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Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis

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Abstract

The welfare of fish is receiving increasing attention and attempts have been made to control welfare in farmed fish through regulation of management practices, including stocking density. However, there is little published information on the influence of stocking density on welfare of fish in marine cages. This present study examined welfare in Atlantic salmon (*Salmo salar*) in cages on a commercial marine farm, exposed to densities ranging from 9.7 to 34 kg m^{-3}. On three occasions over a period of 10 months, fish were sampled from each cage, weighed and measured; their fin condition assessed and blood samples taken for measurement of glucose and cortisol. A multivariate analysis was used to combine four commonly used measures of fish welfare (condition of body and fins and plasma concentrations of glucose and cortisol) into a single welfare score. As well as objectively reflecting a coherence within the data, this score was consistent with the evaluation of welfare by experienced farmers. A generalized linear model indicated that the median welfare score for each cage was significantly related to sampling period, to stocking density (mean over the previous 3 months) and to location of the cage. A model with all the data from individual fish proved to be more robust and also identified sample period, stocking density (mean over 3 months) and position of the cage as significant predictors of the welfare score. There was no significant association between the welfare score and the length of time since grading or lice treatment. Further analysis of the relationship between stocking density and the welfare score suggested that there was no trend up to an inflection point ca. 22 kg m^{-3}, after which increasing stocking density was associated with lower welfare scores. This study suggests that, while stocking density can influence the welfare of Atlantic salmon in production cages, this is only one influence on their welfare and on its own cannot be used to accurately predict or to control welfare.

Keywords: Stocking density; Welfare; Atlantic salmon; Multivariate analysis

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1. Introduction

There is a trend towards increased concern for the welfare of animals at all levels where they come into contact with humans and recently this concern has expanded to include the welfare of fish. Welfare is a complex concept, this complexity being reflected in the diversity of definitions (Dawkins, 1998; FSBI, 2002).

Whichever definition is used, and for welfare to have any real meaning, the animal concerned must have the capacity for suffering and in the case of fish this is controversial (Rose, 2002). However, recent evidence suggests that external stressors and painful stimuli elicit aversive states in fish, as they do in birds and mammals (Sneddon et al., 2003; Braithwaite and Huntingford, 2004; Chandroo et al., 2004), even though these may differ in degree from those experienced by higher vertebrates. In any event, a wide range of organisations in the United Kingdom and Europe now have fish welfare on their agenda including the Department of the Environment, Food and Rural Affairs, the Scottish Executive, Environment and Rural Affairs Department, the Royal Society for the Prevention of Cruelty to Animals, the Scottish Society for the Prevention of Cruelty to Animals, the Fisheries Society of the British Isles and the Council of Europe. The Farm Animal Welfare Council report (Farm Animal Welfare Council, 1996) made a number of recommendations regarding the welfare of fish under production conditions, but also identified areas where scientific investigation was necessary to provide the information upon which to base welfare guidelines and legislation. Stocking density was identified as one such area, since it is often suggested that farmed fish are held at higher densities than prevail in the wild. However, it would appear that some species of fish elect to shoal at very high densities in the wild (Trevorrow and Claytor, 1998) although estimating the density of wild fish is problematic unless the population has low density and uniform distribution (Parkinson et al., 1994).

Many studies have demonstrated an effect of stocking density on various aspects of the welfare of farmed fish (Wedermeyer, 1997), though the results depend on the species concerned; for example, Arctic charr (*Salvelinus alpinus*) suffer less physical damage and grow more rapidly at high density (Jorgensen et al., 1993), whereas sea bass (*Dicentrarchus labrax*, Vazzana et al., 2002) and gilthead seabream (*Sparus auratus*, Montero et al., 1999) show evidence of reduced welfare at high densities. Studies of the relationship between welfare and stocking density are further complicated by interactions with other variables such as food availability (Robel and Fisher, 1999) or water quality (Ellis et al., 2002). The measurement of stocking density in production cages is in itself difficult for a number of reasons. For example, estimating biomass requires accurate information on growth and survival and converting biomass to density requires knowledge of the volume of space available to the fish, which is constantly changing due to deformation of the net. Furthermore, the fish do not occupy all of the available space (Juell and Fosseidengen, 2004).

To date, there have been no other published studies of the effects of stocking density on welfare in marine production cages. Soderberg et al. (1993) reviewed stocking densities for the “satisfactory growth and health” of juvenile Atlantic salmon (*Salmo salar*) and found acceptable welfare at a wide range of densities. In the absence of robust empirical data, the Farm Animal Welfare Council (1996) recommended a maximum stocking density of 15 kg m^{-3} based on the current practice and expert opinion, but called for further research.

Mellor and Stafford (2001) argued that welfare should be improved in an achievable incremental manner rather than aspiring to an unachievable ideal. It is only possible to improve welfare if it can be measured or assessed, but despite recent progress in the evaluation of welfare in terrestrial animals (Spoolder et al., 2003), there is no current consensus on the best way to objectively measure welfare in fish. It has, so far, been difficult to apply systems for assessing welfare in terrestrial animals to fish; this has been partly due to the problems of observing fish especially in production systems. Previous attempts to assess fish welfare have largely concentrated on measurement of individual aspects of welfare such as growth, stress or damage. Such parameters have been used as proximate indicators of welfare in fish (Etscheidt, 1995), but, because they are influenced by factors other than welfare, they are often imprecise or noisy indicators. One possible solution is to use the statistical tool of multivariate analysis to combine simultaneously recorded measures of welfare, on the basis of the observed statistical relationships among them (Manly, 1994). Principal components analysis (PCA) allows trends in multivariate data to be reduced to scores that can then be used as dependent variables in subsequent analysis, without requiring a priori judgements of the relative value of individual variables. Such scores offer the possibility of providing an integrated and objective reflection of diverse measured welfare indicators. This approach to the analysis of complex data sets is only suitable for the research context but may be a very powerful approach for the identification of robust but simple on-farm welfare assessment methods.

This study was initially set up as a balanced design, with replicated cages at three stocking densities. However, although we were successful in generating a range of randomly allocated stocking densities extending beyond those commonly used in salmon aquaculture, the constraints associated with a commercial production site made it impossible to maintain the required densities with sufficient accuracy. We therefore used stocking density as a continuous rather than categorical, independent variable and related this (and other possible influential factors) to the welfare score.

Our specific aims were:

1. To relate a welfare score in Atlantic salmon, derived by multivariate analysis of four commonly used measures of fish welfare, to assessment by farm workers of the health status of their fish.
2. To use this score to examine the relationship between welfare and stocking density in farmed Atlantic salmon.
3. To examine additional effects of time from the start of the study, cage position and time since potentially stressful husbandry event (grading or lice treatment) on welfare.

2. Materials and methods

2.1. Subjects and husbandry

The study was conducted in commercial on-growing cages in Loch Duich on the West Coast of Scotland between January and October 2000. The group of cages consisted of 30 cages (estimated 15 m^2 and 7 or 8 m deep), of which 24 were stocked. These cages were at the northwesterly end of the loch and comprised two adjacent rows of 15 cages located parallel to the shore and orientated with the long axis of the loch (15 "near-shore" and 15 "off-shore") (Fig. 1). The fish were of Marine Harvest L70 stock, 1997 year class, stocked into sea cages in April 1998. The standard operating procedures for husbandry practices were recorded and found to be similar to those throughout the industry at that time (Scottish Quality Salmon, pers. comm.).

2.2. Experimental regimes

Cages were monitored from January 2000 until October 2000 and fish were sampled on three occasions: in late March, early August and late October 2000 (sample periods 1, 2 and 3). Production data recorded included growth, disease treatments, grading and survival. Farm feeding records were not appropriate for analysis. Dissolved oxygen and temperature were recorded on a TPS data logger (TPS, Springwood, Australia) every 30 min on two days for each cage during each of the three sampling periods. Due to the number of fish in these production cages (from 3327 to 24,325), it was only

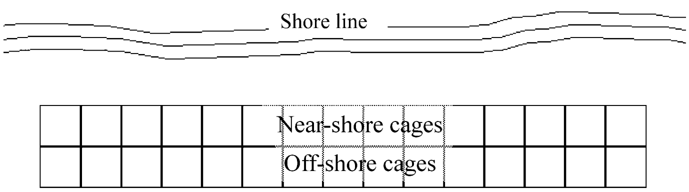

Fig. 1. Diagrammatic representation of the raft of cages used during the study.

possible to obtain estimates of the mean growth in the cage.

Initially, three stocking densities were established (15, 25 and 35 kg m^{-3}), with three replicates of each density, allocated randomly to the available cages. Subsequently, these densities changed as fish grew and as a result of routine husbandry procedures, for example splitting populations. In order to summarise the density experienced by each fish prior to sampling, we took the mean stocking density over the 3 months prior to sampling. Stocking density was calculated from the biomass of fish (from farm records) divided by estimated volume of the net (from manufacturers information). The calculated cage density ranged from 9.7 to 34 kg m^{-3}, a wider range than was normal within the industry at the time (Scottish Quality Salmon, pers. comm.).

2.3. Sampling protocol

On each sampling day, 12 fish (sample size determined by the practicalities of sampling with available resources) were caught from the cage using a cast net, usually catching at least 12 salmon in one throw. This was found to be the most rapid means of obtaining a sample of the population and minimising bias following the recommendations of Cameron (2002). The fish were quickly removed from the net and killed by a sharp blow to the head. The time from capture to death was always less than 5 min. A 2-ml blood sample was immediately drawn by caudal venipuncture using a 24-gauge needle, placed in a heparinised eppendorf tube and stored on crushed ice until all 12 samples had been collected. The samples were then spun at 1000 g for 5 min in a microcentrifuge. Plasma samples were transported ashore on ice for storage at −20 °C. These samples were transferred to −70 °C on arrival at the Institute of Aquaculture, Stirling, within 2 days of sampling. They were analysed for both cortisol and glucose; plasma cortisol by radioimmunoassay using Amerlex™ kits manufactured by Ortho-Clinical Diagnostics, Amersham, UK. Plasma glucose concentration was quantified spectrophotometrically by the Trinder enzymatic reaction (Trinder, 1969) using a Sigma glucose diagnostic kit (No. 315) obtained from Sigma-Aldrich, Fancy Road, Poole, Dorset, UK. Although neither of these assays were originally designed for use in fish, they have been widely used in aquatic species including Atlantic salmon (e.g. cortisol, Wiik et al., 1989; Espelid et al., 1996, and glucose, Pottinger and Carrick, 1999; Ackerman et al., 2000). The effect of storage on these samples was found to be negligible (Bell, 2003).

The fork length and weight of each fish was recorded and used to calculate the condition factor:

$$K = \frac{\text{mass (g)} \times 100}{\text{fork length (cm)}^3}$$

All fins were assessed for the degree of erosion, splitting and thickening using a rating scale (Turnbull et al., 1998; MacLean et al., 2000). While the fish were also examined for other injuries, this produced no significant data.

2.4. Measuring welfare

The outcome (dependent) variables were combined by means of multivariate analysis. First, a PCA was conducted on the fin data and the first component (with significant positive loadings for all measures of

Table 1
(a) The first component generated by principal component analysis of the 12 fin condition scores. (b) The first component derived from principal component analysis of the four welfare indices (n=219, with case-wise deletion for missing data)

Variable	Loadings on component 1
(a)	
Dorsal loss	0.312
Dorsal splitting	0.286
Dorsal thickening	0.326
Caudal loss	0.272
Caudal splitting	0.223
Caudal thickening	0.258
Left pectoral loss	0.320
Left pectoral splitting	0.256
Left pectoral thickening	0.289
Right pectoral loss	0.343
Right pectoral splitting	0.248
Right pectoral thickening	0.306
(b)	
Fin condition	0.456
Condition factor	0.257
Plasma glucose	−0.728
Plasma cortisol	−0.443

fin condition, Table 1a) was used as a score of general fin condition. Combining all the fin measures into a single overall PCA with the other measures (cortisol, glucose and condition factor) produced components biased by the larger number of fin variables. Therefore, a further principal components analysis was carried out using the variables listed in Table 1b. This generated a first principal component accounting for 29.9% of the total variance in the data set, which loaded fin condition and condition factor positively and plasma glucose and cortisol negatively (Table 1b). This principal component incorporated both physical indicators of welfare (fin condition and condition factor) and physiological measures of stress (high plasma cortisol and glucose, Pickering, 1998). The factor scores provided a single, integrated value, which increased with improving welfare.

To examine further the validity of using this score in farmed fish, the median welfare score for two cages that had been reported by the farm staff to be a cause of concern (based on the behaviour, feeding response and gross appearance of the fish) was compared with that of fish from other cages during the same sampling period (period 1) and fish from all the other cages in the study using a Mann–Whitney rank sum test (SigmaStat© 3.00, SPSS 1992–2003). The median welfare score for each cage was also compared with the mean mortalities per month per 1000 fish and the mean weight gain per month (estimated from a sample of 100 fish).

2.5. Data analysis

A generalized linear model analysis (GLZ) (Statistica© 6.0, Statsoft 1984–2001) was carried out to investigate the relationship between the dependent variable (welfare score) and four independent variables, “cage position”, “average stocking density” (over 3 months), “days since last stressful husbandry event” (grading or lice treatment) and “sample period”. Analyses were conducted for both cage median and individual fish welfare scores. Following examination of the data, a normal distribution and log link function ($f(z)=\log(z)$) were employed for the GLZ (McCullagh and Nelder, 1989).

To identify the best model (best subset regression), the Akaike Information Criterion (AIC) was used to optimise the number and combination of predictive variables included. To validate the proposed models, the Wald statistic was used to check the significance of the regression coefficient for each parameter, a likelihood ratio test was used to evaluate the statistical significance of including or not including each parameter and model goodness of fit was checked using deviance and Pearson χ^2 statistics. Residual plots for each model were assessed visually to exclude remaining un-attributed structure indicative of a poor model fit.

Having identified that there was an association between the welfare score and stocking density, we further examined that association in a univariate analysis and found it to be best modelled by a piecewise linear regression with Quasi-Newton estimation of breakpoint (Statistica© 6.0, Statsoft 1984–2001). To scrutinise more precisely the position of a break point, the same analysis was carried out on the welfare score of all the individual fish sampled, again with the individual fish from each sample period and all the individual fish in the off-shore cages. Using data from all individual fish in these analyses made the assumption that each fish represented an independent data point. We regarded this assumption as justified in this case, since individuals within a single large cage (840 m^3) containing up to 26,555 fish are likely to experience a wide range of conditions. The use of data from individual fish is further justified since the variability in welfare score within the cage is as large as, or larger than, that between cages (see Fig. 3).

3. Results

The first component produced by the PCA was biologically consistent with welfare and accounted for 29.9% of the variability in the data. The additional principal components did not have any biological interpretation relevant to welfare and did not warrant further analysis according to the scree test (Cattell, 1966).

3.1. The welfare score and industry assessment of fish status

Fish in the cages identified by the farm staff as problematic on the basis of feeding behaviour and

Table 2
Median (and interquartile ranges) of the welfare scores from fish in cages identified as problematic and from fish in all other cages, both during sample period 1 and over the whole study, compared by Mann–Whitney sum rank tests

	Median	25%	75%	*n*
Fish from problem cages compared with	−1.416	−2.512	−0.407	23
Other fish in sample period 1	0.0393 T=591.0	−0.619 P≤0.001	0.482	82
All other fish in the study	0.248 T=1098.0	−0.413 P≤0.001	0.722	279

general appearance of the fish had significantly lower welfare scores than those in all other cages, regardless of position or time of sampling (Table 2).

Using linear regression, the average number of mortalities per 1000 fish per month was not significantly correlated with the welfare score (R^2=0.0263, P=0.419), but mortalities were very low (overall mean mortalities 2.2 fish 1000^{-1} month^{-1}). Neither was there any significant correlation between the mean monthly weight gain and the welfare score (R^2=0.0783, P=0.207). While there was considerable variation in weight gain between cages, all growth rates were considered to lie within normal production ranges by the farm management. The mean weight of the fish in the study was 3.52 kg (S.D. 0.543) at the first sample, 4.89 kg (S.D. 1.115) at the second and 6.553 kg (S.D. 1.297) at the third. Lice treatment, when necessary, was conducted in all cages over a period not exceeding 3 days. Grading or division of the population was conducted mostly at the start of each sampling period, in May and August, minimising its effect on the population at sampling. Grading is generally conducted to maintain stocking densities, to remove grilse (early maturers) or fish for harvest. In this study it was also used in an attempt to maintain a wide range of stocking densities within the trial cages. The populations in the trial cages were divided with the aim of maintaining the same distribution of sizes, but there were changes in average weights associated with grading. Table 3 contains details of the monthly average weights before and after grading during the

Table 3
Change in average weight in trial cages over the May and August grading, derived from a sample of 100 fish, expressed in kg and as a percentage of the average starting weight

Change in weight from the month before to the month after grading				
Cage ID	April–June		July–September	
	kg	%	kg	%
17	0.538	16	0.947	23
20	−0.299	−9	1.185	34
27	−0.410	−11	0.412	11
32	0.668	21	0.135	3
33	0.367	16	1.110	38
34	−0.004	0	1.056	29
35	0.509	20	0.469	13
36	−0.197	−5		
40	1.410	51	−0.500	−11
Mean	0.287	11	0.602	18
S.D.	0.578	20	0.587	17

Table 4
Description of two GLZ models with median cage welfare score as the dependent variable and sample period (1, 2 or 3), mean stocking density in the cage over the 3 months prior to sampling and location in the row of cages near-shore or off-shore as independent variables

Independent variable	Wald statistic	*P*
(a) Three-variable model, n=27		
Sample period	13.33000	**<0.001**
Mean stocking density	4.20513	**0.040**
Near-shore or off-shore	1.50065	0.221
(b) Three-variable model with one outlier removed, n=26		
Sample period	12.98941	**0.001**
Mean stocking density	1.81336	0.178
Near-shore or off-shore	3.50271	0.061

(a) Model using median values from all cages. (b) Model with one extreme point removed. Bold indicates P<0.05.

Table 5
The output of two GLZ models with individual fish welfare score as the dependent variable and sample period (1, 2 or 3), mean stocking density in the cage over the 3 months prior to sampling (kg m^{-3}), and location in the row of cages near-shore or off-shore as independent variables

Independent variable	Wald statistic	*P*
(a) Three-variable model		
Sample period	36.461	**<0.001**
Mean stocking density	17.510	**<0.001**
Near-shore or off-shore	6.183	**0.013**
(b) Three-variable model with all data from worst cage removed		
Sample period	22.453	**<0.001**
Mean stocking density	4.124	**0.042**
Near-shore or off-shore	12.319	**<0.001**

(a) Model using all data. (b) Model with all data from one cage with the lowest median welfare score removed. Bold indicates P<0.05.

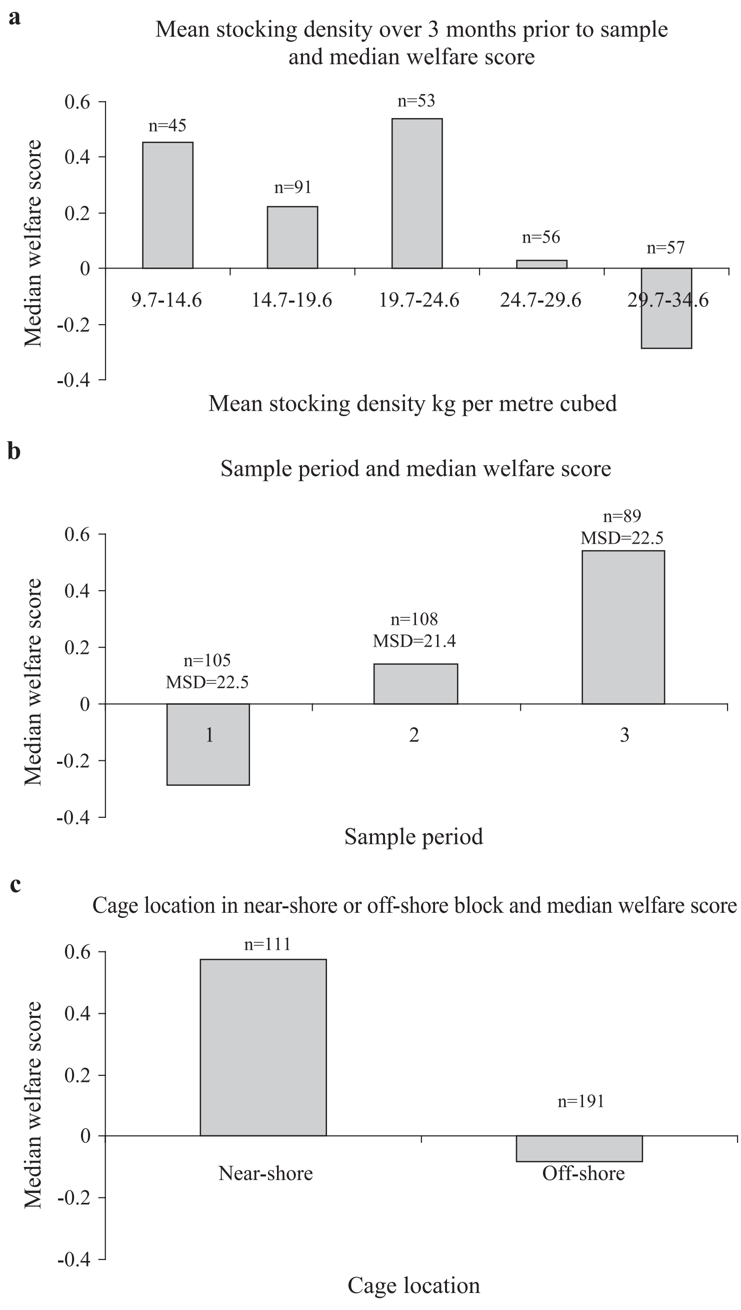

Fig. 2. (a) Median values of the welfare score for all fish in the study, categorised according to mean stocking density over 3 months prior to sampling. (b) Median welfare score for all fish in each of the three sampling periods, MSD = mean stocking density during that period. (c) Median welfare score for all fish in the near-shore cages and off-shore cages. *n* = number of fish.

trial. All of the cages were graded in May and August and therefore there were no figures for change in weight in non-graded cages.

3.2. Predictors of welfare

A GLZ carried out on the median welfare score for each cage and the four independent variables produced a model with only “sample period” and “mean density” showing significant fits, this remaining true when “days since last stressful husbandry event” was removed to provide the best three-variable model (Table 4a). Using median data for the model, however, made it highly sensitive to changes in individual data points. For example, only “sample period” remained significant after removal of a single extreme point identified through a plot of chi-squared versus predicted values (Table 4b).

The use of data from all individual fish produced models that were more robust. The most reliable model was a three-variable model with “sample period”, “mean density” and “cage position” as independent variables and with all these being significant (Table 5a), and with “days since last stressful husbandry event” having been excluded as a non-significant factor. Even with all the data from the cage with the worst welfare score (median and total) removed from the model, these same three variables were still significant in the best solution model (Table 5b). For the purpose of illustration, the median welfare scores for the three significant independent variables without adjustment for interactions are presented in Fig. 2.

Fig. 3 shows the relationship between stocking density and welfare score for all the fish sampled and Table 6 summarises the results of piecewise linear regression with breakpoint analysis using cage medians, data from all fish and individual fish data divided by sample period and in the off-shore cages only. Data from the near-shore cages were not

Table 6

Results of piecewise linear regression with breakpoint estimation for the welfare score on mean stocking density (a) using medians from every cage in the study, (b) using all fish for all sampling periods, (c) using all fish sampled in period 1, (d) using all fish sampled in period 2, (e) using all fish sampled in period 3, (f) using the medians from all cages with the worst cage removed and (g) using fish in the off-shore cages only

	Variance explained (%)	Final loss	Break point (kg m^{-3})
(a) Cage medians	78.9	265	22.11
(b) All fish, all periods	82.5	868	22.47
(c) All fish, period 1	76.2	1402	22.47
(d) All fish, period 2	76.2	1402	21.43
(e) All fish, period 3	71.8	1274	22.60
(f) Cage medians with worst cage removed	79.2	231	21.65
(g) Off-shore cages	83.3	1127	25.31

The final loss, calculated by the least squares method, is a measure of the discrepancy between the observed data and data “predicted” by the fitted function, it provides a means of evaluating the fit of the model.

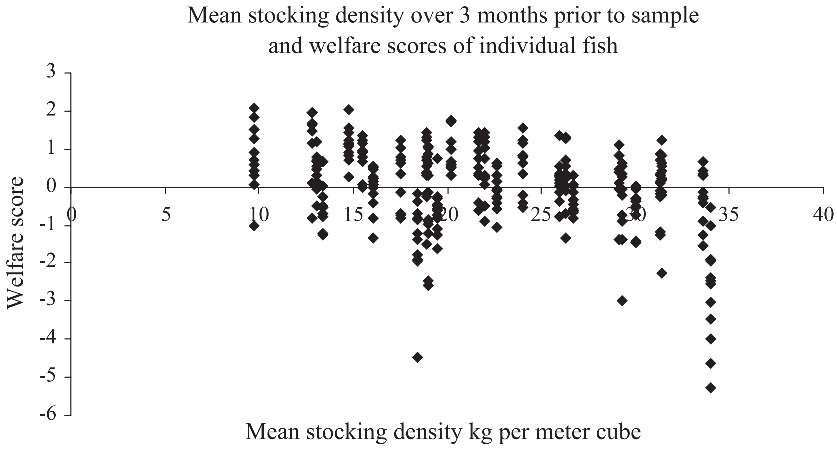

Fig. 3. Relationship between welfare score and mean stocking density over the preceding 3 months for all fish.

Table 7
The mean and standard deviation of dissolved oxygen and temperature from two days recorded in each cage during each sample period

Sample period	Dissolved oxygen mean±S.D. (mg l^{-1})	Temperature mean±S.D. (°C)
1	11.1±0.7	8.0±0.1
2	9.2±0.5	11.7±0.1
3	7.7±2.5	13.3±0.1

analysed since only part of the stocking density range was represented in these cages (9.7–24 kg m^{-3}), this was as a result of routine farm management following initial randomised distribution of stocking densities.

Considerable variability was found at most densities, some fish with poor welfare scores being found at low stocking densities and some fish with high welfare score being found at high stocking densities. In both analyses, the welfare score remained stable with stocking density up to an inflection point at ca. 22 kg m^{-3}, after which it began to fall.

Recorded temperature was within acceptable limits and remained stable (Table 7). Dissolved oxygen levels only fell below the recommended minimum of 6 mg l^{-1} (Shepherd and Bromage, 1992) in the third sample period and then only for periods of less than 30 min after feeding. However, data from four cages was lost during the third sampling period due to a technical fault.

4. Discussion

4.1. Measuring welfare

The complex nature of welfare is reflected in many physiological and behavioural aspects of an animal, so to evaluate welfare it is necessary to obtain information from a wide range of sources. Although single measures of welfare, such as plasma cortisol, can give useful information about fish status with respect to a single biological system, by integrating a number of relevant measures into a reduced number of values, multivariate analysis potentially provides a useful and important tool for investigating the complexity of welfare in real environments. Such welfare scores give breadth by considering different aspects of welfare and derive power by combining them objectively. As in a companion paper (Adams et al., submitted), principal components analysis identified a single dimension in the data (with condition factor and fin condition contributing positively and glucose and cortisol concentrations contributing negatively) accounting for ca. 30% of the total variance in the data set.

A series of univariate analyses were examined in this study in order to exclude the possibilities that the welfare score either reflected a single univariate trend or masked important trends in the univariate data. For example, whilst it might be suggested that improving condition factor in larger fish largely drove improvement in the welfare score over the duration of the experiment, this was not found to be the case. A comparison of all univariate analyses and analyses using the welfare score indicated that no single measure reflected all the associations found with the welfare score and that there was no evidence of masking of univariate effects. Even had there been significant univariate trends it would have been difficult to relate these directly to welfare, since univariate parameters are notoriously imprecise reflections of welfare.

It is difficult to validate the welfare score conclusively, since there is no absolute standard for determining welfare status. However, the welfare score agreed with the independent opinion of experienced fish farm staff; people whose daily job necessitates evaluation of the health and welfare of fish. While the opinion of such people should not be taken as a definitive measure of fish welfare, the agreement between such opinion and our objectively derived welfare score supports the use of this approach. Additional validation will require the application of the welfare score in other circumstances and comparison with as wide a range of alternative measures of welfare as possible (e.g. growth, food consumption, gill damage).

Since it was impossible to use individually marked fish in working production cages, the measure of growth available was a simple mean weight gain per month at a cage level and therefore it was only possible to compare this with the median welfare score for each cage. Any relationship could have been obscured by the relatively small size of the data set and the large number of factors influencing such a crude measure of growth. The significant association between a similar welfare score and growth observed by Adams et al. (submitted) was derived from individually marked fish under controlled conditions in tanks. It is not surprising that there was no significant association between the welfare score and mortalities in this cage study, since mortalities were low throughout the study and were unlikely to have reflected population level health or welfare problems.

4.2. Predictors of welfare

A number of models were developed to describe the data (not all presented here), the most reliable model including the entire individual fish data and three independent variables all of which were significant predictors of the welfare score. Rigorous diagnostics confirmed the fit of this model to the data. The models based on cage median welfare score were highly sensitive to changes in the individual data points and removal of a single data point was found to change the model, as one would expect with such an unavoidably small sample size (n=27). Distribution of variability within and between cages suggested that the individuals expressed significant independence in the large populations in these production cages (G. Ruxton, pers. comm.). The conclusions from the analysis of the individual fish data were consistent with those from the cage medians but should nevertheless be treated with caution given the risk of pseudoreplication.

From the best fit model using this multivariate welfare score, one important result to emerge from this study is that high stocking densities over the 3 months prior to sampling are associated with reduction in welfare of farmed salmon, but only above a threshold density. Under the conditions prevailing in the present study, the threshold was ca. 22 kg m^{-3}. This outcome was consistent when the breakpoint analysis was carried out on the welfare score of individual fish over the whole study, in individual sampling periods, with the omission of a single cage with particularly poor welfare, in off-shore cages only and also using cage median data. Therefore, it would appear that this is a robust conclusion from the data collected in this study. These results are in partial agreement with the companion study carried out in tanks (Adams et al., submitted), where welfare deteriorated in fish held at densities above 25 kg m^{-3} (at least in the relatively undisturbed tanks).

We found a high level of variability within and between cages at a given density, both above and below the 22 kg m^{-3} breakpoint; clearly, factors other than stocking density were influencing the status of the fish. The present study was designed specifically to identify and characterise the relationship between stocking density and welfare in Atlantic salmon, so different densities were allocated randomly among the available cages. Consequently, the design did not provide the data necessary to fully explore other potential influences. Even so, it was possible to examine the effects on welfare of cage position and time since grading or lice treatment, some environmental conditions and behavioural observations. The location of the cage (near-shore or off-shore) was a significant predictor of welfare, even when adjusted for stocking density and sample period. There is no clear explanation for this, although factors that might be involved are disturbance level, effect of wind or water energy. The time since a stressful husbandry event was not significantly associated with the median cage welfare score when adjusted for stocking density and sample period, but there was relatively little variability in these data and this conclusion should be treated with caution.

Water temperatures and dissolved oxygen measured during the study were within the recommended ranges for Atlantic salmon in sea cages for the vast majority of the time. Therefore impaired welfare at high densities in this study did not appear to be dependent on reduced water quality in any simple way, as suggested by Ellis et al. (2002) for rainbow trout (*Oncorhynchus mykiss*). We elected to manipulate stocking density by using same-sized fish stocked in different numbers in cages of equivalent volume. Stocking density and fish number were therefore tightly correlated and thus we could not tease apart effects of biomass and absolute number of fish. Behavioural observations during this study did not show systematic variation in the frequency of jumping and rolling at the water surface, swimming speed or aggressive interactions. This suggests that the negative effects of high density on welfare were not due to an increased rate of social interactions; the same conclusion was reached by Ellis et al. (2002) in a review of the literature relating to rainbow trout.

4.3. Implications for aquaculture

These results have a number of implications for salmon aquaculture. They confirm other studies showing that the conditions in which farmed fish are cultured do indeed influence their welfare (Wedermeyer, 1997) and demonstrate that, as recognised by the Farm Animal Welfare Council (1996), stocking density has an influence on welfare. However, the considerable variability within cages, within sample periods and within ranges of stocking densities in this study also shows that concentrating on stocking density alone will not allow us to predict or control welfare accurately. A similar conclusion was reached in a large-scale study of hens in commercial battery systems, in which details of housing were more important than stocking density in predicting variation in welfare between rearing units (Dawkins et al., 2004). The variability in the welfare of individuals in the cages studied is not surprising, since variability in health or diseases, often following a negative binomial distribution, is a recognised property of animal populations.

The non-linearity of the relationship between welfare and stocking density suggests that, below a critical point around 22 kg m^{-3}, increasing density did not reduce welfare under the conditions studied on a commercial farm. Therefore, the maximum stocking density of 15 kg m^{-3} recommended by the FAWC (Farm Animal Welfare Council, 1996) based largely on current farming practice at the time is not supported by our findings. Our data do not allow us to suggest an alternative single threshold stocking density that will ensure the welfare of the fish concerned, since stocking density combines with other factors that will vary from time to time and place to place, such as the energy of the site and cage deformation due to water flow. It is clear from this study that good welfare can be maintained at high densities and that conversely low densities are no guarantee of good welfare. Arguably, these results suggest that setting a threshold density is not the most effective way to ensure the welfare of farmed fish. Instead, within a specified broad range of acceptable densities, farmers could be required to ensure that their stock meet a set of welfare criteria. Minimising other stress factors such as lice burden or cage deformation, particularly at higher densities, are equally likely to promote the welfare of farmed Atlantic salmon.

Welfare is a complex multifaceted condition and is reflected in many different aspects of the animal's behaviour and biology. The approach adopted in this study was to measure as many biological indicators of fish welfare as practically possible under commercial production conditions. The resulting welfare score reflected a real coherence in the data and was consistent with growth in a related study (Adams et al., submitted) and the opinion of experienced farm staff in this study.

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