Development of practical 'on-farm' cod welfare indices

SARF021

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EXECUTIVE SUMMARY

The aim of the contract was to identify, develop and appraise potential welfare indices that are practical and meaningful for farmed Atlantic cod, *Gadus morhua.*

The results of crowding trials and post-harvest studies lead us to recommend a welfare standard for pre-handling procedures (grading or harvest) of raising the cage net depth by up to 2 metres/day until the maximum depth prior to handling is equal to or less than 10 metres. Air-lifting or pressure vacuum pumping for harvesting is recommended rather than lift nets, in view of the welfare benefits.

Investigations of welfare indices lead to recommendation of:

- monitoring ventilation depth, using in-cage cameras and a validated scoring scheme,
- assessment of muscle mass, using a condition index,
- monitoring blood lactate using a hand-held lactate meter, when blood samples are available,
- counting ventilation rates and numbers of surface fish during pre-handling grading and harvesting,
- application of a fin erosion key and scoring scheme to identify welfare implications of erosion.

Further information on the development of welfare indicators and the main outcomes is summarised below.

Fin erosion

- We developed a photographic key and instructions for a 4-point fin erosion scale that allows rapid non-invasive assessment of fin erosion in farmed cod. Scoring is potentially subject to errors of judgement, but after appropriate training, substantial agreement can be achieved.
- Use of the scoring system in three separate trials showed low levels of fin erosion. We found no evidence that fin erosion reduced condition or increased the sensitivity to acutely stressful practices such as grading or crowding pre-harvest. Indeed, cod with the highest levels of fin erosion had lower blood lactate and plasma cortisol during crowding. Fin erosion scores were not correlated with blood cortisol, glucose, lactate, haematocrit, splenic:somatic index or hepatosomatic index.
- Industry application of the photographic key and scoring scheme is recommended in order to determine how environmental conditions influence fin erosion, e.g. whether density and food availability affect erosion. This could help identify points where welfare improvements can be made.

Behavioural indices

- A simple method of measuring the time taken for 5 or 10 ventilation beats was employed for cod at the surface, during crowding. The high rates agreed with physiological measurements for the cod and support the use of this rapid index of aerobic status where husbandry procedures allow.
- A categorical scale for ventilation depth was developed. Allocation of scores has a subjective element, and regular retraining was found to be important. For this, a library of categorised video could be employed. Provided appropriate and regular training is in place, reproducible scores can be achieved.
- During routine activity, virtually all cod were in category 0 (shallow breathing) in April, but in July a larger percentage were category 1 (moderate depth), probably due to increased water temperature, and oxygen use. Nevertheless, scoring of ventilation depths, monitored using in-cage camera technology, can provide a welfare index if measurements are made against expected norms, and could be developed for other species.
- Ventilation scoring showed a rapid decrease in the proportion of cod using shallow ventilation during crowding practices. Higher levels of distress occurred in cod in the corner than in cod the centre of the sweep, when the cage was suspended at 14 metres. The benefits of raising the cage prior to pre-handling procedures were shown and informed the recommended welfare standard for pre-handling practices.

Condition indices and muscle mass

- Two body condition indices were assessed. The commonly used condition index assesses whole body weight relative to body length. An alternative, based on body weight after removal of viscera, gave a better index of muscle mass, particularly for mature fish in which gonad adds to body weight.
- Low values for the muscle mass index in female cod (2006 studies), suggests that maturation can have adverse effects. Female cod in surface waters with a high gonadosomatic index (GSI) and low muscle mass index showed poor homeostasis. This supports use of the muscle mass index in monitoring welfare.

Cod in surface waters

- Surface location in cod, when not feeding, can be taken as indicative of poor welfare. We found small numbers at the surface in 2006. Their poor avoidance responses (captured in hand nets) and poor condition were coupled with severe osmoregulatory, endocrine and metabolic disturbances.
- We developed a photographic method to count the percentage of cod at the surface and showing their white underbelly during a series of crowding events, in different conditions. We believe that over-inflation of the swimbladder causes this behaviour and the accompanying disturbed physiology. Surface bound cod are conspicuous, but represented <3% of cod in the cage. The percentage of cod showing their underbelly provides a meaningful non-invasive measure of welfare during grading or harvesting.
- The number at the surface was lowered by raising the cage prior to crowding or by reducing crowd density. We recommend a welfare standard for pre-handling procedures (grading/harvesting) of raising the cage net depth by up to 2 metres/day until the maximum depth prior to handling is less than or equal to 10 metres.

Skin colour and lightness

- Direct observations of skin darkening in cod at the surface (2006) with low muscle mass and disturbed physiology suggests that skin colour can act as a valid welfare indicator.
- Non-subjective measurements of skin lightness and colour were made using a colorimeter. Readings were taken beneath the dorsal fin, and on the ventral surface to avoid the influence of the characteristic patterning of cod. Some trends between skin hue and lightness and fin scores were identified, but there is a high level of unexplained variability in skin lightness and colour, which at this stage precludes use of these parameters as reliable welfare indicators.

On-farm monitoring of blood lactate by meter readings

- We found that the Lactate Pro^{TM} meter (Arkray Inc) is reliable for rapid on-farm measurement of blood lactate in cod, and gives an excellent correlation with laboratory measurements. Application in other species is recommended, once checks of reliability have been undertaken. Alternative meters may be equally reliable, but need assessing on a case-by-case basis.
- A meter reading of 'Lo' (<0.8 mM) will provide a rapid index of health and welfare in relation to gill and cardiovascular function and provision of oxygen for aerobic tissue function. Line-caught cod sampled rapidly, with minimal time on line or muscular activity, consistently gave meter readings of 'Lo'.

Gill colour as a surrogate for haematocrit

- A high haematocrit in cod caught in hand nets (2006) and significant increase in haematocrit in crowded cod, prompted investigation of gill colour, as an on-farm index of blood haematocrit. A chart based on normal variability in gill colour was prepared from Pantone colours.
- Haematocrit and the gill colour score were correlated, but the high variability of haematocrit at any colour score indicates other influences. Furthermore, rapid assessment is essential to avoid colour changes and on-farm monitoring will be affected by light conditions, so we do not recommend this method.

Crowding studies and discrimination of line-caught cod and crowded cod

- Statistical analysis was used to investigate which of the parameters measured allow discrimination of cod caught pre-crowding (line-caught) and the two behaviourally different groups of crowded cod (in the corner and centre of the sweep net). The analysis resulted in correct allocation of 68% to 86% of cod. Blood lactate, haematocrit, and cortisol were important discriminators and condition (muscle mass), skin lightness and colour also helped discrimination, suggesting that these are useful indicators.
- Plasma lactate, cortisol, glucose and blood haematocrit were significantly increased during crowding. Differences in cage depth and crowd density affected the timing of responses and differentiation of cod in the centre and corners of the sweep. The results reinforced our recommendation of low cage depths prior to grading and crowding, to minimise impacts.

Welfare in harvesting

The effects of altering crowding and harvest procedures, by employing air-lifting instead of wet brailing to deliver fish from the crowd to the harvesting table, were investigated. Air-lifting from the corner of the sweep was found to avoid the increase in blood lactate and glucose and reduced the increase in plasma cortisol normally seen during the crowding and brailing procedure. In line with current standard industry practice we recommend that air-lifting or vacuum pumping is routinely used to move fish in preference to lift nets.

CONTENTS

1. CONTRACT OBJECTIVES

The main aim of this project was to identify, appraise and develop welfare indices that are practical, meaningful and specific to commercially farmed Atlantic cod, *Gadus morhua*. Initial contract objectives and amendments after annual monitoring and under novation to Aquatonics Ltd (02/12/08) were:

1. Undertake a replicated pilot study of a current crowding and pre-harvest handling procedure to investigate an initial set of welfare indicators and establish the level of variability in key physiological, environmental and behavioural parameters, so that subsequent experiments take account of these.
2. Organise a preliminary workshop on cod welfare to appraise the findings of the pilot study, to develop an agreed set of welfare indicators for investigation in the later replicated trials and revise the sampling protocol if required.
3. Run a series of replicated trials during commercial harvests that will further investigate and validate the chosen welfare indicators (derived through assessment of the results of the pilot study and the initial workshop), so that they are most valuable to the industry.
4. At the Project Annual Monitoring (Interim Report Form), a change to year 2 studies was agreed with SARF to examine the effects of depth of sweep and position in the sweep net, instead of the series of manipulations initially proposed.
5. Under the novation (02/12/08) it was agreed that Aquatonics Ltd will not be held responsible for prior changes to the project beyond the contractor's control, specifically lack of cage replication in year 2 studies, lack of assessment of eye damage using fluorescein staining, and lack of assessment of burrowing in the sweep net.
6. Produce and present a report of cod welfare indices and recommend pre-harvest handling protocols to the industry.
7. Under the novation, (02/12/08), the original objective to organise a final workshop to disseminate the results of the project to the wider industry and other interested parties was no longer required.

All contract objectives (as modified by the Annual Monitoring and novation) have been met in full. The scope of the studies, results, outcomes and recommendations are outlined below.

2. SCOPE OF STUDIES

The studies were carried out at NoCatch Ltd (formerly Johnson Seafarms), in Vidlin Voe, Shetland, using cod held in suspended sea cages. During the contract a series of potential indices of cod welfare were explored including:

- behavioural indices (swimming rate; ventilation rates; ventilation depth) monitored by video recording, photography and visual observations
- physical indices (fin damage, condition indices, organ mass (liver, gonad, spleen) relative to body mass, skin colour/lightness, gill colour)
- physiological/biochemical indicators of homeostasis, stress or exertion (swimbladder pressure, haematocrit, blood and plasma lactate concentrations, plasma osmolality, plasma concentrations of cortisol, glucose, sodium and chloride).

Over the 2 years of studies in Shetland, four categories of cod were investigated:

- cod captured by rod and line fishing (with minimal time on line)
- cod showing abnormal behaviour (swimming in surface waters) caught in hand nets
- cod crowded using industry standard practices
- cod from which blood samples were obtained immediately post-slaughter

3. PILOT STUDIES (2006) OF BLOOD PARAMETERS TO GUIDE DEVELOPMENT OF OPERATIONAL WELFARE INDICES

In the first year of the studies (2006), four sea cages stocked with cod that had reached harvest size were used. In two of the cages, more than 100 cod were captured by rod and line fishing, over a period of up to 3 hours. Cod in the other two cages were subjected to crowding in a sweep net, using current harvesting practices. An initial group of cod were taken from these cages by rod and line fishing, and further groups of cod were blood sampled at timed intervals during crowding.

A range of blood parameters were measured to assess physiological and biochemical health and well-being (Arlinghaus et al., 2007) and to guide our development of welfare indices. Blood measurements falling outside the normal homeostatic range could be indicative of poor welfare, particularly if coupled with physical damage or behavioural changes. However, the occurrence of physiological responses to stress is not necessarily equivalent to suffering and responses may be beneficial, at least in the short term (Huntingford et al., 2006). Nevertheless, repeated exposure to acute stressors or prolonged exposure to poor conditions has adverse effects (Wendelaar Bonga, 1997; Pickering, 1998) and monitoring multiple components of stress responses has given valuable insights into the welfare of various species of fish (Turnbull et al., 2005; Adams et al., 2007). For example, fish with low plasma cortisol, glucose and lactate and normal plasma osmolality, as well as adequate growth and condition index are usually considered to be in good health and experiencing good welfare (Adams et al., 1993; Turnbull et al., 2005).

To obtain meaningful data for blood parameters, it was critical to ensure that the cod were landed rapidly and immediately blood sampled, because an increasing duration on line causes physiological disturbances (Cooke et al., 2008). Capture of cod from a vessel moored alongside the cage minimised the time on the line and muscular activity before percussion killing, which was followed immediately by rapid blood sampling (within 1.5 min from hooking).

The results for plasma lactate showed that our procedures avoided the notorious problem of elevation of blood/plasma lactate seen after line capture in fish that are not immediately landed (Gustaveson et al., 1991; Pottinger, 1998; Cooke et al., 2008). In our studies, the mean plasma lactate concentrations of cod caught by rod and line (0.44 mM and 0.35 mM in the two cages studied) agrees with published values for routinely-active or resting cod, monitored in the laboratory, where capture is more straightforward (Nelson et al., 1996; Herbert and Steffensen, 2005; Johansen et al., 2006). Similarly, blood haematocrit and plasma concentrations of cortisol, glucose, sodium and chloride were all realistic compared to those previously published for undisturbed cod held in tanks (Staurnes et al., 1994; Olsen et al., 2008; Herbert and Steffensen, 2005), which demonstrates the validity of our methods.

Routine farm activities, our presence and sampling of cod, and natural diurnal cycles over the timescale of our studies had little or no effect on the measured blood parameters (Brown et al., 2009). This leads to the conclusion that rod and line capture of cod held in commercial sea cages can provide meaningful measures of physiological well-being for a caged population, provided that time on the line is minimized and muscular activity after capture is avoided.

Examination of the physiological responses to crowding (Brown et al., 2009) was used to select those blood parameters that could be most informative in further studies and guide the development of welfare indices. For example, only slight changes in plasma osmolality and sodium occurred, and there was no change in plasma chloride during crowding, so we discontinued monitoring the osmoregulatory parameters. In contrast, there was a clear increase in plasma cortisol, demonstrating physiological disturbance during the sweeping and crowding (Figure 1). Blood glucose also gave useful information and was retained in later studies. The variability in these blood parameters and statistical power analysis was used to inform the design of later studies.

Figure 1 Plasma cortisol concentrations before and during crowding of cod

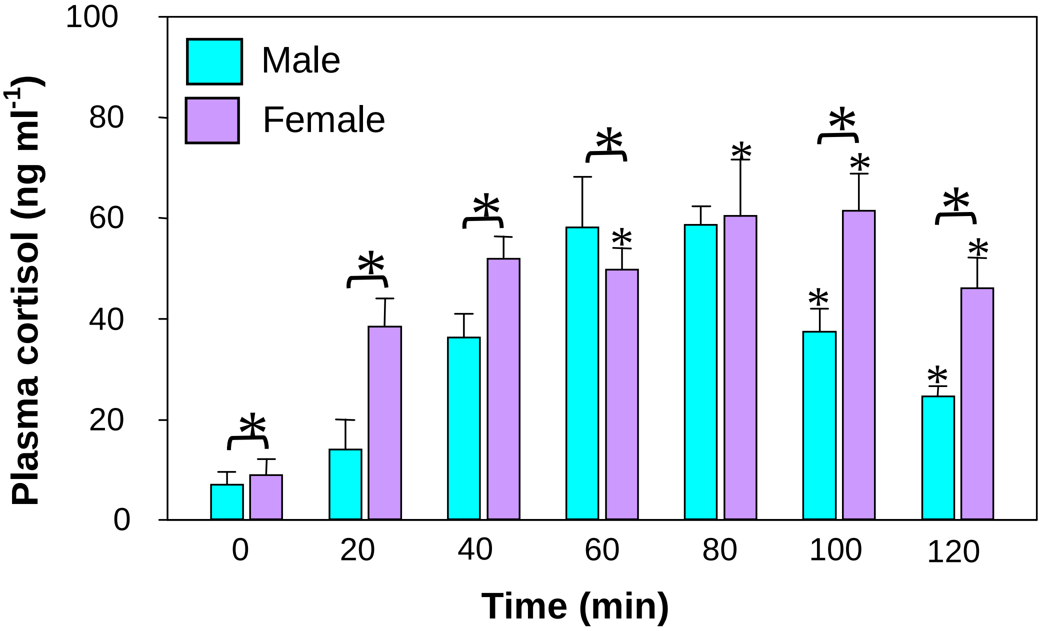


Fig 1. Data are mean + SE at each time point. Large asterisks show where values in male and female cod differed significantly; small asterisks show groups that differ significantly from pre-crowding control values; 3-way ANOVA and *post-hoc* multiple comparisons.

Blood lactate rapidly increased in cod crowded in the sweep net (Brown et al., 2008), and indicated increased muscular exertion, probably due to the adverse implications of rapid swimbladder inflation during sweeping cod into surface waters. Cod with an over-inflated swimbladder face the choice of tolerating the increased buoyancy, or struggling against it, and the latter could compromise their aerobic scope (Lapointe et al., 2006) and would account for the increase in blood lactate during crowding. In later studies, we assessed the possible use of a tonometer (TonoVet® Tiolat Oy, Helsinki, Finland) to measure swimbladder pressure (section 9).

The idea that crowding in a sweep net resulted in over-inflation of the swimbladder was supported by the presence of cod with fins out of water, particularly in the corner of the net, while those in the centre of the sweep net were more mobile. The welfare of the corner fish was of concern to everyone involved in the project, including RSPCA staff present during these studies. We hypothesised that the responses to crowding of corner fish differ from those in the centre of a sweep, and that these differences may reflect differences prior to crowding. These hypotheses were explored in 2007. We also assessed the effects of cage depth and the potential benefits of a reduced cage depth prior to crowding/harvesting (sections 6.2, 6.3 & 12).

Duplicate cages were set up for crowding trials in 2007, but unfortunately, due to unforeseen technical difficulties, one cage was lost from the study, so cage duplication was not feasible. In April 2007, the cage was held at 8 metres for an initial trial and then lowered to 14 metres and re-swept, two weeks later. In July 2007, at a reduced density, the cage was again swept from 14 metres. On each occasion, cod were initially captured by rod and line to provide a control group and then cod were sampled at 30 min intervals from the centre and corner of the sweep net.

4. CONDITION, MUSCLE MASS AND MATURATION

Condition index (K), calculated using the standard formula: K = 100 x [whole body mass (g)/length (cm)^3] has been suggested as a measure of energy reserves (Lambert and Dutil, 1997; Lloret and Rätz, 2000). However, maturation increases gonad mass relative to body mass, increasing the gonadosomatic index (GSI), and will increase whole body mass and apparent condition, but muscle mass relative to body mass may actually decline. An alternative condition index, $K_{no\ viscera}$, calculated using body mass after removal of the viscera (Rideout et al., 2006), offers a better measure of muscle mass and may therefore be considered as a more useful welfare indicator in farmed cod.

In the 2006 trials (June), values for $K_{no\ viscera}$ were significantly lower in female cod than male cod, suggesting that maturation has adverse effects on muscle mass of female cod. Furthermore, a small number of cod that were located in surface waters and easily caught in hand nets had low values for $K_{no\ viscera}$ and showed poor physiological homeostasis (see section 6.3). These cod were mainly female, with a higher gonadosomatic index (GSI) than line-caught female cod from the same cage (mean $\pm$ SE: net-caught cod; 18.53 $\pm$ 2.31 (n=8); rod and line cod: 10.72 $\pm$ 2.33 (14); P = 0.04, Student's t test). These data support the use of $K_{no\ viscera}$, a measure of muscle mass relative to size, as a useful indicator of cod welfare.

Several elements of data obtained in 2007 suggested that maturation has adverse chronic metabolic effects on farmed cod. Plasma glucose of line-caught cod significantly declined as GSI increased (General linear model (glm) mixed model, trial as random effect: χ_1^2, 5.28, P= 0.02), but female cod had higher plasma glucose than male cod (χ_1^2, 5.57, P= 0.02) suggesting higher levels of stress. A similar effect was also apparent in data from crowded cod. Although plasma glucose was increased during crowding, there was relatively lower plasma glucose as GSI increased (χ_1^2, 9.77, P = 0.002) and females had higher glucose (χ_1^2, 5.57, P = 0.03). The haematocrit response to crowding was also reduced by maturation and a high GSI (χ_1^2, 9.67, P = 0.002), but without any effect of gender. However, plasma cortisol responses to crowding were not affected by GSI.

5. FIN DAMAGE

Confinement of fish in aquaculture can lead to aggression and physical damage from biting causing fin damage (Moutou et al., 1998; Turnbull et al., 1998; Hoyle et al., 2007), and levels of fin damage have been used as an indirect measure of aggression in salmonids (Moutou et al., 1998; MacLean et al., 2000). There has been much less work on cod, but recent work has identified the potential importance of fin damage in juvenile cod, particularly when food is restricted (Hatlen et al., 2006). These researchers reported a correlation between bite incidence on fins and weight loss, but found only sporadic evidence of fin damage in 450 g cod. However, their experiments used juvenile cod, held in tanks for relatively short periods, which has limited relevance to commercial rearing conditions. Damage during handling in husbandry procedures such as vaccination could add to any fin damage, but has not been assessed in cod. Our aim was to develop a qualitative measure of fin damage for application as a potential welfare indicator in cod reared in sea cages.

In 2006, all signs of acute and chronic damage to fins, skin or eyes were systematically recorded for more than 500 cod of 1.34 to 7.62 kg, using a pre-agreed set of criteria. Damage to the eyes and skin was infrequent and was not aggravated by crowding for grading/harvest, and was not investigated any further in later studies. The initial studies of fin damage revealed difficulties in reliably distinguishing acute and chronic damage, which prevented detailed analysis of the data. However, a valuable library of photographs had been collected, and these were used to devise a simplified scoring system for percentage area of fin erosion, based on a system used in trout (Hoyle et al., 2007). Initially, 5 categories of damage were described, from insignificant to severe, but a team exercise to test this categorical scale on a further 243 cod led to reduction of the categories to a 4-point scale, scored 0 to 3, as shown in Table 1.

Table 1 Scale for assessment of fin erosion in cod

Score	% Fin erosion	Description
0	0-5%	insignificant fin erosion
1	6-20%	minor/moderate fin erosion
2	21-50%	significant fin erosion
3	>51%	severe fin erosion

The reliability of fin scoring was assessed in two exercises in which independent operators assessed fin erosion prior to any training (30 fins) and after training (40 fins). Each operator made three assessments for each fin, in randomised order. The kappa statistic, which is based on the difference between observed agreement and that expected by chance was calculated for both intra- and inter-observer agreement. Table 2 shows a suggested qualitative scale of agreement (Landis and Koch, 1977), although it has been noted that the number of categories and subjects affects the magnitude of the value, and that the kappa statistic is higher when there are fewer categories.

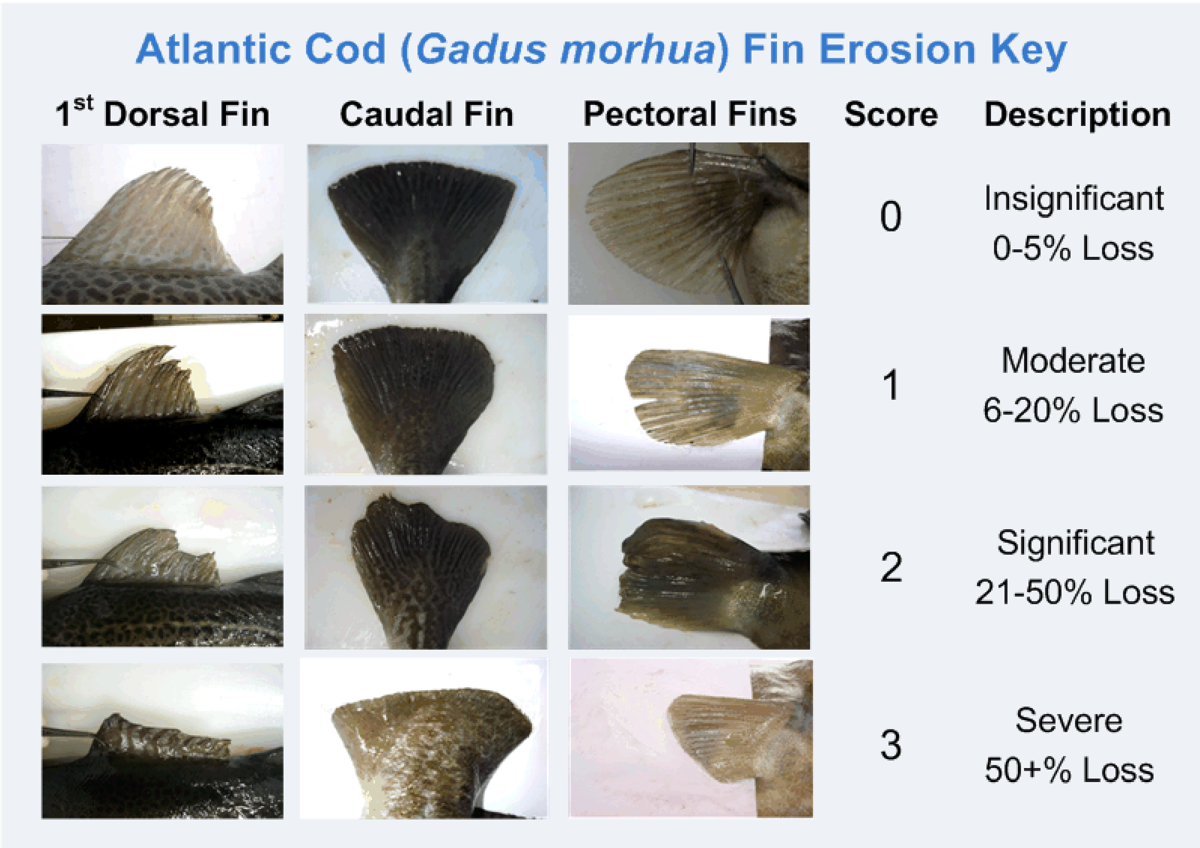
Table 2 Description of strength of agreement for kappa statistic

Kappa statistic	Strength of agreement
<0.0	Poor
0.0 – 0.20	Slight
0.21 – 0.40	Fair
0.41 – 0.60	Moderate
0.61 – 0.8	Substantial
0.81 – 1.00	Almost perfect

Initially, scores varied between observers and discrepancies were discussed. This training and discussion improved agreement. A post-training test exercise showed ‘substantial agreement’ between observers (kappa statistic = 0.695), and substantial to almost perfect intra-observer agreement (kappa statistics = 0.754 and 0.813).

Figure 2 shows the photographic key developed during the contract. This key allows rapid non-invasive assessment of fin damage in farmed cod. In rainbow trout, wild fish had to be employed to identify fins in pristine condition (Hoyle et al., 2007), as these are rare on trout farms. In cod, however, assessment of fin erosion in 2007 for 222 fish, showed insignificant fin erosion in 7.7% of cod. Furthermore, a small sample of wild cod (n = 13) from the fish market (Lerwick) showed a high level of apparent fin erosion, particularly in the caudal fin and right and left pectoral fins, although the possibility of damage during trawling and post-capture handling cannot be excluded. Those few wild specimens with pristine fins, had more elongate and sail-like fins, presumably because of the different environmental conditions experienced and the greater age of the wild cod examined, which were 4 to 8 kg in weight.

Figure 2 Photographic key for scoring fin erosion in Atlantic cod



The index of fin erosion in farmed cod allows regular monitoring whenever husbandry procedures provide the opportunity for close examination, such as during stocking, grading, vaccination, or at slaughter, and hence could enable identification of particular points in the production cycle where welfare improvements can be made. Assessment of fin erosion scores for groups of cod in individual cages could allow differences between hatchery origins or farm site locations to be identified and erosion to be monitored over time. Application of the erosion key will also allow assessment of how environmental conditions influence fin erosion and, for example, whether cod densities and food availability affect aggressive interactions that may cause fin erosion.

In 2007, the fin scoring system was employed in three separate trials. Many cod showed insignificant damage (score 0) or minimal erosion (score 1) to particular fins, but the 1st dorsal fin typically showed some erosion, with scores often 1 to 3. There was a significant correlation of erosion between fins: 1st and 2nd dorsal fins (χ^2_1 = 20.45, P <0.001), 1st dorsal fin and right pectoral fin (χ^2_1 = 4.36, P <0.037), 1st dorsal fin and left pectoral fin (χ^2_1 = 7.48, P <0.006), 2nd dorsal fin and caudal fin (χ^2_1 = 30.57, P <0.001) and 2nd and 3rd dorsal fin (χ^2_1 = 50.13, P <0.001).

We added scores for the 8 fins, which gave total scores of between 0 and 15. Overall, the amount of fin erosion seen in the farmed cod examined was relatively slight compared to that described in rainbow trout, where more than 70% loss can occur (Hoyle et al., 2007), and this raised the question: does fin erosion in farmed cod affect their welfare?

We examined this question by investigating the correlates of fin damage in line-caught cod, using generalised multiple mixed model regressions of total fin score and 1st dorsal fin scores against other measured parameters (trial date as a random effect). The statistical analyses showed that the low levels of fin erosion had no deleterious impact on condition. Indeed there was a significant positive relationship between total fin score and $K_{no\ viscera}$ (χ_1^2 = 8.93, P = 0.0028). This suggests that fish with the highest muscle mass have the highest levels of fin erosion. However, total fin score and $K_{no\ viscera}$ in the larger sample of cod obtained after crowding, showed no significance relationship (χ_1^2 = 0.44, P = 0.51), so we must remain cautious in interpretations of links between fin erosion and muscle mass.

5.1 FIN EROSION, BLOOD PARAMETERS AND ORGAN:SOMATIC INDICES

Once the key for assessing fin erosion was developed, we were able to investigate whether cod with high levels of fin damage were physiologically compromised. Our statistical analyses showed that the total fin damage of 27 line-caught cod sampled in the three 2007 trials was not significantly correlated with plasma concentrations of cortisol, glucose, or lactate, blood haematocrit, splenic:somatic index or hepatosomatic index. This suggests little impact of fin damage on homeostasis. However, plasma cortisol and plasma lactate were significant negative correlates of the scores to the 1st dorsal fin, which usually showed the highest levels of erosion (χ_1^2 = 10.276, P = 0.001 and χ_1^2 = 4.469, P = 0.035 respectively). These negative correlations indicate that cod with the highest levels of fin damage and higher $K_{no\ viscera}$ have lower blood lactate and lower plasma cortisol. Again, this paradoxically implies that fish with highest levels of fin damage show lower levels of stress and physiological disturbance.

5.2 FIN EROSION AND RESPONSES TO CROWDING

In pilot studies, during the normal harvesting and grading processes, crowded cod increased their haematocrit and plasma concentrations of cortisol, lactate and glucose compared to values in line-caught cod (Brown et al., 2009). During the second year of the project, we were able to assess whether these responses were increased in cod with higher levels of fin damage, i.e. whether fin erosion increased the sensitivity to stressful husbandry practices. Statistical analysis showed no significant correlation between plasma cortisol and total fin score or 1st dorsal fin score during crowding (glm mixed models, trials as random effect: (χ_1^2 = 1.64, P = 0.22; χ_1^2 = 0.55, P = 0.46 respectively). Similarly, there was no significant correlation between total fin scores and blood haematocrit or plasma lactate and plasma glucose (P = 0.819, P = 0.504, P = 0.206 respectively). Therefore, we found no evidence that fin damage exacerbates the effects of acutely stressful practices.

6. BEHAVIOURAL INDICES

In the pilot studies, continuous surface and sub-surface video recording was used to explore cod behaviour and investigate unusual behaviour, such as surface breaking by fins (during crowding for harvesting), swimming behaviour, and respiratory frequency and ventilation depth as potential welfare indices. Attempts were made to monitor swimming activity (body lengths min^{-1}) from sub-surface video recordings, but differences in the direction of movement made the method unreliable.

6.1 VENTILATION FREQUENCY

Monitoring ventilation frequency in individual cod by remote video recording proved impossible for commercial cages, and was not pursued in further studies. However, timing of 7 to 10 ventilatory beats in cod resting at the surface, during crowding, was achieved. The number of trials and measurements were limited, but showed that 26 of 27 cod monitored had ventilation rates above the normal reported range (16 to 21 breaths per min^{-1}) for resting cod held in normoxic conditions (Claireaux & Dutil, 1992; Nelson et al., 1996). Ventilation rates typically rise to 28 breaths min^{-1} during exercise, reaching up to 38 breaths min^{-1} during exhaustive exertion (Nelson et al., 1996). We monitored a mean ventilation rate of 28.4 $\pm$ 1.5 (n = 9) after 70 min of crowding and 30.7 $\pm$ 0.93 (n = 11) after 104 min crowding when sweeping a cage suspended at 8 metres, and 31.66 $\pm$ 1.9 (n = 7) after 90 min in the crowd for a sweep from 14 metres depth. There was no evidence for significant differences in ventilation rates after 70 min crowding or between cages swept from 8 metres and 14 metres (ANOVA: 1.35, P = 0.279). Nevertheless, the increased ventilation rate compared to resting levels indicates that in some husbandry procedures, measurement of the time taken for 10 ventilation beats could give a rapid and non-invasive index of aerobic status, and has potential use as a simple index of welfare.

6.2 VENTILATION DEPTH

Ventilation volume of fish is regulated according to environmental conditions (Holeton & Randall, 1967) and can be visualised as more pronounced opercular movement. Our initial video footage of cod (pilot studies) suggested differences in ventilation depth of routinely-active cod and crowded cod. We suspected that the increase in ventilation depth could occur because of an increase in buoyancy, resulting from swimbladder inflation, causing increased exertion, and because of the increase in fish density in the crowd. Initially, sub-surface recording was made difficult by fish coming too close to the lens. However, the available video footage was sufficient to allow development of a recognisable categorical scale of ventilation depth (Table 3). For later studies, a cowling was built around a VideoRay® Pro3E ROV to maintain at least 30 cm between the fish and the lens.

Table 3 Categorical scale of ventilation depth

Score	Description
0	shallow breathing = normal
1	moderate depth of ventilation
2	deep/laboured ventilation

Later studies scored ventilation depths of routinely-active cod from video recording collected for a 20 min period before crowding and at 30 min intervals during crowding in a sweep net. Before crowding, we were only able to score a small number of cod captured on video in the 20 min allowed, but this would be less of a problem if applied during routine on-farm monitoring of ventilation depth.

In developing the method for ventilation scoring, we assessed the level of agreement between observers or by individual observers. To do this, two observers independently categorised ventilation depth, three times (after randomisation of the footage). A kappa statistic (Landis & Koch, 1977) was calculated for inter-observer and intra-observer agreement for 3 sequential independent assessments by each observer of 40 cod pre-numbered on video footage. The observers showed substantial agreement for the first two runs through the video footage (kappa statistic = 0.73, 0.67), and moderate agreement for the third assessment (kappa statistic = 0.54). There was almost perfect intra-observer agreement (kappa statistics observer 1 = 0.898, 0.948, 0.851; observer 2 = 0.691, 0.697, 0.634). These results demonstrated the reliability of the method. However, the allocation of a ventilation score has a subjective element and during development of the technique, it became clear that regular 'retraining' is important to achieve reliable categorising of ventilation depths. For this, a library of categorised video could be employed.

We employed ventilation scoring from video records in the three 2007 trials. Figure 3 shows the distribution of ventilation scores in a cage that was initially suspended at 8 metres (April 2007), then lowered to 14 metres for further study two weeks later and finally studied again in July, at 14 metre depth when there were fewer cod in the cage and a reduced crowd density. Accurate densities are difficult to measure, because of the difficulties in estimating the volume of the sweep net, but we estimated densities of ~220 kg m^{-3} in July compared to ~480 kg m^{-3} in April). During crowding, there was a rapid immediate decrease in the proportion of cod showing shallow ventilation (Figure 3). Statistical analysis of the ventilation depth scores for the separate trials, showed no evidence of different patterns of ventilatory behaviour through time, of cod swimming in the centre of the sweep and cod in the corner of the sweep ($\chi^2_8 = 10.7687$, $P = 0.2151$, $\chi^2_8 = 4.0962$, $P = 0.8483$, $\chi^2_8 = 5.5082$, $P = 0.7021$ in the three trials respectively). However, at fixed time points there were significant differences in the percentage of cod in each category of ventilation depth at the two locations (generalised linear models of replicated data at fixed time points, ignoring controls). Results showed:

(i) Fewer corner cod than cod in the sweep centre with score 0 in the 8 metre trial ($\chi^2_1 = 29.2$, $P < 0.001$).

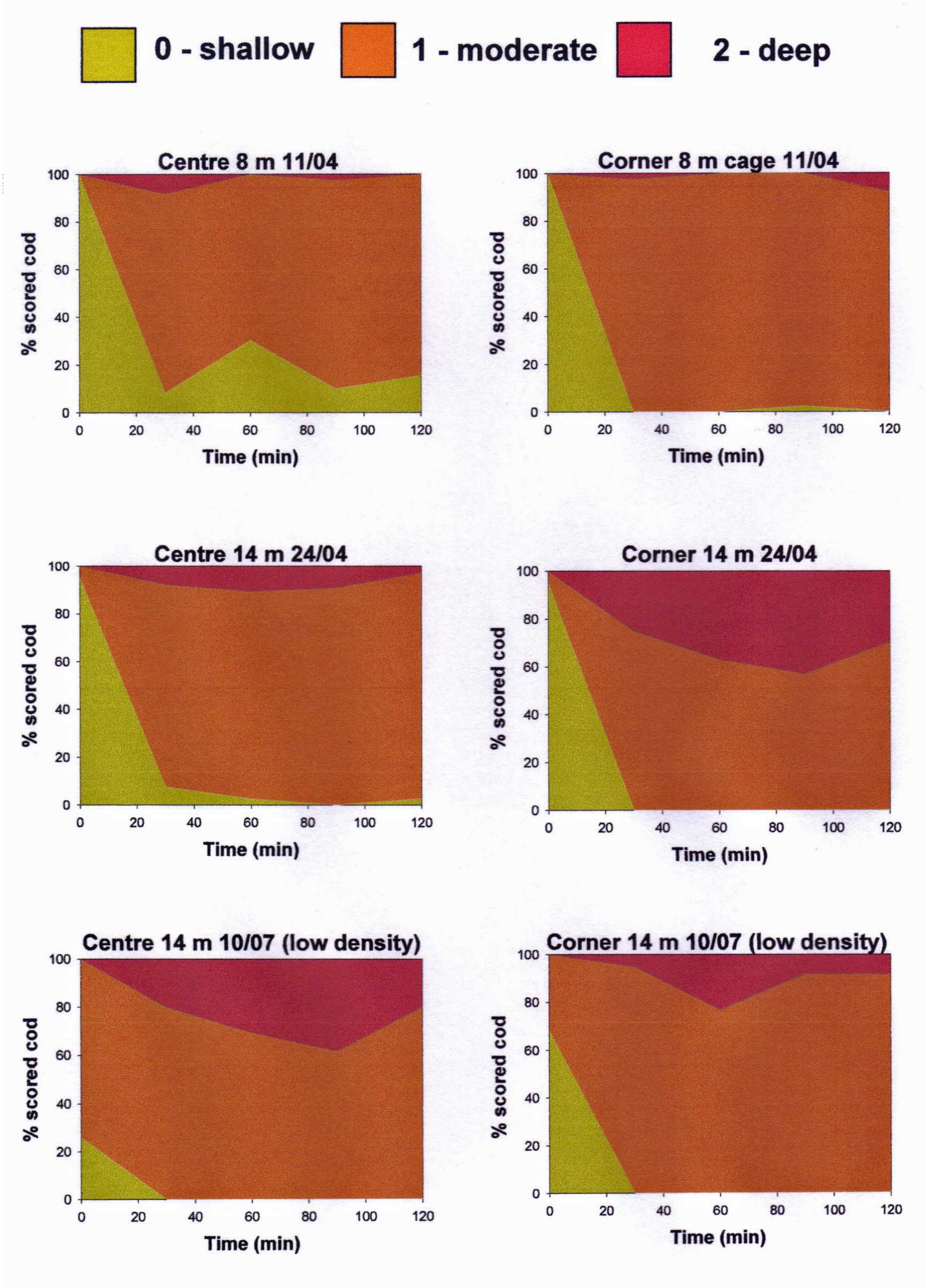
(ii) More corner cod than cod in the sweep centre with score 2 in the 14 metre April trial ($\chi^2_1 = 26.1$, $P < 0.001$).

(iii) Fewer corner fish compared to cod in the sweep centre with score 2 in the 14 metre July trial ($\chi^2_1 = 19.2$, $P < 0.001$).

These results suggest a higher level of aerobic stress in cod in the corner than those swimming in centre of the sweep. These behavioural indices are backed up by physiological measurements (see section 12), which further validate the behavioural methodology. In the third trial (July), corner fish were less affected by crowding at a lower density and again this agrees with data for the measured physiological parameters.

Our results show the viability of using ventilation depth as an index of aerobic welfare in cod. However, seasonal influences need to be considered. During routine activity prior to crowding in the two April trials, virtually all cod were in category 0 (shallow breathing), but in July a large proportion were category 1 (moderate), probably reflecting seasonal increases in water temperature and effects on oxygen availability and use. This complicates the routine application of ventilation depth as a welfare indicator. Nevertheless, scoring of ventilation depths monitored remotely using drop-down videos could be usefully employed to monitor on-farm welfare, provided measurements are made against expected norms.

Figure 3 Distribution of ventilation depth scores pre- and during crowding



6.3 COD IN SURFACE WATERS

Cod held in on-growing cages are not usually present at the surface (Rillahan et al., 2009), but small numbers were located here during 2006 and warranted closer inspection. Their location and poor avoidance responses (allowing capture in hand nets) proved to be indicators of poor welfare. These cod had low $K_{no\ viscera}$ (muscle mass) and showed severe osmoregulatory, endocrine and metabolic disturbances (Table 4; Brown et al., 2009). Their dark colouration suggested that skin colour might be a valid welfare indicator (see section 7).

Cod at the surface were also identified during the crowding studies. We made the prediction that a rapid rise from depth could lead to over-inflation of the swimbladder, and cause difficulties in normal swimming, particularly as the density of fish in the crowd increased. One way of examining such difficulties was to monitor the number of cod with fins out of water or belly visible (due to positive buoyancy). To allow counting of the proportion of cod in the cage that were at the surface, a rapid sequence of photographs was taken at 30 min intervals during crowding. Each photograph was taken from the same position, at the corner of the sweep, looking along the float line, to include the whole surface of the sweep (see Figures 4A & 4B). Most of the cod floating at the surface were in the corners of the sweep.

Table 4 Blood parameters of line-caught cod from two cages and cod caught at the surface in a hand net

Parameter	Line-caught			Surface caught Hand Net	
	Cage A	***P*** Cage A & B	**Cage B**	**Cage A**	***P*** line vs netted
Plasma Cortisol (ng/ml)	8.13 ± 3.43	NS (0.096)	6.82 ± 0.86	88.54 ± 20.61	<0.001
Plasma Glucose (mg/100ml)	53.40 ± 1.81	0.047	48.23 ± 1.77	69.73 ± 12.43	NS (0.118)
Plasma Lactate (mM)	0.44 ± 0.035	NS (0.053)	0.35 ± 0.022	2.59 ± 0.52	<0.001
Haematocrit (%)	20.11 ± 0.36	0.001	22.09 ± 0.49	28.69 ± 1.39	<0.001
Osmolality (mOsm kg^{-1})	338.55 ± 0.73	0.002	343.08 ± 1.25	383.27 ± 13.03	<0.001
Plasma Sodium (mM)	169.07 ± 1.17	0.01	165.09 + 0.90	181.26 ± 2.01	<0.001
Plasma Chloride (mM)	147.32 ± 0.54	0.046	145.74 ± 0.55	156.56 ± 3.11	<0.001

Data are means ± SE; line-caught from Cage A: *n* =54, line-caught from Cage B: *n* =44, Hand net: Cage A: *n* =9. *P* values (Student's t test or Mann Whitney Rank Sum test) comparing data between Cage A and B for line-caught cod, and line-caught and netted fish in Cage A. NS: not significantly different

To make counts, three photographs at each time point were selected and given a coded label. Three assessors then made counts of surface bound cod on each photograph, marking the belly of each counted cod on the photo. Figure 5 shows the mean percentage count of cod from the cage at each time point.

There were no cod at the surface prior to crowding. Gathering the fish with a sweep net from a 14 metre deep net, with cod at an initial density of <15 kg m^{-3}, and crowding them to a density of 480 kg m^{-3} in April, increased the number of cod with visible underbellies at the surface (Figure 5). More than 2% of cod in the cage were at the surface within 30 min. Figure 5 compares this response to that seen when the cage was suspended at the same depth in July, at a crowd density of less than half that in April. Although seasonal effects cannot be excluded, it seems probable that the reduced density had a beneficial effect: less than 0.15% of the cod in the cage became surface bound.

Figure 4A Example of photograph used to count surface cod with visible white belly

Our counts of cod at the surface demonstrated the beneficial effects of reducing cage depth. Figure 5 allows comparison of the number of cod with a visible white belly in cages initially suspended at 8 m and 14 m depth, at a similar crowd density (~ 480 kg m^{-3}). At the lower depth, the percentage of the cod in the cage that were at the surface and showing their underbelly was significantly reduced ($P < 0.001$).

Although these results are based on single trials in each set of conditions, they showed that assessment of the number of surface cod is feasible and could provide a meaningful non-invasive measure of welfare during crowding. The cod at the surface are conspicuous, as shown by the photographs in Figures 4A & B, but represented at maximum 2.74% of the cod in the cage. Their appearance suggests fatigue, which may be triggered by an exceedance of a physiological threshold, but there is evidence in some species that fatigue represents a behavioural decision (Peake and Farrell, 2006). The decision may act as a protective mechanism to avoid excessive activity or activity that is energetically inefficient. In this case, we suspect that the cod had inflated swimbladders, causing swimming difficulties, particularly at high densities. Once the sweep net was released the cod rapidly righted themselves. Almost all swam away within 1.5 minutes, and all within 5 minutes. Numbers at the surface were improved by reducing the depth of the cage prior to crowding or by reducing the density in the crowd. We therefore suggest a welfare standard for pre-handling procedures (crowding for grading or harvest) of raising the cage net depth by no more than 2 metres per day until the maximum net depth prior to handling is no greater than 10 m.

Figure 4B Example of photograph used for counts of cod at the surface with visible white bellies

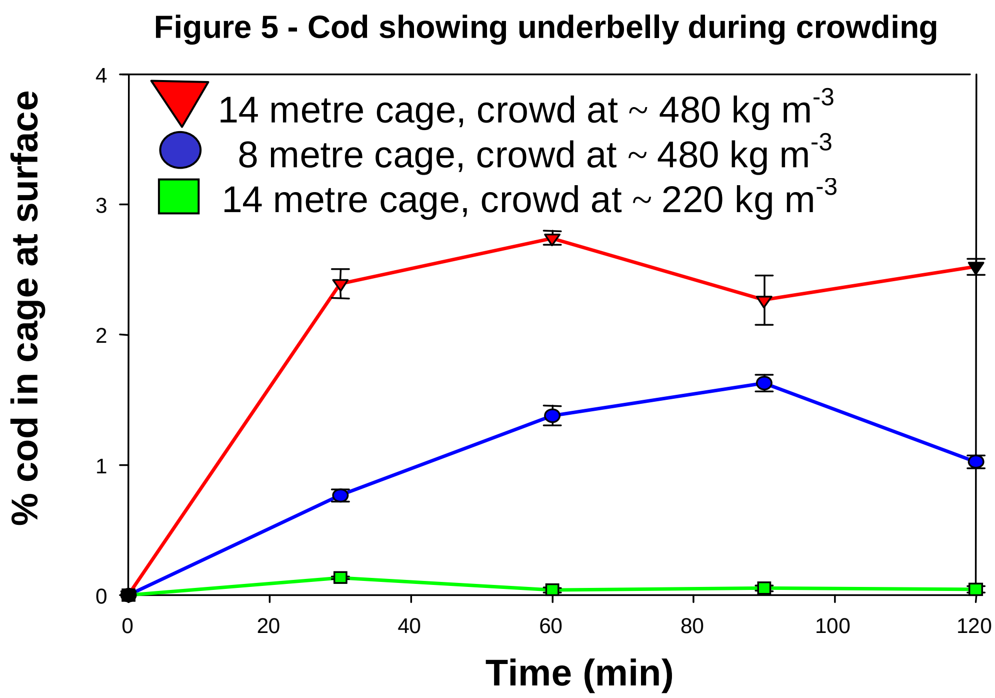


Fig 5. Data are mean % counts (n = 3 photographs, $\pm$ SE) resulting from counting white underbellies of cod at the surface, by 3 observers. ANOVA on Ranks showed significant differences (H = 31.15, P <0.001). *Post hoc* Tukey tests identified significant differences between each pair (q: 3.946, 3.946 and 7.891, P <0.001 in each case).

7 SKIN COLOUR

In 2006, the cod caught at the surface of the cage in a hand net were noticeably darker than other cod in the cage. These cod were subsequently found to be in poor condition (low $K_{no\ viscera}$). Although background colour is a major factor in determining skin colour (Doolan et al., 2007), other factors such as density, diet and stress have also been reported to affect skin colouration of farmed fish (van der Salm, 2004; Doolan et al., 2008). Therefore, skin colour or skin lightness/darkness could act as practical welfare indices.

In the 2007 trials, we measured skin lightness/darkness using a colorimeter (Minolta Chroma Meter: CR- 410; measurement area 63 mm) to avoid subjective errors. The colorimeter directs a pulse of light at an object and measures reflected light to calculate chromaticity and lightness on the international colour scale (Commission Internationale L'Eclairage, CIE) of Lightness (L*), red-green (a*) and yellow-blue (b*). An average of three readings were obtained for each fish. In the first trial, we took readings immediately beneath the dorsal fin, but in later trials, readings were also taken on the ventral surface, between the pelvic fins, where the characteristic patterning of cod cannot influence readings. From the readings, hue and chroma (colour intensity) were calculated using the following equations: chroma = $\sqrt{(a^{*2} + b^{*2})}$; hue (in degrees) = arctan (b*/a*) for positive values of a* and b* and appropriate adjustments when a* or b* <0 (Robb, 2001; Stein et al 2005).

In line-caught cod, dorsal skin hue and lightness were identified as correlates of total fin damage (P = 0.0018 and 0.001 respectively: glm mixed model regressions of total fin damage against all other parameters, with trial as a random effect), which suggests that dorsal skin hue or lightness may be useful welfare indicators when stocks are closely inspected. However there was a high degree of variability in the readings. Dorsal lightness/darkness of salmonid species signals social status (Hoglund et al., 2000) and this could complicate interpretation if it also occurs in cod. Social stress increases skin darkness in salmonid species and triggers hormonal responses, including an elevation in blood cortisol (Hoglund et al., 2000). We found no evidence of a correlation between plasma cortisol of line-caught cod and their dorsal or ventral lightness or hue and chroma readings (*P* values 0.11 to 0.95), so the variability in readings remains unexplained, and further work is required before considering use of lightness or colour as welfare indicators.

8. ON-FARM USE OF LACTATE PRO™ METER

When oxygen availability for tissue function is compromised, glycolysis gives rise to increased lactate production as a by-product of anaerobic carbohydrate metabolism in white muscle cells. The muscle lactate enters the blood, so measurements of blood lactate are commonly used to assess the impacts of aquacultural procedures such as crowding and pre-harvest handling, or the metabolic consequences of exhaustive swimming and fatigue (Gustaverson et al 1991; Nelson et al., 1996; Hopkins and Cech, 1992; Johansen et al., 2006; Peake and Farrell, 2006). Therefore, the measurement of blood or plasma lactate has the potential to be used as an index of physiological and behavioural welfare, and can provide valuable insights into the aerobic status of fish in aquaculture, where both oxygen availability and use may vary. Lactate meters offer the possibility of rapid on-farm results without the need for costly and delayed laboratory analysis. We employed the Lactate Pro™ meter (Arkray Inc) in measuring blood lactate of cod. This meter gives a reading of 'Lo', when blood lactate is less than 0.8 mM.

Meter readings of 'Lo' were obtained for all cod caught by rod and line. This low blood lactate was confirmed by laboratory assays, for which the mean (± SE) blood lactate of 34 line-caught cod was 0.45 ± 0.22 mM. This leads to the conclusion that a meter reading of 'Lo' can provide a rapid index of health and welfare in relation to gill and cardiovascular function and the provision of sufficient oxygen for aerobic tissue function.

Where it is necessary for practical reasons to delay reading of blood lactate, collection of blood into tubes containing an anticoagulant and antiglycolytic mix (sodium fluoride and oxalate) ensures stability of blood lactate readings, for at least 8 hours (Brown et al., 2008), which increases the practical use of blood lactate as an operational welfare index.

Pilot studies suggested that sweeping and crowding brings up fish from their preferred position towards the bottom of the net. When brought up into surface waters, the swimbladder inflates and increases buoyancy, so that more energy is used in swimming, leading to a potential oxygen shortage and use of anaerobic pathways generating muscle lactate. The diffusion of muscle lactate into the blood means that blood lactate is an indicator that can be employed in conditions such as crowding or where there is disturbance or increased exertion. In these conditions, meter readings for whole blood lactate showed an excellent correlation with laboratory measurements of plasma lactate (R^2 = 0.94) or whole blood lactate (R^2 = 0.93). These data showed the reliability of inexpensive meters, such as the Lactate Pro, for on-farm application to assess the aerobic status of farmed cod. Alternative meters may be equally reliable, but need assessing on a case-by-case basis.

Lactate meter measurements can provide an index of aerobic welfare that is relevant during any farm procedure that affects physical activity, and provides information on welfare in a more general sense. Table 4 shows that cod caught in surface waters by hand netting had elevated lactate concentrations, as well as other indications of poor physiological homeostasis.

9. SWIMBLADDER PRESSURE

The possibility of measuring swimbladder pressure, as a welfare index in cod, was investigated using a rebound tonometer (TonoVet® Tiolat Oy, Helsinki, Finland) that is marketed as a veterinary tool for measuring pressure in the eye. In practise, it proved difficult to obtain consistent readings. The tonometer gave variable results depending on where the probe hit the bladder and so measurements were discontinued in the third crowding trial.

10. GILL COLOUR AS A POTENTIAL SURROGATE FOR HAEMATOCRIT

Our pilot studies showed that haematocrit is one of the physiological indicators allowing recognition of welfare and well-being (Arlinghaus et al., 2007), but measurements require careful handling of capillary tubes and centrifugation which is unlikely to be easy on-farm, at the cage side. A high haematocrit in cod caught in hand nets (Table 4) as well as the significant increase in the haematocrit of cod crowded for harvesting prompted us to investigate the possibility of assessing gill colour, as a surrogate for blood haematocrit.

A series of Pantone colours that covered the spectrum of gill colours were identified in trial examinations of gills of harvested cod and collated as a series, from the lightest to the darkest, with allocated scores of 0.5 to 7. This colour chart was then used to rapidly assess gill colour, immediately before blood sampling.

There was a significant correlation between haematocrit and the gill colour score of cod crowded in a net suspended at 14 metres, which gives some support for use of the colour chart as a surrogate for haematocrit. However, the low correlation coefficients (r = 0.223, P=0.046 in April; r = 0.377, P<0.001 in July), indicates other influences and poor predictive potential. Other difficulties for reliable on-farm monitoring of gill colour are likely to limit the value of gill colour as a practical welfare index. These are:

- (i) Ambient light conditions may affect colour matching
- (ii) Blood oxygenation and circulation will affect gill colour
- (iii) Very rapid assessment of colour is essential to avoid the effects of circulatory changes after death.

11. DISCRIMINATION OF LINE–CAUGHT COD AND CROWDED COD, IN CENTRE AND CORNER OF THE SWEEP NET

There were differences in ventilation depths of mobile cod in the centre of the sweep and cod in the corners of the sweep net (Figure 3), which may relate to different levels of aerobic distress in the two groups. Data for blood parameters and physical parameters, skin colour and fin damage for 231 cod in the three trials were used to investigate which measurements best identified the cod as: (i) line-caught controls, (ii) cod in the centre of the sweep net during crowding, or (iii) cod in the corners of the sweep net during crowding. For this analysis, Fisher's Linear Discriminant Function Analysis (LDFA) was employed. This method relates to Principal Components Analysis, but LDFA deliberately finds combinations of explanatory variables that maximally separate experimental units into pre-defined groups. The analysis weighted the importance of each measurement while maximally separating the three groups of cod to determine which combination of features best separates the groups. In analyses with three groups of cod (line-caught, centre and corner fish), two linear discriminant functions were used to separate the groups. The first function, LD1, separated line-caught cod from the other two groups. The second function, LD2, helped to separate centre and corner fish. When only two groups were analysed (centre vs corner fish), only one linear discriminant function was used. As there were gaps in the datasets, eight discriminant analyses were employed, looking at the 3 trials separately and excluding measurements where there were many missing values. In each case, the success rate in allocating cod to the known groups was assessed.

Figure 6 shows one example of the separation achieved for the three groups. In this case, there was 86% correct allocation of line-caught cod, which have low values of LD1. Separation of cod caught in the centre and corners of the crowding sweep is less easy, and is determined by both LD1 and LD2. Corner fish tend to have larger values of LD1 and LD2. The analysis resulted in correct allocation of 65% of cod in the centre of the sweep and 69% of cod in the corner of the sweep, giving overall 68% correct allocation. Table 5 summarises the pattern of identification of the various parameters as contributors to LD1 and LD2 to allow identification of the three groups.

Figure 6. Separation of cod into line-caught (0), centre (1) and corner (2) categories by Linear Discriminant Function Analysis, using all data for all trials.

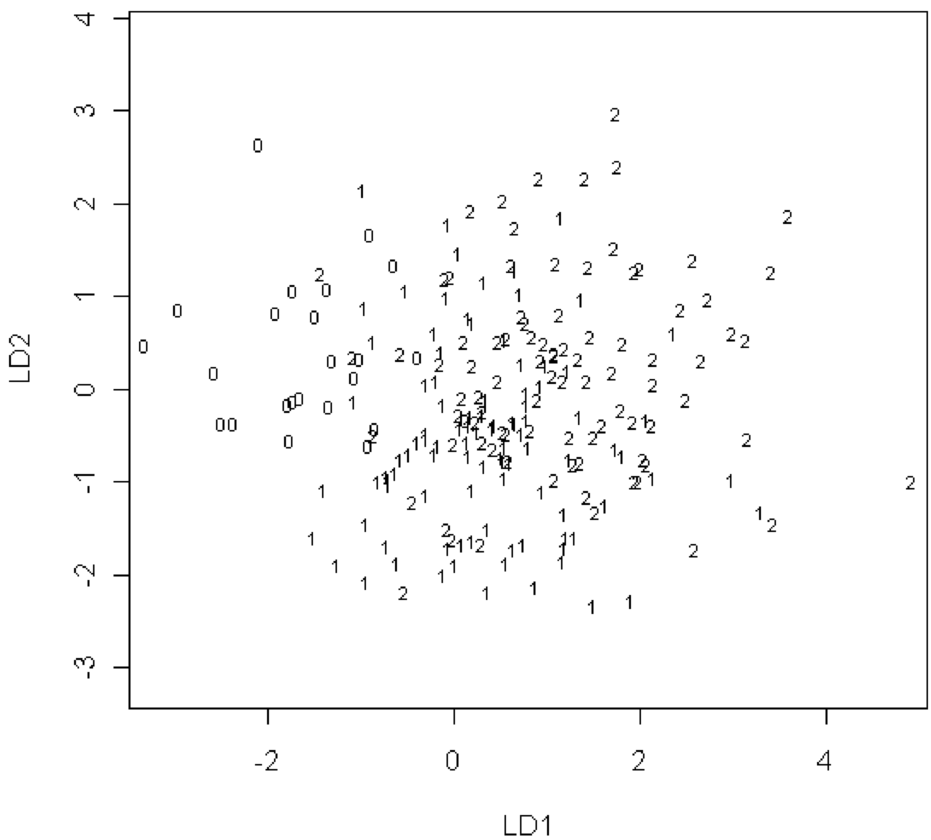


Table 5. Summary of 8 discriminant analyses for separation of line-caught and crowded cod: in centre and corner of sweep net

Variable loading >0.5	1a	1b	2a	2b	3a	3b	4a	4b
Haematocrit	+LD1	+LD1				+LD1 -LD2	+LD1	+LD1
Plasma lactate	+LD1	+LD1	+LD1	+LD1	+LD1	+LD1		
Plasma glucose	+LD2	+LD2					+LD2	+LD2
Plasma cortisol			+LD1	+LD1			+LD1 -LD2	+LD1 -LD2
Dorsal hue			+LD1	+LD1	+LD1			
Gonadosomatic index			-LD2	-LD2				
Dorsal lightness			-LD2		-LD1		+LD1	
Dorsal chroma			+LD2		+LD1	+LD2		-LD2
Swimbladder pressure					-LD1			
Ventral chroma					-LD1	-LD2		
Ventral lightness					-LD2	-LD2		
Ventral hue					-LD1 -LD2	-LD1		
Splenic:somatic index				-LD2				
$K_{no\ viscera}$			-LD1	-LD1	-LD1	+LD2		
Hepatosomatic index						-LD2	+LD1	+LD1

Plus sign identifies positive effects on LD1 or LD2; negative sign identifies negative effects on LD1 or LD2
1a = data from all three trials, excluding ventral colour (not measured trial 1), swimbladder pressures (not measured trial 3) and fin scores (missing values trial 3), 68% correct allocation.
1b = data from all three trials, including fin scores, 70% correct allocation
2a = trial 1 data (11/04/07), no measurements for ventral colour, 83% correct allocation
2b = trial 1 data (11/04/07), no measurements for ventral colour, excluding swimbladder measurements where many gaps in data set, 81% correct allocation.
3a = trial 2 (24/04/07), 86% allocation success.
3b = trial 2 (24/04/07), excluding bladder pressure where some missing values, 82% accuracy
4a = trial 3 data (10/7/07), no data for bladder pressures, 73 % correct allocation
4b = trial 3 data (10/7/07), no data for bladder pressures without fin damage, 64% correct allocation

Table 5 shows that plasma lactate was a good discriminator of the line-caught cod and the two groups of crowded cod, except in the 3rd trial (July), when the density in the crowd was less than half that of the April crowds. Blood haematocrit is another contender in discriminating between the three groups, but was not useful in the first April trial, when the cage depth prior to sweeping was lower than in the other trials. Plasma concentrations of cortisol were sometimes useful. There was also evidence that condition index ($K_{no\ viscera}$ as a measure of muscle mass), skin lightness, hue, and chroma can contribute to the discrimination of the three groups of cod.

Further analyses were performed, excluding data for line-caught cod, to assess which parameters allow discrimination of cod in the centre and corner of the sweep net. The percentage correct allocation varied from 63 % to 87 %. Dorsal chroma, condition ($K_{no\ viscera}$) and GSI were useful discriminators. Plasma lactate and glucose were good discriminators, with higher levels suggested for corner fish in both April trials, which suggests physiological differences linked to the behavioural patterns of the two groups identified in sections 6.2 and

6.3. Therefore, we examined whether cod caught in the centre of the sweep showed significantly different responses in these discriminators from those in the corner of the sweep net.

12. RESPONSES TO CROWDING

The changes of the candidate discriminators of line-caught cod, and cod in the centre and corners of the sweep, were examined for each of the 2007 crowding trials. This involved statistical analysis of the effects of time, location and gender on dorsal chroma, $K_{no\ viscera}$, plasma glucose, plasma lactate, plasma cortisol and haematocrit.

Condition ($K_{no\ viscera}$) was not significantly different in male and female cod, or in cod in the two locations during crowding (*P* >0.05 in all cases). This suggests that condition is unimportant in influencing the different behavioural responses to crowding. Dorsal chroma was not affected over the period of crowding in any of the trials i.e. it was unaffected by the stress of crowding, however, in the first trial (April) there was a significantly higher dorsal chroma in cod in the corner of the sweep than those sampled from the centre (*P* = 0.02). This lends support to further work on the importance of dorsal colour as a welfare index.

Figure 7 and 8 summarise the results for plasma lactate in the 3 crowding trials. Plasma lactate concentrations were significantly increased above the basal concentrations of 0.24 to 0.76 mM, as in the pilot studies. Cod in the centre and corner of the sweep net differed in their plasma lactate in the 14 m trial at high density (April). These data support the continued use of blood lactate as an index of physiological welfare in conditions that increase physical activity.

Figure 7 Effect of crowding 8 metre and 14 metre cages (April) on plasma lactate of cod in center and corner of net

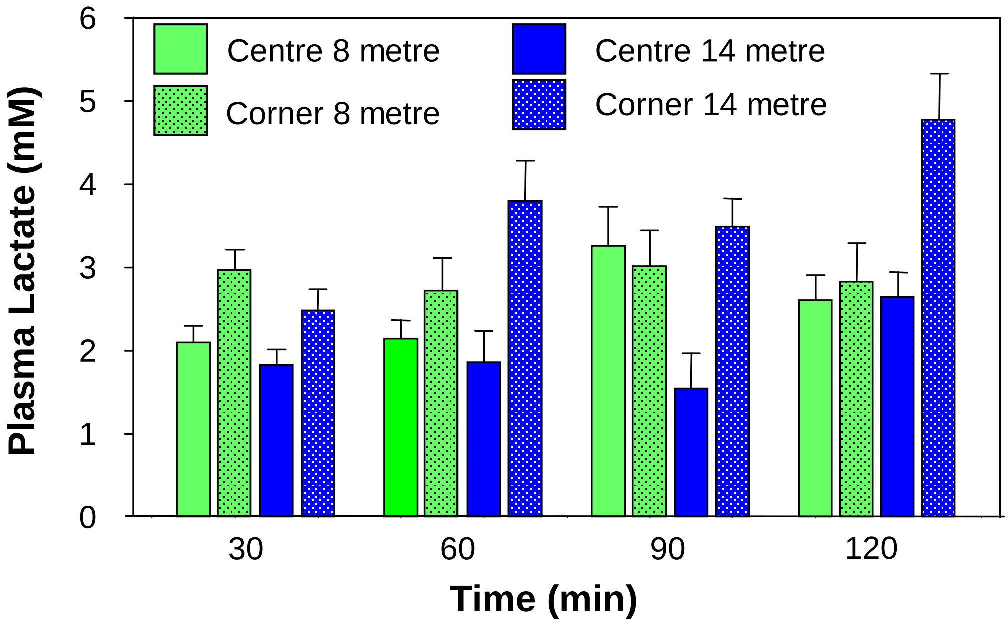


Fig 7. In the 8 m trial, corner and centre cod showed a similar and sustained increase in plasma lactate during crowding, within 30 min. Female cod showed significantly higher plasma lactate than male cod during this trial (*P* = 0.04). Crowding from a cage depth of 14 m resulted in both time-related and location-related differences in plasma lactate (*P* <0.001). Cod in the corner had significantly higher plasma lactate throughout this trial, and all fish showed a significant increase at 30 min compared to time 0 min values in line-caught cod (*P* = 0.04), and a further increase between 90 min to 120 min (*P* <0.001)

Figure 8 - Plasma lactate of cod crowded in 14 m cages at low and high density

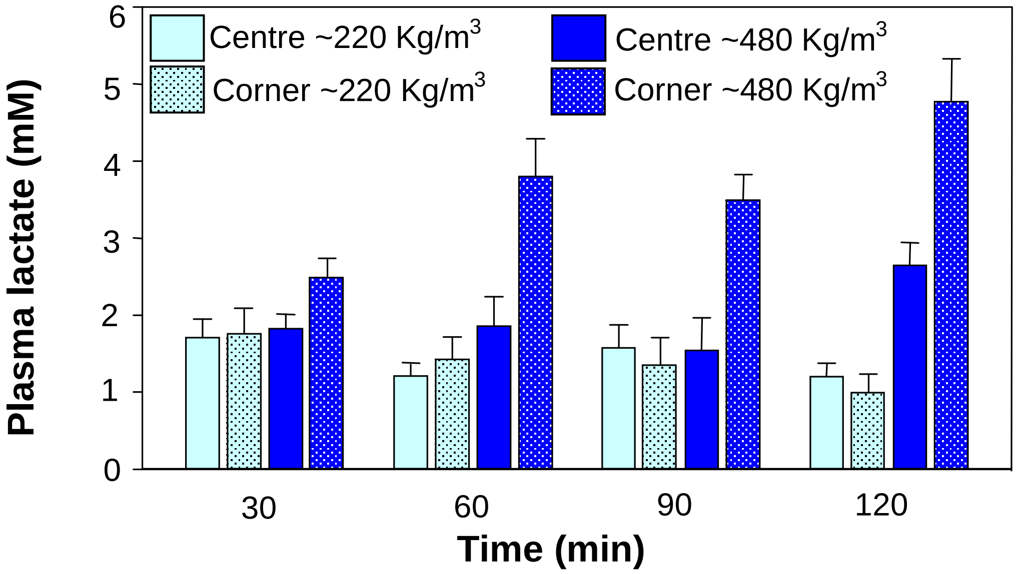


Fig 8. In the low-density crowd (July 2007), within 30 min plasma lactate was increased above basal concentrations found in line-caught cod (P=0.004) but then showed some recovery (decrease between 30 min and 120 min, P = 0.01), and no apparent differences between cod in the two locations. Crowding at a higher density (April 2007) resulted in both time-related (P<0.001) and location-related (P<0.001) differences in plasma lactate.

Plasma cortisol was significantly increased above basal concentrations during all 3 crowding trials (Figures 9 to 11), but showed different patterns of responses.

Figure 9 Plasma cortisol of cod crowded in 8 metre cage (April) 2007

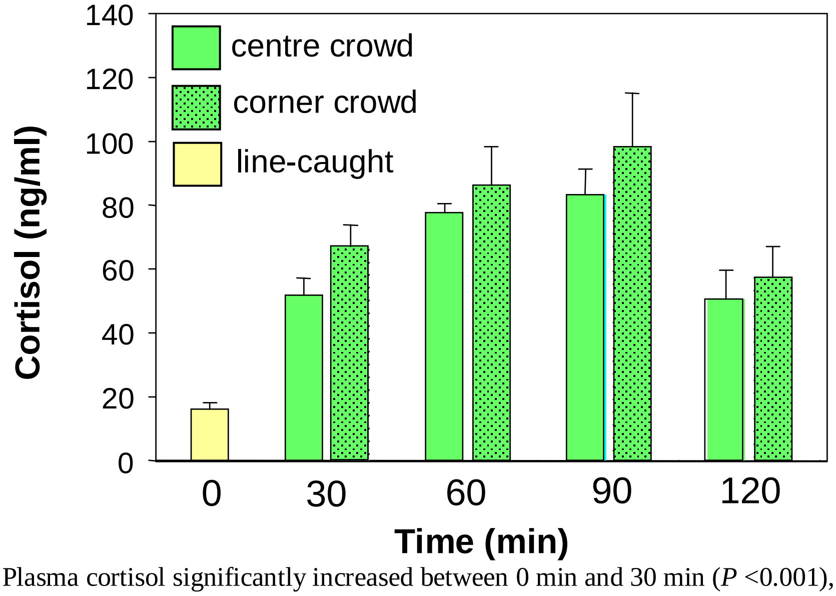


Fig. 9. Plasma cortisol significantly increased between 0 min and 30 min (P <0.001), and 30 to 60 min (P <0.001). There was some recovery between 90 min and 120 min (non-significant comparison of 30 min vs 120 min, P = 0.43).

Figure 10 Plasma cortisol of cod in 14 metre cage (April) 2007

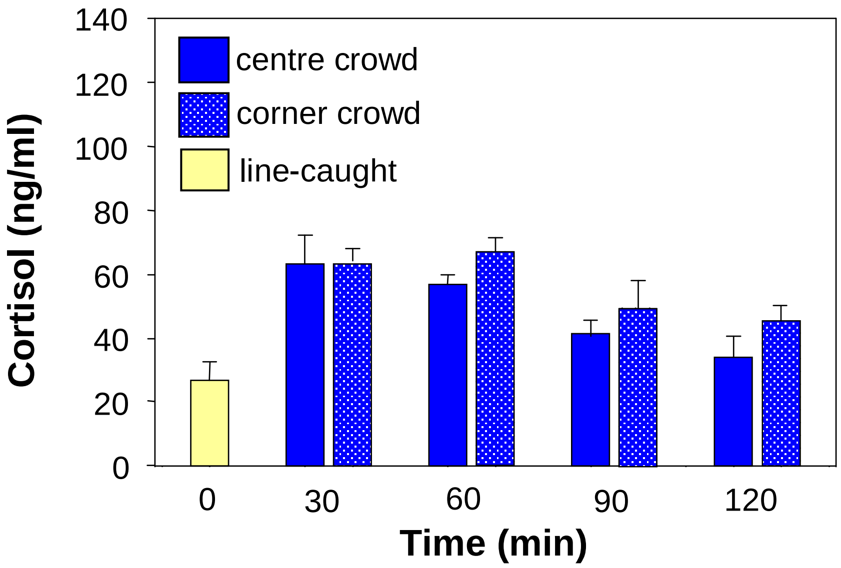


Fig. 10. Plasma cortisol showed a significant increase between 0 min and 30 min (*P* <0.001), was maintained at 60 min, and showed some recovery after 60 min (*P* = 0.002), with no significant differences between cod in the corner and centre of the sweep, throughout the trial (*P* = 0.18).

In the two April trials, gender affected cortisol responses to crowding. Female cod showed a higher plasma cortisol concentration than male cod in both crowding trials (P = 0.02 and 0.004 respectively. This is reminiscent of the effects noted in the pilot studies (run in June: see Figure 1).

Figure 11 Plasma cortisol of cod in 14 metre cage (July) 2007

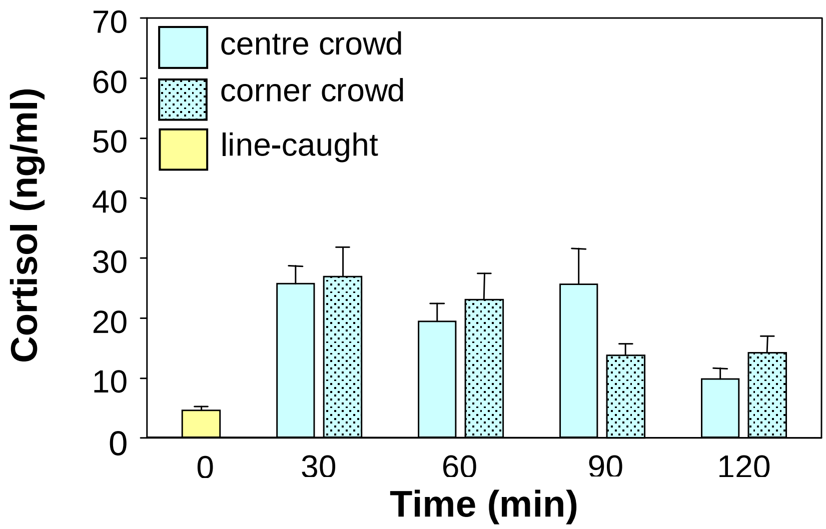


Figure 11. Plasma cortisol prior to crowding was significantly lower in July than in April (Figs 9 & 10). In July, there was no effect of location in the crowd or gender on the cortisol responses. There was a significant increase at 30 min in crowd (P<0.001) and then recovery (comparison of 30 min vs 120 min, P<0.001).

Data for plasma glucose during crowding showed significant effects of crowding in all three trials, but there were complex time relationships and differences between the responses of cod located in the corner and centre of the sweep that did not follow the same pattern as cortisol responses. In all three trials, there was a significantly higher plasma glucose concentration in cod located in the corners than in cod located in the centre of the sweep. Since cortisol was not significantly different in cod from the corner and centre of the sweep in either of the 14 metre trials, differences in glucose are likely to reflect an increase in catecholamines due to oxygen shortage. This conclusion is supported by the significant correlation between plasma lactate (triggered by oxygen shortage) and plasma glucose (χ^2_1 =56.34, P < 0.001). Interestingly, correlation analyses suggested that female cod had higher plasma glucose (χ^2_1 =8.24, P = 0.004), but no gender effects were apparent in glm analysis of the changes in each trial.

The suggested increase in circulating catecholamines during crowding can be predicted to stimulate splenic release of erythrocytes and an increase in haematocrit. In all 3 of the 2007 crowding trials, there was a sustained increase in blood haematocrit, with a significant increase by 30 min of crowding. The location of cod (centre and corner) had no effect on haematocrit in the 8 metre April trial, but splenic:somatic index (SSI) was significantly lower (P = 0.015) in cod in the corner of the sweep net, which suggests that erythrocytes were released into circulation. Therefore, SSI may be a more sensitive index of a neural/catecholamine response to oxygen shortage than blood haematocrit. This idea is supported by evidence of a negative correlation between SSI and blood lactate in the three trials (χ^2_1 =7.94, P = 0.005).

In the 14 metre April trial, when there was a high crowd density, cod in corner of the net showed a further increase in haematocrit (P = 0.03) at 60 min of crowding. This agrees with analysis of SSI for this trial, which identified an effect of location (P = 0.004), with SSI lower in cod in the corner of the sweep net. In July at lower density, the sweep from 14 metre had no significant effect on SSI, while haematocrit was increased. The lack of a significant difference between cod in the two locations agrees with other data for these fish and suggests that lower levels of physical activity are beneficial (see section 6.3). Our data for blood haematocrit and SSI support the continued use of these parameters as indices of physiological well-being and welfare during crowding or other husbandry procedures.

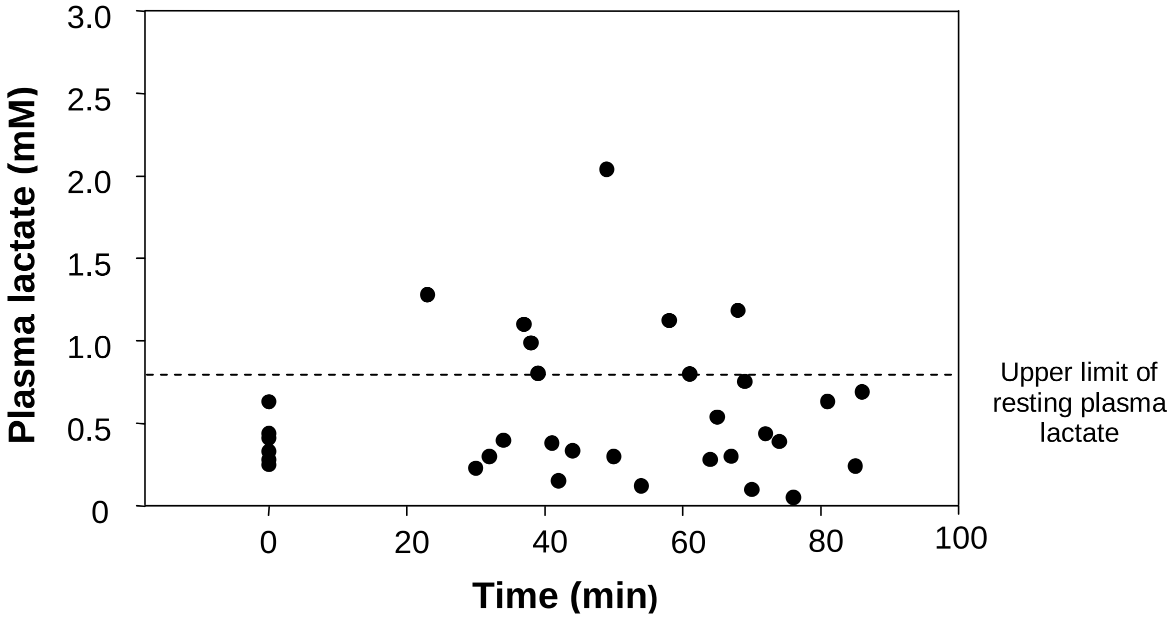
The results from our studies lead to the conclusion that a low cage depth and a low density are beneficial for cod during crowding. However, the practicalities need to be considered, and low fish density will reach a point where harvesting is unrealistic. Lower depths of crowding are a more realistic option and can reduce the number of surface cod that tend to be in the corners of the sweep net (see section 6.3).

13. WELFARE IN HARVESTING

During the contract period, harvesting practises changed, with the aim of reducing adverse implications that were shown in the pilot studies. The introduction of air-lifting from the corners of the sweep net, where cod could be most readily accessed is of significance, since there were higher levels of disturbance in these cod in the crowding trials (sections 6.2 & 12). We therefore investigated the impacts of the new crowding and harvest process, which was complete in 90 min. Blood samples were collected immediately after a cod was stunned with a MT4 Pneumatic Fish Stunner (Richard Bass Ltd), followed by exsanguination after cutting through three gill arches, by catching the spurts. Blood was collected from individual cod at known times after starting the sweep.

The practise of air-lifting from the side of the harvest cage avoided a significant increase in the mean plasma glucose or plasma lactate normally seen during crowding. Figure 12 shows the plasma lactate values of individual cod over the 90 min of harvesting, 78% of which had lactate concentrations within the normal range for resting cod. Plasma cortisol concentrations of these cod was increased from 1.51 $\pm$ 0.49 ng/ml in line-caught cod (n = 6) prior to harvesting to 10.10 $\pm$ 1.46 (n = 25) during crowding (P < 0.001, Mann Whitney Rank Sum test), but the increase was less than in previous crowding trials.

Figure 12 Plasma lactate of cod prior to and during harvesting by air-lifting from cage suspended at 8 metres



14. RECOMMENDATIONS AND FURTHER WORK

A photographic key and scoring scheme for assessment of fin erosion key has been developed. We recommend application of the scoring scheme to determine how environmental conditions influence fin erosion, for example, whether stocking density affects erosion, and to assess the welfare implications of fin erosion.

Hand-held lactate meters are recommended for use in monitoring blood lactate, as a means of assessing husbandry practices and their impacts on welfare in cod. This approach can also be readily used in other farmed species. The work on cod focused on one meter and it is important to emphasise the need for validation if alternative meters are employed.

We recommend use of in-cage cameras to monitor cod behaviour. We developed and validated a ventilation depth index for cod, but seasonal norms need fuller consideration. This scheme could be adapted for other species. Direct counts of ventilation rates are also recommended when circumstances allow this.

A condition index, based on eviscerated body mass as a measure of muscle mass, is recommended for assessment of chronic impacts on cod welfare.

Assessment of the numbers of cod at the surface, when not feeding, is recommended as an index of chronic welfare. We have also developed a method for assessment of acute behavioural changes leading to surface location during crowing. Based on the results using this method, and other behavioural and physiological indices, we recommend a welfare standard for pre-handling procedures (grading or harvesting) of raising the cage net depth by up to 2 metres per day until the maximum net depth prior to handling is equal to or less than 10 metres.

In line with current standard industry practice, air-lifting or vacuum pumping fish at key points in the production cycle (harvesting, grading etc) is recommended in view of the clear benefits over the use of lift nets.

For cod, swimbladder inflation was identified as a reaction to standard handling procedure. While changes in husbandry procedures went significantly towards reducing the incidence, and reduced the number of fish at the surface, further investigations of the causes and significance of this behaviour are required.

Maturation, and its effects on the welfare of farmed cod, has been identified in this study as an area of significant concern. Further investigation into strategies to minimise and/or prevent maturation in farmed Atlantic cod is therefore warranted.

The link between pre-slaughter handling stress and end-product quality, which was beyond the scope of this project, is another key area for future work.

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16. REFERENCES

Adams, S.M., Brown, A.M., Goede, R.W., (1993). A quantitative health assessment index for rapid evaluation of fish condition in the field. Trans. Am. Fish. Soc., 122, 63-73.

Adams, C.E., Turnbull, J.F., Bron, J.E., Huntingford, F.A. (2007). Multiple determinants of welfare in farmed fish: stocking density, disturbance, and aggression in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci. 64, 336-344.

Arlinghaus, R., Cooke, S.J., Schwab, A., Cowx, I.G. (2007). Fish welfare: a challenge to the feeling-based approach with implications for recreational fishing. Fish & Fisheries 8, 57-71.

Brown, J.A., Watson, J., Bourhill, A., Wall, T (2008). Evaluation and use of the Lactate Pro, a portable lactate meter, in monitoring the physiological well-being of farmed Atlantic cod (*Gadus morhua*). Aquaculture 285: 135-140.

Brown, J.A., Watson, J., Bourhill A., Wall, T. (2009). Physiological welfare of commercially reared cod and effects of crowding for harvesting. Aquaculture (submitted to Aquaculture).

Claireaux, G. Dutil, J-D. (1992). Physiological response of the Atlantic cod (*Gadus morhua*) to hypoxia at various environmental salinities. J. Exp. Biol. 163, 97-118.

Cooke, S.J., Suski, C.D., Danylchuk, S.R., Aanychuk, A.J., Donaldson, M.R., Pullen, C., Bulté, G., O'Toole, A., Murchie, K.J., Koppleman, J.B., Schulz, A.D., Brooks, E., Goldberg, T.L. (2008). Effects of different capture techniques on the physiological condition of bonefish *Albula vulpes* evaluated using field diagnostic tools. J. Fish Biol. 73, 1351-1375.

Doolan, B.J. ,Booth, M.A., Jones, P.L., Allan, G.L. (2007). Effect of cage colour and light environment on the skin colour of Australian snapper *Pagurus auratus* (Bloch & Schneider, 1801). Aquaculture Res. 38, 1395-1403.

Doolan, B.J. Allan, G.L. Booth, M.A. Jones, P.L (2008). Effect of cage netting colour and density on the skin pigmentation and stress response of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801). Aquaculture Res. 39, 1360-1368

Gustaveson, A.W., Wydoski, R.S., Wedemeyer G.A. (1991). Physiological response of largemouth bass to angling stress. Trans. Am. Fish. Soc. 120, 629-636.

Hatlen, B., Grisdale-Helland B., Helland S.J. (2006). Growth variaation and fin damage in Atlantic cod (*Gadus morhua* L.) fed at graded levels of feed restriction. Aquaculture 261, 1212-1221.

Herbert, N.A., Steffensen, J.F. (2005). The responses of Atlantic cod, *Gadus morhua*, to progressive hypoxia: fish swimming speed and physiological stress. Mar. Biol. 147, 1403-1412.

Holeton, G.F., Randall, D.J. (1967). The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. Exp. Biol. 46, 317-327.

Hoglund, E., Balm, P.H.M., Winberg, S. (2000). Skin darkening, a potential social signal in subordinate Arctic charr (*Salvelinus alpinus*): the regulatory role of brain monoamines and pro-opiomelanocortin-derived peptides. J. Exp Biol. 203, 1711-1721.

Hopkins T.E., Cech J.J. (1992). Physiological effects of capturing striped bass in gill nets and fyke traps. Trans. Am. Fish. Soc.121, 819-822.

Hoyle, I., Oidtmann, B., Ellis, T., Turnbull, J., North, B., Nikolaidis, J., Knowles, T.G. (2007). A validated macroscopic key to assess fin damage in farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture 270: 142-148.

Huntingford, F.A., Adams, C., Braithwaite, V.A., Kadri, S., Pottinger, T.G., Sandøe, P., Turnbull, J.F. (2006). Review paper. Current issues in fish welfare. J. Fish Biol. 68, 332-372.

Johansen, J.J., Herbert, N.S.A., Steffensen, J.F. (2006). The behavioural and physiological response of Atlantic cod *Gadus morhua* L. to short-term acute hypoxia. J. Fish Biol. 68, 1918–1924.

Lambert, Y., Dutil, J-D. (1997). Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of Atlantic cod (*Gadus morhua*)? Can. J. Fish. Aquat. Sci. 54: 104-112.

Landis, J.R., Koch, G.G. (1977). The measurement of observer agreement for categorical data. Biometrics 33: 159-174.

Lapointe, D., Guderley, H., Dutil, J.D. (2006). Changes in condition factor have an impact on metabolic rate and swimming performance relationships in Atlantic cod (*Gadus morhua* L.). Physiol. Biochem. Zool. 79, 109-119.

Lloret, J., Rätz, H-J. (2000). Condition of cod (*Gadus morhua*) off Greenland during 1982-1998. Fisheries Research 48, 79-86.

MacLean, A., Metcalfe, N.B., Mitchell, D. (2000). Alternative competitive strategies in juvenile Atlantic salmon (*Salmo salar*): evidence from fin damage. Aquaculture 184, 291-301.

Moutou, K.A., McCarthy, I.D., Houlihan, D.F. (1998). The effects of ration level and social rank on the development of fin damage in juvenile rainbow trout. J. Fish Biol. 52, 756-770.

Nelson, Y.A., Tang, Y., Boutilier, R.G. (1996). The effects of salinity change on the exercise performance of two Atlantic cod (*Gadus morhua*) populations inhabiting different environments. J. Exp. Biol. 199, 1295-1309.

Olsen, R.K. Sundell, K., Ringø, E., Myklebust, R., Hemre, G-I, Hansen, T., Karlsen, Ø. (2008). The acute stress response in fed and food-deprived Atlantic cod, *Gadus morhua* L. Aquaculture 280, 232-241.

Peake, S.J., Farrell, A.P. (2006). Fatigue is a behavioural response in respirometer-confined smallmouth bass. J. Fish Biol. 68, 1742-1755.

Pickering, A.D. (1998). Stress responses of farmed fish. In: Black, K.D., Pickering, A.D. (Eds.), Biology of Farmed Fish, Sheffield University Press, pp 222-255.

Peake, S.J, Farrell, A.P. (2006). Fatigue is a behavioural response in respirometer-confined smallmouth bass. Journal of Fish Biology 68, 1742-1755.

Pottinger, T.G., 1998. Changes in plasma cortisol, glucose and lactate in carp retained in anglers' keepnets. J. Fish Biol. 53, 728-742.

Rideout, R. M., Morgan, M. J., Lilly, G. R. (2006). Variation in the frequency of skipped spawning in Atlantic cod (*Gadus morhua*) off Newfoundland and Labrador. ICES Journal of Marine Science, 63 (6): 1101-1110.

Rillahan, C., Chambers, M., Howell, W.H., Watson III, W.H. (2009). A self contained system for observing and quantifying the behaviour of Atlantic cod, *Gadus morhua*, in an offshore aquaculture cage. Aquaculture 293: 49-56.

Robb, D.H.F. (2001). Measurement of fish flesh colour. In: Farmed Fish Quality (eds Kestin, S.C., Warriss, P.D.) pp 298-307. Wiley Blackwell.

Staurnes, M., Sigholt, T., Pedersen, H.P., Rustad, T. (1994). Physiological effects of simulated high-density transport of Atlantic cod (*Gadus morhua*). Aquaculture 119, 381-391.
Stein, L.H., Hirmas, E., Bjørnevik, M., Karlsen, O., Nortvedt, R., Røra, A.M.B., Sunde, J., Kiessling, A. (2005). The effects of stress and storage temperature on the colour and texture of pre-rigor filleted farmed cod (*Gadus morhua* L). Aquaculture Research 36: 1197-1206.
Turnbull, J., Bell, A., Adams, C., Bron, J., Huntingford, F. (2005). Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis. Aquaculture 243, 121-132.
Van der Salm, A.L., Martinez M, Flik G., Wendelaar Bonga S.E. (2004). Effects of husbandry conditions on the skin colour and stress response of red porgy, *Pagrus pagrus.* Aquaculture 241 371-386.
Wendelaar Bonga, S.E.W. (1997). The stress response in fish. Physiol. Rev. 77, 591-625.