of 100 to yield numbers of similar magnitude and for consistency with the usual calculation of Fulton's K.

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2 | MATERIALS AND METHODS

Data were collected from two different environments: a hatchery at Ardtoe in Scotland and in salmon sea cages in the Faroe Islands and Scotland. The morphometric data were collected across all sampling sessions and conditions (hatchery and sea cages). See Supporting Information Figure S1 for timeline of the experiment and sampling dates. External appearance was also recorded and body or sucker deformities, skin damage or illness monitored (such as cataracts, bacterial infections, operculum or jaw damage). The care and use of animals at hatchery and sea farms complied with animal welfare regulations in the UK and the Faroe Islands, sampling complied with the UK Animals (Scientific Procedures) Act 1986 and the experiments were performed with approval of the University of Stirling's Animal Welfare and Ethical Review Body [number AWERB (16 17) 200].

2.1 | C. lumpus at hatchery

C. lumpus stock origin was from the Stofnfiskur hatchery in Iceland (http://stofnfiskur.is), hatched in June 2016. The experimental stock was transported from Iceland by sea 10 weeks post hatch (at 1 g) to Gairloch in the north-west highlands of Scotland and then by road to the FAI hatchery at Aultbea (FAI Aquaculture Ltd, http://www.faifarms.com) for the on-growing phase. After 1 month settling time the *C. lumpus* stock was transported to the FAI Aquaculture Marine Research Facility at Ardtoe (FAI Aquaculture Ltd). The stock arrived at Artoe on 4 October 2016. At Ardtoe Marine Research Facility, *C. lumpus* stock ($n \ge 7000$) was split between two flow-through larval rearing tanks. The holding

tanks were 1.3 m³ round PVC black tanks of 150 cm diameter and 80 cm water depth at stocking densities of 10%-15% (c. 12 kg/m^3). The hatchery fish were fed GEMMA 800 µm (Biomar Inicio Plus, Marine diet, Grangemouth, UK) for 1 month and this was increased to 1 mm afterwards at 5% initially, decreasing to 3% of body weight. Water temperature and dissolved oxygen concentration were measured twice each day, with a manual (OxyGuard, Farum, Denmark) handy polaris probe. The average temperature over the total sampling period was $10.93 \pm 1.49^{\circ}C$ (mean ± s.p.), min. 8.2°C (January), max. 14.2°C (October). Mean salinity levels were from 30.8 to 32.1 ppt. Mean pH and dissolved oxygen values were 7.7 ± 0.22 and 11.3 ± 1.44 mg/l (90%-100% saturation). After 8 weeks (9 December 2016), the C. lumpus were vaccinated for Vibrio anguillarum and atypical furunculosis with Alpha Marine micro 4 (Pharmaq, Bergen, Norway) at a dose rate of 0.5 ml, needle length 5-6 mm according to weight and then graded into six tanks. Mortalities were recorded daily.

The *C. lumpus* were sampled a total of four times at 2-weekly intervals (17 October, 1 November, 17 November and 12 December 2016). On the first two sampling occasions 20 individuals from each tank were randomly caught using a small hand net from varying locations within the tanks. Nets were soft fine nets with a mesh size of 200 μ m. This was reduced to 10 fish per tank for the third and fourth samplings. All sampled fish (*n* total = 130) were euthanized with an overdose of MS222 (tricaine methane sulfonate, TMS; Sigma, Munich, Germany) at 200 mg/l followed by dissecting the spinal cord and destroying the brain with a needle thereafter (schedule 1 of the UK ASPA method). The weight of each *C. lumpus* was measured using an electronic scale (Escali, Burnsville MN, USA) and recorded in grams (to 0.1 g). A fixed camera on a tripod was set up with the camera always fixed at the same distance



FIGURE 1 Fin damage in *Cyclopterus lumpus* (top, caudal; bottom, dorsal). The score has four numeric categories from 0-3: 0 = no visible damage, 1 = marginal biting or fin splitting, 2 = major distal fin ray loss, 3 = complete removal of fin and tissue damage (from Astier, 2016; after Goede and Barton 1990)

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from the fish (50 cm) to capture ventral (with sucker adhered to glass), lateral and dorsal images against the background of a 5 cm scale (see Supporting Information Figure S2). Morphometric measurements were taken from the images and fins were assigned a fin damage score (see Figure 1). This was a four-point scale: 0/zero = no visible damage, $1 = \text{marginal biting or fin splitting}, 2 = \text{major distal fin ray loss}, 3 = \text{com$ $plete removal of fin and tissue}) fin damage from the dorsal, caudal and$ anal fin were recorded. However, the anal fin was not used for analysisdue to the lack of fin damage and for consistency with sea farm datawhere this was less practicable to monitor.

To measure hatchery *C. lumpus*, the software tpsDig2 (Rohlf, 2015) was used to calculate the standard length from the photographs taken during sampling by marking landmarks on the lateral images at the snout tip and end of caudal peduncle as well as two points on the scale 50 mm apart. Standard length (mm) was measured from the tip of the premaxilla to the end of the caudal peduncle (see Figure 2).

2.2 | C. lumpus sampling in sea cages

C. lumpus were sampled from three Faroese salmon farms (sampled in June 2017) and 12 Scottish sea farms (sampled September 2017 to March 2018) using a circular net on a 2.5 m pole to collect the fish that were near the cage net. The fish were immediately euthanized with an overdose of MS222 (TMS; Sigma) at 200 mg/l with death confirmed, and followed by dissecting the spinal cord and destroying the brain with a needle thereafter (schedule 1 of the UK ASPA method) before taking size measurements and dissection.

Individual adult fish were measured to assess condition and size from standard length (cm), height, width and weight (g). Standard length was measured from the tip of the premaxilla to the end of the caudal peduncle, height from the lowest part of the ventral area to the highest point of the dorsal crest and width on the broader part of the body, as shown in Figure 2. These measures were taken to 0.1 cm precision using waterproof graph paper with a printed measurement scale. The total weight was measured to the nearest gram using an electronic scale (Escali). The fish were placed in a labelled sample bag and transported to shore, where they were sampled in an onsite health laboratory space within 1 h of collection.

Dorsal and caudal fin damage were assessed for adult fish with the same categorical scoring system as for hatchery fish, shown in Figure 1.

2.3 | Different size-based condition indices used

We tested four different condition indexes based on the size measurements and divided them into two classes: (1) the weight of the fish relative to a standard length-weight relationship; and (2) the weight of the fish relative to its peers in the same tank. See Table 1 for illustrations and calculations of the indices. In class (1) there are two indices: 1a, deviation from a species-specific growth curve obtained from our data set; 1b, Fulton's K. In class (2) condition is measured relative to peers in two ways: 2a, individual weight relative to tank mean expressed as a proportion (*e.g.*, weight is 95% of the mean); 2b, individual weight expressed as a multiple of the tank s.p. of weight (*e.g.*, 0.5 s.p.s below the mean). These four indices were correlated with the fin damage index observed in the fish studied.

To calculate index 1a (variation from the species-specific lengthweight relationship), log-log regression was performed against the data set with hatchery fish in a tank versus sea cage fish as an additional explanatory factor and the model refined stepwise by removing



FIGURE 2 Photograph of sampled specimen: (a) lateral view: (*) snout, (**) hypural plate; white solid double arrow, standard length; white dashed double arrow, position and direction of fish for fin measurements: (1) dorsal fin, (2) caudal fin, (3) anal fin, (b) dorsal view: (white double arrow) width

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nonsignificant terms. To ensure consistency in the data, this regression was performed using data collected by the research team and did not use data provided by the companies. Using the resultant lengthweight relationship and the tank or cage mean and s.D. of weight, each of the four condition indices was calculated for the hatchery and the sea farm fish for which we had all the necessary data recorded.

2.4 | Selection of morphometrics condition index using model comparison

The best condition index to indicate the potential for fin damage at each life stage (hatchery and at sea) was established by fitting separate mixed-effect logistic regression models to explain fin damage using each of the four indices. Then we used a model comparison (Anova) to select the best fit model in each case and compare to a null hypothesis.

These models were fitted to data consisting of observations of each fin (*i.e.*, two observations per fish, distinguished by the 'dorsal' or 'caudal' categorical variable). To allow for individual variation between fish and variation between containment units, a mixed-effect model was used in all cases, with random effects grouped by sample (identified by sample date and containment unit) and individual fish.

The null-hypothesis model was a mixed-effect ordinal logistic regression with fin damage (on a 0–3 scale) as a response variable, differing between fins (dorsal and caudal) expressed with a categorical explanatory variable, but not affected by any condition index, with

random effects grouped by both sample (identified by the containment unit and date of sampling) and individual fish. Hence this nullhypothesis model allowed different levels of damage between the dorsal and caudal fin, but not between fish of different conditions.

The model for each index was a mixed-effect ordinal logistic regression with fin damage (on a 0-3 scale) as a response variable, differing by fin ('dorsal' or 'caudal' as a categorial variable) and the condition index (1a, 1b, 2a or 2b), with random effects grouped by both sample (identified by the containment unit and date of sampling) and individual fish. The models predict the expected damage index for both of the two fins based on the condition index.

For the hatchery, two tanks were sampled on each of 3 days and four tanks on the final day (10 samples in total). For the sea cages, a total of 32 cages from 11 farms were each sampled on a single occasion. In total 10 models were fitted to the data: null hypothesis and indices 1a, 1b, 2a and 2b for hatchery *C. lumpus*, and null hypothesis and the four indices for *C. lumpus* in sea cages. The mixed-effect ordinal logistic regression was fitted using the ordinal library (version 10 December 2019) in R (version 3.5.0) (Christensen, 2019; Core Team, 2014); full code for the analysis can be found in the Supporting Information.

The models using each of the condition indices were compared in two ways. Anova (with a chi-square test for model comparison) was used for pairwise model comparison, first for each of the four condition indices *versus* the null-hypothesis, and the Akaike information criterion (AIC) was used to select between the condition indices that were better than the null hypothesis (se Table 2).

TABLE 2 Model comparison to compare the four condition indices as predictors of fin damage for Cyclopterus lumpus

Condition index	Comparison with null hypothesis (Anova with chi-square test)	Odds ratio for caudal being in higher damage category than dorsal (95% Cl)	Effect of condition index expressed as odds of moving to higher damage category with 10-point decrease in condition (95% CI)	Model AIC (* indicates
Hatchery		(,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		iowest,
Null hypothesis		1.34-3.70		632
1a	P = 0.34			634
1b	P = 0.10			632
2a	P < 0.001***	1.32-3.64	1.09-1.33	619*
2b	P < 0.001***	1.31-3.63	1.03-1.11	622
Sea cage				
Null hypothesis		1.32-2.56		1390
1a	P = 0.15			1390
1b	P = 0.09			1389
2a	P < 0.009**	1.32-2.55	1.02-1.15	1385
2b	P = 0.002**	1.31-2.54	1.01-1.05	1382*

Note. All models are mixed-effect multilevel logistic regressions with fin damage class as response variable. Data are individual fins (two data points per fish). Separate models are fitted to hatchery and sea cage data. In the null hypothesis model the only predictor is the identity of the fin (categorial variable: dorsal or caudal). In the models for each index, the index is included as a linear predictor. The sampling unit (defined by the containment unit and date of sampling) and the unique fish identifying number are both used as random effects in the models. 95% confidence intervals (CIs) for odds ratios are calculated from 95% CIs for model coefficients, and only shown for the condition indices if the model AIC or chi-square test shows this was a better fit than the null hypothesis model.

2.5 | Validation of method using a data set on S. salar

A comparable data set for *S. salar* parr (morphometrics and fin damage) held in freshwater tanks was obtained from a Scottish salmon farm (Ellis, 2020). A total of n = 2021 *S. salar* were sampled on 23 separate occasions over an 8 month period and were analysed in the same way as the *C. lumpus*.

3 | RESULTS

3.1 | Hatchery study: fin damage and fish condition as OWIs

A summary of the timeline of the hatchery is given in Figure 3. This shows changes in size distribution of the different damage classes and the frequency of the different damage classes over time.

3.2 | Length-weight relationship

Inspection of the *C. lumpus* size data showed different trajectories between sites. To ensure consistency in the data, stepwise log-log regression of weight against length with hatchery fish in tank *versus* sea cage fish as an additional explanatory factor was performed using data collected from the research team and not using data provided by companies. This data consisted of 130 fish sampled in the hatchery in Scotland and 96 fish sampled in the Faroes. The log-log regression did not have a significant interaction effect for the difference in slopes

between hatchery and sea farm (P = 0.07). As this was limited to a subset of the data and it is likely that changed conditions in transfer from hatchery to sea will alter growth, we used the different slopes in the calculation of condition indices. This is the equivalent of fitting the curves separately to the hatchery and the sea farm. The data and resultant model coefficients are shown in Figure 4, which indicates the slightly different curvature and apparent discontinuity between hatchery and sea cage fish.

3.3 | Model selection to compare condition indices

The four condition indices were calculated for all fish that had length, weight and status of both fins, and that were sampled with at least two other peers to calculate the standard deviation for condition index 2b (see Table 1). This yielded 130 hatchery fish and 326 sea cage fish. Distributions of the resultant condition indices, and the relationship between these and fish length are shown in Figure 5. The distributions show that all four condition indices have roughly symmetric distributions. The plots of condition against length indicate whether there is a trend in condition with size. For condition 1a (species-specific length-weight) there is no trend due to this being the residuals from the length-weight regression, and this is the expected pattern for a condition index based on length and weight: for condition 1b (Fulton's K) the trend is due to the exponent of 3 in the index being inappropriate for this species. For indices 2a and 2b (size relative to peers on the same containment unit) there is a trend in condition with length because individuals



FIGURE 3 Timeline in hatchery. Top, weight distribution for each damage class (bars labelled 0–6 are sum of dorsal and caudal, each on 0–3 scale, darker shading indicates worse damage) on each sampling date; bottom, relative frequency of each damage class on each sampling date



FIGURE 4 Stepwise regression on log(weight) against log(length) was performed using data from the hatchery (triangles) and sampled by the research team in the Faroe Islands (circles), with juvenile in tank versus adult in cage as an additional explanatory variable giving curves expected for hatchery weight = $0.11 \times \text{length}^{2.75}$ and cage weight = $0.05 \times \text{length}^{2.91}$



FIGURE 5 Distributions of condition indices (left panels) and relationship with length (right panels). Circles are juvenile fish from tanks; triangles are adult fish from sea cages. Indices 2a and 2b are expected to correlate with length because fish that are heavier than their peers (and likely also to be longer) are always given a higher value

larger than their peers (therefore with high index values) are generally both heavier and longer.

The relationship between the four indices and fin damage is shown in Figure 6. For the length-weight relationship-based indices 1a and 1b, a trend in condition with fin damage is not clear, but for indices 2a and 2b (based on size relative to peers) worse damage (index 3) is associated with lower condition index for fish in both hatchery tanks and sea cages.

Multilevel logistic models were fitted to predict fin damage from each of the four condition indices, with variation between samples included as a random effect. A single logistic regression was performed for each condition index for tanks and cages separately; these logistic regressions were compared to the null hypothesis model. The logistic regressions are fitted to both caudal and dorsal fin damage simultaneously, resulting in a coefficient representing the odds of higher damage for the caudal fin than the dorsal fin. The results of the model comparison are shown in Table 2.

For the hatchery, condition index 2a (size relative to peers expressed as percentage difference) was the best explanatory variable for fin damage. Fin damage was rated as worse for the caudal fin compared with the dorsal fin. In condition index 2a, a 10-point decrease in condition corresponds to a reduction in size of 10% of the mean size; the model shows that in tanks this led to the odds of being in a worse damage category increasing between 1.08 to 1.23 is the range of the 95% CI. The deltaAIC (the difference between the lowest AIC and another model AIC) for the condition index 2b is 4, and this indicates that the models would generally be regarded as equally good (Burnham & Anderson, 2004).

In sea cages condition index 2b was a slightly better predictor of fin damage than 2a (percentage difference from mean weight). Index 2b differs from 2a in that it uses size difference measured in standard deviations, therefore high index values are possible even when the overall range in size is small. This suggests that size differences within a cage may lead to welfare differences even when fish are of similar size. However as the delta-AIC of the model for index 2a is 4, the models would generally be regarded as equally good, so the evidence that this is definitely the case is weak.

3.4 | S. salar data set

To ensure consistency with the *C*. *lumpus* analysis above, a length-weight relationship was fitted to the data set of n = 2021 parr. This resulted in the curve expected weight = $0.0075 \times \text{length}^{3.13}$.

The four condition indices were calculated in the same way as for *C. lumpus.* Figure 7 shows each of the indices in comparison with the five-point fin damage scale. Multilevel logistic models were fitted to predict fin damage from each of the four condition indices, and the results of comparing these models are shown in Table 3.

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790 REY ET AL. **FISH** BIC ٦G tank tank cage cage condition1a condition1a 300 300 200 200 100 100 0 0 2 2 0 2 3 0 0 3 3 n 1 1 2 dorsal caudal tank cage tank cage condition1b condition1b 250 225 1050 2505050 2 3 0 0 3 0 n 1 1 2 3 2 1 2 3 caudal dorsal tank cage tank cage condition2a condition2a 250 200 150 100 50 250 200 150 100 50 2 3 2 2 3 0 0 n 2 dorsal caudal tank tank cage condition2b cage condition2b 400 300 700 100 -100 n 2 3 0 2 3 0 2 3 0 2 3

FIGURE 6 Relationship between the four condition indices and fin damage. Separate plots for hatchery (tank) fish and adult (cage) fish as different behaviours may lead to different patterns of fin damage with condition. There are separate plots for caudal and dorsal fins as the average levels of damage are different

By the AIC model comparison, the best predictor of S. salar fin damage is condition 2a. The two condition indices 1a and 1b (deviation from the length-weight relationship and Fulton's K) are rejected as predictors of fin damage as for this data set better condition was associated with worse fin damage.

dorsal

DISCUSSION 4

4.1 Fin damage in C. lumpus

Fin damage or fin erosion is a fish welfare issue as injury to tissue containing nerves and blood vessels can lead to poor condition and death (Ellis et al., 2008). It is usually an outcome of some aggressive interaction between individuals, such as fin biting or nipping, that causes chronic social stress and can be detrimental to the fish, hampering growth and increasing the size differences in fish populations (Abbott & Dill, 1985, 1989). Fin damage can also affect larger dominant fish due to aggressive interactions and not be directly related to poor welfare but to dominance hierarchies (MacLean et al., 2000), mainly in bigger groups of S. salar.

C. lumpus is a highly aggressive fish species during their early live stages (larvae and juveniles) (Treasurer et al., 2018). The overall welfare of C. lumpus could be improved by reducing intraspecific aggression and consequently the rate of fin damage. The application of the OWI of fin damage to size and condition data was important in identifying the impact fin damage had on C. lumpus. These results indicated significant variation in fin damage with size and life stage of the C. lumpus, which is to be expected of a species being held under a variety of husbandry conditions (hatchery and sea cage) in environments that differ from its natural habitat.

caudal

Hatchery C. lumpus held in tanks prior to deployment at sea suffered from greater levels of fin damage, particularly early in the production cycle (see timeline in Figure 3). Three hypotheses of how size impacts on fin damage were tested in the study by using model selection to compare size-based condition indices. The hypotheses were that the fin damage was due to fish failing to meet the expected growth curve for the species (suggesting that other impacts on health may be contributing to fin damage) or from fish being smaller than their peers (suggesting aggression and other behavioural effects contribute to fin damage) or that it affected all fish uniformly independent of size, but declines with age.

Selection of size-based condition index for 4.2 C. lumpus in aquaculture

Two sets of condition indices were compared using model selection. The first set compared individual fish to a species-specific expected

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length-weight relationship (or, more generally, Fulton's K where expected weight = $a \times \text{length}^3$). When this type of index is linked to overall health, it implies that growth of the fish (in terms of putting on weight with somatic growth) and energetic reserves are critical to



FIGURE 7 Relationship between the four condition indices and fin damage in salmon

TABLE 3	Model comparison for
salmon	

other aspects of health. This type of condition index has proved valuable in both fisheries and aquaculture, especially as a proxy for reproductive status, but also in relation to other aspects of health (Davidson & Marshall, 2010; Lloret & Planes, 2003; McPherson *et al.*, 2010; Rätz & Lloret, 2003). It is likely that *C. lumpus* in captivity retain overall good nutritional status (*e.g.*, there is high food availability), so there is little variation in this type of condition index in captivity driving variations in health, therefore it proves less important in this particular aquaculture context (Treasurer *et al.*, 2018, Eliasen *et al.*, 2020.

Our second set of condition indices compared individual fish weight to the average of the peers in the same tank or cage. This index made no use of species-specific information. Two variations of this type of index were used: index 2a was the percentage variation from the average; index 2b re-expressed this as the number of standard deviations variation from the mean. The critical difference between the two indices is that when the overall range of size in a tank is small, the fish have a small range of variation in index 2a, but (because of the scaling by standard deviation) they always retain the same range of variation in index 2b.

The main finding was that the relative weight of an individual within the local population is most important in predicting the severity of fin damage, *i.e.*, the condition index that best predicted fin damage was 2a, which is the size of a fish relative to its peers. The same result held for *C. lumpus* and *S. salar*. The wider implication of this for aquaculture is that the welfare of each individual depends on how it compares to its peers, rather than its individual characteristics (as would be the case for a solitary free-living fish). It also demonstrates the importance to aquaculture of considering the population within a containment unit instead of using the classic approach of comparing with a condition index from a standard population like the first set of condition indexes.

This index showed that for hatchery *C. lumpus* in tanks, a 10% decrease in size was predicted to have a 7%–22% probability of having worse damage; for fish in sea cages, a 10% decrease in size was associated with a 1%–11% chance of increased damage [95% confidence intervals (CI) from Table 2]. Importantly, index 2b (scaled to standard deviation) was a slightly poorer predictor of fin damage, suggesting that when the overall range of variation of size in a

Condition index	Comparison with null hypothesis (Anova with chi-square test)	Effect of condition index expressed as odds of moving to higher damage category with 10-point decrease in condition (95% CI)	Model AIC (* indicates lowest)
Null hypothesis			5876
la		Negative effect	5873
1b		Negative effect	5876
2a	<i>P</i> < 0.001	1.08-1.18	5848*
2b	<i>P</i> < 0.001	1.01-1.03	5857

Note. For indices 1a and 1b, the correlation is in the wrong direction for a condition to serve as a predictor of fin damage in which poorer condition correlates with worse damage (Figure 7 shows this relationship).

population (tank or cage) is small the overall damage is reduced (as there are less extreme small values of index 2a in these circumstances) hence the importance for grading as a welfare measure (Benhaïm *et al.*, 2011; Huntingford *et al.*, 2006). Had index 2b been a better predictor of damage then index 2a, this would have implied that even when overall size variation is small, dominance hierarchies still result in damage to the smaller individuals (something that may occur in other species) (Martins *et al.*, 2012).

Although the overall level of damage was less severe in fish deployed in sea cages, the index 2b (scaled by standard deviation) was a better predictor of fin damage, suggesting that a dominance hierarchy exists whether or not fish have a wide range of sizes (although the effect was small). The life history of *C. lumpus* indicates dominance hierarchies related to feeding and space availability (Treasurer *et al.*, 2018). In sea tanks and sea cages they have preferred areas (shelters, feeding areas, *etc.*) that might be a source of competition leading to aggression and fin damage (Johanensen *et al.*, 2018; Huntingford *et al.*, 2006.

Notably, even in the less controlled environment of sea cages, with a greater chance of variation in feed or external factors affecting fish energetics and growth, the length-weight relationship indices did not serve as better predictors of fin damage. There was no indication that fish that were lighter for their length suffered additional welfare issues. However, recent studies still record high mortalities for *C. lumpus* during autumn/winter seasons, meaning some undercurrent issue (poor nutritional status, aggression, environmental conditions, *etc.*) is hampering their welfare (Geitung *et al.*, 2020).

4.3 | Management implications of the selected index

This selection of the index based on size relative to peers highlights the importance of early grading in hatchery facilities where tanks are used to house hatchery *C. lumpus*. Grading should start from when *C. lumpus* are 2 g approximately, as damage was observed even at this small size. Not only should grading occur regularly in stationary facilities it is important that when *C. lumpus* are transported, they are graded before they are placed into transportation tanks.

Grading is common practice in aquaculture to ensure that fish of a similar size are stocked together as a measure to improve growth by reducing food competition and aggression due to social hierarchies (Abbott & Dill, 1989; Treasurer *et al.*, 2011). Fish grow and develop at different rates, resulting in greater morphological variation within populations (Nakamura, 1955; Wallace & Kolbeinshavn, 1988). At hatchery level grading procedures are regularly implemented based on the idea that when larger fish are removed from a tank the smaller fish will grow better (Gunnes, 1976; Treasurer *et al.*, 2011). Grading fish changes social hierarchy, removes competition and allows for suitably sized food to be given to match the fish size. Grading of fish populations has indicated a positive increase in the welfare of fish. It was observed in hatchery *S. salar* (Gunnes, 1976), Arctic charr (*Salvelinus alpinus*) (Wallace & Kolbeinshavn, 1988), white sturgeon (Acipenser transmontanus) (Georgiadis et al., 2000a, 2000b) and cod (Gadus morhua) (Treasurer et al., 2011) that growth rates increased when fish were graded and that there was a reduction in cannibalistic and agonistic behaviour. In general, few of those studies indicate there is no effect on fish growth through the process of grading, with the majority indicating positive growth outcomes (Jobling & Wandsvik, 1983). Grading of *C. lumpus* may therefore reduce the severity of fin damage in tanks and cages. In contrast, grading can be a stressful event and a source of fin damage itself by the netting and handling of the animal, so adequate nonstressful passive grading systems must be developed to mitigate this negative effect. Soft nets should be always used to avoid damage to the fins.

In sea cages our results suggest that smaller fish suffer more damage, regardless of the range of sizes in a cage. This conclusion was drawn because condition index 2b (size relative to peers, scaled by standard deviation so that in a small range of sizes the smallest fish still has a low value of the index) was a slightly better predictor of fin damage, but we are cautious in drawing firm conclusions about this because of the small difference in model AIC. Sea cages present a complex environment to *C. lumpus*, in which they interact with *S. salar* and possibly other species (*C. lumpus* are commonly stocked with other cleaner fish like wrasse). It is difficult to observe behaviour and the netting procedure we used to obtain samples may have biases if bold or proactive fish are more likely to be captured.

Mature *C. lumpus* show pronounced sexual dimorphism. If *C. lumpus* were to be used as cleaner fish after maturation, then this is likely to alter the expected weight and length between sexes and patterns of aggressive behaviour, hence the implications of size to this are also likely to change.

Our study is limited to *C. lumpus* aquaculture in two geographic areas within a single year. As the species becomes more established and understanding of husbandry evolves (such as developing feeding practices and habitat improvements in both hatchery and sea cages), we are likely to see changes to behavioural intra- and interspecific behavioural interactions, and also possibly changes to the expected growth of *C. lumpus* in captivity. These may have impacts on the prevalence of fin damage, and also allow development of improved estimates of length-weight relationships.

4.4 | Model selection for choosing condition indices

The study made use of statistical model selection to choose the best index. This depended not only on a sufficient data set of size measurements with corresponding fin damage observations, but also a statistical model to predict fin damage from each condition index. The aim was to make explicit the link between the OWI that directly observes health and the condition index calculated from size measurements that serves as proxy for the status of the fish. It is possible that other measures of condition might relate better to other OWIs, even in the same species. For example, comparing the same four indices to an OWI derived from nutritional status may yield different results, but there are no examples in the literature to confirm this. Similarly, there is no reason to expect other species and environmental conditions to yield similar results, but the analytic framework used here allows us to select an index.

As we had fin damage observations for both caudal and dorsal fins, we used a logistic model to predict fin damage from the condition index with the identity of the fin (caudal or dorsal) as an additional predictor. This avoided either arbitrarily weighting the two fins equally (if the sum of the scores was used as a response variable) or treating the two fins as completely independent (if separate models were used for each fin). An additional outcome of this model is a prediction of the different levels of damage between the fins with a CI [for hatchery fish the caudal fin is between 1.17 and 2.77 times (95% CI) more likely to be in a worse damage category than the dorsal].

We chose to model the effect of condition on fin damage separately for the tank containing hatchery fish and the fish deployed in sea cages. This decision was based on the high possibility that the different environments that the fish are housed in or the different behaviour at different life stages could have led to different processes being involved in generating fin damage.

4.5 | Concluding remarks

In conclusion, this study highlights the importance of choosing a correct condition index to understand the impact of size variation and welfare implications. We demonstrated that traditional condition indices were not predictive of fin damage in captive *C. lumpus* and *S. salar*. The implications of this study are highly important for the aquaculture industry both for hatcheries and sea cages, and can be applied to multiple different farmed fish species. There is no one-size-fits-all solution for evaluating welfare and condition, but our method of comparing indices could be used to select indices for wild fish, for fisheries and conservation, and in aquaculture systems.

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CONTRIBUTORS

S.R., B.J.M. and J.T. proposed and designed the study and obtained funding. J.T. was responsible for husbandry in the hatchery, with S.R., B.J.M. and C.P. collecting data. Collection of data at sea was coordinated by S.R. Data analysis was performed by B.J.M. All authors contributed to writing the manuscript. http://hdl.handle.net/11667/177.

DATA AVAILABILITY STATEMENT

The datasets and Supporting Information generated during the current study are available in the University of Stirling DataSTORRE repository (persistent web link to datasets). http://hdl.handle.net/ 11667/177.

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