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Fluctuating sea-cage environments modify the effects of stocking densities on production and welfare parameters of Atlantic salmon (*Salmo salar* L.)

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Abstract

Stocking densities are commonly used to set limits for the production of fish in sea-cages, yet limited information exists to assess how environmental fluctuations modify the effects of stocking densities on the production and welfare of fish. Atlantic salmon (*Salmo salar* L.) of average size 1.3 kg were held at high (15.7-32.1 kg m^3) or normal (5.6-14.5 kg m^3) stocking densities in triplicate 2000 m^3 sea-cages from August to December. Intense crowding within both the high (189 kg m^{-3}, 10 × stocking density) and normal (147 kg m^{-3}, 17 × stocking density) density cages occurred when sub-optimal temperatures limited the amount of vertical space available. In addition, when stocking density in the high treatment exceeded 26.5 kg m^{-3}, feed intake, growth rate and feed utilisation declined and a greater number of cataracts, fin erosions and skin lesions developed. Fish with cataracts on both eyes were smaller than fish with only one or no cataracts. High stocking densities have significant detrimental effects on production and welfare, particularly when they are exacerbated by environments that drive crowding. Stocking densities should therefore be based on the characteristics of each location, to account for the influence of environmental variability.

Introduction

Intensive salmonid farming is four decades old and global annual production of Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Onchorhynchus mykiss*) now exceeds 1.4 million tons live weight (Kjønhaug, 2009). Within the on-growth phase in sea-cages, the industry has often used stocking density to plan production and monitor performance and the authorities have used stocking density to set production limits (e.g. Norway; 25 kg m^{-3}, Norwegian Ministry of Fisheries and Coastal Affairs, 2008). Recently, stocking density has been discussed as a tool to ensure acceptable welfare (e.g. Ellis et al., 2002; FSBI, 2002; Turnbull et al., 2005; Adams et al., 2007; Ashley, 2007; Huntingford and Kadri, 2008; Turnbull et al., 2008). However, it has been argued that the use of stocking density alone is insufficient to ensure welfare of farmed salmonids (e.g. Ashley, 2007; Huntingford and Kadri, 2008; Turnbull et al., 2008).

Stocking density *per se* may not determine welfare outcomes, rather the underlying consequences of a high or low degree of social interactions (e.g. Adams et al., 2007) or more importantly; the degradation of water quality with increasing density (e.g. Ellis et al., 2002). For example, hypoxia is regularly observed in sea-cages (Johansson et al., 2007; Vigen, 2008). Oxygen consumption increases with density and more hypoxic conditions have been observed at high compared to normal stocking densities (Johansson et al., 2006). Social interactions may also alter with stocking density, rates of aggression in Atlantic salmon peaked at 15 kg m^{-3} in seawater tanks (Adams et al., 2007). Ashley (2007) stated that a complex matrix of factors influences the effects of stocking density and the relative importance of these is case specific. As recommendations for specific stocking density limits have not emerged from tank-based studies (e.g. Adams et al., 2007), alternate investigations within commercial sea-cages have been attempted. A welfare score based on multivariate analysis of body and fin condition and plasma concentrations of glucose and cortisol indicated negative effects of stocking densities above 22 kg m^{-3} (Turnbull et al., 2005). However, stocking density was only one of several factors that affected the welfare score. In addition, environmental parameters such as temperature and oxygen were not monitored in time and depth; other studies have documented that these parameters fluctuate widely in sea-cages and their impact upon welfare is believed to be substantial (Johansson et al., 2006; 2007).

An underestimated aspect of the welfare of fish in sea-cages is the swimming density of the fish (hereafter called observed fish density, OFD). This is the density at which fish choose to school at in sea-cages and is a response to a variety of environmental and internal behavioural drivers (see review by Oppedal et al., 2011). This differs from the stocking density, which is a simple average calculated by dividing the biomass of the fish in the cage by the total cage volume. Several authors have argued that a better approach than using only stocking density would be to develop husbandry systems that maximise welfare through observations of fish behaviour and monitoring of water quality (e.g. Ashley, 2007; Huntingford and Kadri, 2008). Similarly, Dawkins (2004) states that the spatial patterns of animals will indicate their social choices and likes or dislikes about the physical aspects of their environment. Thus, changes in such patterns with stocking density or degree of crowding will be particularly important in helping us to decide whether animals want more space. In addition, Volpato (2009) emphasizes the wants of fish as important criteria for assessing welfare and it is generally accepted that choice and preference tests are one of the keys to set standards and manage welfare in aquaculture production. Therefore, we contend that the observed fish density and the stocking density must be considered together in assessments of welfare.

Normally, salmon in sea-cages do not distribute evenly throughout the water column but congregate at certain depth intervals in OFDs 1.5 to 20 times the stocking density (see review:

Oppedal et al., 2011). These studies of group behaviour using high spatial and temporal resolution echo-sounders (Bjordal et al., 1993) suggest that swimming depth and schooling densities are modulated by photo- and thermoregulatory behaviour traded off against motivational factors such as feed and perceived threats. However, comparisons between the published studies with emphasis on stocking density effects has been inadequate as stocks, sites, cages, year and most importantly seasonal variations in the environment have confounded comparisons. Salmon show clear depth preferences (e.g. Johansson et al., 2006; Dempster at al., 2008; 2009; Korsøen et al., 2009), but the extent to which they are able to fulfil them in sea-cages at high and low stocking densities remains unknown. Therefore, we studied the combined effects of stocking density and observed fish density, and how this was mediated by spatial (depth-related) and temporal variability in sea-cage environments.

The aim of the present study was to test if the maximum and median observed fish density (kg m^{-3}) and preference index differed: (i) between high and normal stocking densities; (ii) with time of day; and (iii) with seasonally changing environments from August to December. In addition, (iv) we assessed the effects of the combined stocking density and observed fish densities on fundamental production parameters, including feed intake, growth rate, feed conversion ratio, and indicators of welfare, including body and fin condition, and the prevalence of cataracts and body lesions. We evaluate the results in a welfare context.

Material and methods

Study site and experimental groups

The experiment was performed at the Cage Environment Laboratory (60°N, 4°E) of the Institute of Marine Research, Matre, Norway; a typical fjord site with brackish layer at surface. On 12 August 2002, 6 cages of 12 m x 12 m wide and 14 m deep were stocked with

NORMAL (5.6 ± 0.3 kg m^{-3}) or HIGH (15.7 ± 0.5 kg m^{-3}) densities in triplicates (Fig. 1). Totals of 26406 and 74213 Atlantic salmon (*Salmo salar* L., NLA strain) of 1.28 ± 0.02 kg (mean ± standard error) were used in the NORMAL and HIGH groups, respectively. These stocking densities were chosen as they represent commercial densities at which salmon are normally farmed. Prior to the trial, the salmon had been transferred to sea-cages as out-of-season smolts in October 2001 and grown under normal farming conditions at stocking densities of < 11.7 kg m^{-3}. Fish were randomly distributed among cages by a well-boat and densities allocated systematically to alternate cages so that each density had three replicate cages without the same treatment as a neighbour (see Johansson et al., 2006). The nets were changed every third week to avoid net fouling. The targeted end densities for late November 2002 were approximately 15 and 35 kg m^{-3} for the NORMAL and HIGH groups, respectively (Fig. 1). Johansson et al. (2006; 2009) have previously published data extracted from short periods within the experiment on the spatial and temporal variation of dissolved oxygen levels in sea-cages (Johansson et al., 2006) and the behaviour of individuals (Johansson et al., 2009). All experimental protocols complied with Norwegian ethical standards for research involving animals.

Fish were fed Biomar 800 Classic 9 mm pellets (Biomar, Myre, Norway) in excess (determined by waste food appearing below the fish viewed by underwater cameras) continuously during two daily feeding periods (09:00-12:00 and 14:00-16:00 hours) using a pneumatic centralised feeding system (AEM, Austevoll, Norway). During the first three weeks, an automatic appetite feeding system was used (AF, Storvik Aqua AS, Sunndalsøra, Norway). This system experienced technical problems which resulted in the HIGH group being underfed compared to the NORMAL group. This period was therefore excluded from all subsequent analyses presented here. Mortality was recorded at least twice per week by emptying the dead fish collectors at the bottom of the cages.

The environment was monitored using a YSI 6600 CTD (Yellow Springs Instruments, Ohio, U.S.A.) with probes for temperature, conductivity, oxygen, depth (pressure) and light intensity (LI192, LiCor, Lincoln, Nebraska, U.S.A.). Continuous profiling was performed at a reference point placed approximately 14 m outside the nearest cage using an automatic winch with data logging at approximately 0.5 m depth intervals. The polarographic oxygen sensor (YSI 6562 DO probe, Yellow Spring Instruments) had large drift at the high frequency sampled and only short periods of reliable data was retrieved (published in Johansson et al., 2006). Salinity was 13 to 28 in the upper 3 m with large fluctuations caused by variable freshwater run-offs. Below, salinity was more stable ranging from 26 to 33 ppt. In general, salinity increased from the beginning to the end of the experiment. Temperature displayed large variations with time and depth (Fig. 2). From August to mid-October, the coldest water occurred either close to the surface or deep down, with a warm peak at depths of 2-5 m. From mid-October onwards, the warmest water was below 4 m with little variation, while the surface waters became colder and more variable. The mean temperature over all depths was around 16 °C in August, rising to 18.5 °C in early September and thereafter declining rapidly to 14 to 12 °C in late September before gradually declining to 10 °C in December.

Observed fish density

The vertical fish distribution was observed continuously by a PC-based echo integration system (Lindem Data Acquisition, Oslo, Norway). A full description of this system is given in Bjordal et al. (1993). Upward-facing transducers with a 42° acoustic beam were mounted in gimbals and positioned at 17 m depth below the centre of each cage. Every 3 weeks a 3-day period was intensively observed and data analysed in detail (periods 1-4, see Fig. 1 and 2).

Sampling, net change and other disturbing activities were performed between these four periods. Echo intensity, which is directly proportional to fish density, was recorded at 0.5 m depth intervals from 0 to 14 m and converted to relative echo intensity for each interval. The mean of the 60 observations min^{-1} was recorded and condensed to hourly averages per depth interval prior to analysis. The relative echo intensity was transformed to observed fish density (OFD) in kg m^{-3} by multiplying with the total biomass in the cage and dividing by the volume of each depth layer. Within each period, the vertical distributions (average of triplicate cages) were contour plotted using the Krieging method of Surfer, ver 8.0 (Golden software, Colorado, USA) for each density group. Subsequently, several parameters were calculated over all depths at a given hour: OFD$_{max}$ as the maximum observed fish density; median OFD as the observed density with half of the fish above and below; and PI (preference index) as the sum of the density above the average density divided by the n depth layers with densities above the average (see Oppedal et al. (2007) for calculations). In order to elucidate the OFD$_{max}$ value, the following example is given: At OFD$_{max}$ equals 100 kg m^{-3}, a total of 7200 kg fish (100 kg m^{-3} × 12 m × 12 m × 0.5 m) is swimming in the 0.5 m depth interval. Given an average size of 1.5 kg, this represents 4800 fish, which is about 19 % of fish in the HIGH group and 54 % of the fish in the NORMAL group.

Growth and production measures

Every third week live body weight (to the nearest 5 g), fork length (to the nearest 0.5 cm), prevalence of cataracts, fin erosion and lesions were measured within each cage (for each replicate cage and sampling time: n = 162-195 fish). For sampling, a cast net of 5 m × 5 m × 5 m was positioned at the cage bottom, left for 15-25 min, and then rapidly pulled up to surface. The fish captured by the cast net were moderately crowded and sampled by randomly dip-netting out of the cast-net during crowding where fish were forced to distribute randomly.

Subsequently, fish were anaesthetised with Benzocain (Norsk Medisinaldepot, Bergen) prior to measurements. Fulton's condition factor (K) was calculated using $K = (W \times L^{-3})\ 100$, where W was the live body weight (g) and L was the fork length (cm) of each fish. Specific growth rate (SGR, % per day) was calculated from the formula: $SGR = (e^{q}-1)\ 100$, where $q = (\ln(W_2)-\ln(W_1)) \times (t_2-t_1)^{-1}$ and W_2 and W_1 were the average live body weights at times t_2 and t_1, respectively. Feed intake was defined as the amount of feed fed each day as a percentage of the total salmon biomass within each cage per day. Feed conversion rate (FCR) was calculated for every three week period between sample dates as: $FCR = (\text{Feed intake})\ (\text{biomass increase})^{-1}$. Sexual maturation was assessed by external examination of sexual characteristics. Eye cataracts (Wall and Bjerkås, 1999) were looked for on each eye and defined as present or absent. Fin condition can be assessed either by subjective classification of the extent of damage or by comparing the lengths of the fins relative to body length (e.g. Hoyle et al., 2007; Ellis et al., 2009). Assessments have variously been made on the dorsal, caudal, adipose, anal, pectoral, and pelvic fins. In this trial, "fresh/ recent" fin erosion on any fin (Latremouille, 2003) and body lesions were defined as present or absent.

Statistics

Within each period, maximum and median observed fish density and preference index were compared between stocking densities and time of day with a 3-way ANOVA with cage nested in stocking density and time of day (day or night) followed by a Student-Newman-Keuls post-hoc test. The analysis was based on the hourly averages with night defined as the hours during which light intensity was below 0.1 µE m^{-2} s^{-1} and day defined as the second hour after night to the hour before dusk. One hour was therefore excluded in both the morning and evening. Feeding periods were excluded since feeding is known to alter the swimming depth of the fish

(Bjordal et al., 1993; Juell et al., 1994). Periods of day lasted 10 to 3 hours and night periods 7 to 15 hours from August to November, respectively.

Feed intake was compared by calculating daily differences between the group means and then testing this against 0, using a t-test for each period (Zar, 1996). Specific growth rates (SGR) and feed conversion rates (FCR) were compared among the HIGH and NORMAL groups by the nonparametric Mann-Whitney U-test (Zar, 1996). Live body weight, fork length and K were compared by means of ANOVA, with replicate cages nested in stocking density (Zar, 1996). Prevalence of eye cataracts, fin erosion and body lesions were compared across HIGH and NORMAL treatments with a χ^2-test for the triplicates combined (Zar, 1996). Size differences between fish with cataracts on no, one or two eyes were compared by ANOVA, with replicate cages nested in stocking density, followed by a Student-Newman-Keuls post-hoc test (Zar, 1996). Effect size, or the relative difference between the HIGH and NORMAL groups, was calculated as HIGH/NORMAL for K, prevalence of eye cataracts, fin erosion and body lesions.

Results

Over the course of the experiment, stocking density rose to the expected 14.5 ± 0.8 kg m^{-3} in the NORMAL cages, but only to 32.1 ± 1.1 kg m^{-3} in the HIGH cages, which was 3 kg m^{-3} less than expected (Fig. 1).

Observed densities

In late summer and early autumn, salmon were mainly distributed at depths in the sea-cages where the coldest water was available, range 14.9-20.0 °C during period 1, and at depths with cooler temperatures, range 10.4-17.2 °C in period 2, with a clear avoidance of the highest temperature layer (17-20 °C) at 2-3 m depth (Fig. 2, 3). A clear diurnal pattern was evident where fish displayed a bimodal distribution, swimming both at the surface and the bottom layer of the cages during the day. At night, fish densities were skewed towards the surface, but clear avoidance of the very highest temperature layer at 2-3 m depth was observed. Significant differences in the observed density parameters OFD_{max}, median OFD (not significant in period 2) and preference index (not significant in period 2) were detected between time of day at P-levels <0.001 (Fig. 4, 5, Table 1). The intense crowding in the 0-1 m depth layer observed during period 1 led to hourly maximum observed densities of up to 189 (10× stocking density) and 147 kg m^{-3} (17× stocking density) within replicate cages of the HIGH and NORMAL groups, respectively. Group averages of maximum densities ranged from 39 to 105 kg m^{-3} during period 1 and from 31 to 53 kg m^{-3} during the less extreme temperatures of period 2 (Fig. 4). The highest observed median density of NORMAL fish (29 kg m^{-3}) occurred in period 1, while no value exceeded 33 kg m^{-3} during periods 1 and 2 (Fig. 5). In late autumn and early winter, the salmon distributed at depths in the sea-cages where the warmest waters occurred during both period 3 (8-15 °C) and 4 (6-12 °C) with avoidance of the colder surface layer (Fig. 2, 3). A distinct diurnal pattern in swimming depth was still detected (P<0.001 for all density measures), with fish swimming deeper and more tightly packed during the day and swimming in more dispersed densities towards the surface at night. At daytime during the last day of period 3, a distinct movement towards the surface concurred with a surface increase in temperature, in particular during feeding. Coincident with a larger volume of optimal temperature available in the sea-cages, preference indexes were lower in periods 3 and 4 compared to periods 1 and 2 and median densities × stocking densities in the NORMAL density group were only 1.1 to 1.3 times in periods 3 and 4 compared to 1.9 to 3.7 times in periods 1 and 2 (Table 1).

HIGH group fish always swam in significantly greater densities ($P<0.001$) when compared at the corresponding time points to NORMAL group fish (Fig. 4, 5). In addition, another prominent group difference in behaviour was that more of the HIGH group fish experienced the 18-20 °C warm water layer that occurred at 2-9 m depth in period 1 (Fig. 2, 3). These fish swam at median densities of 28-33 kg m^{-3} and a maximum density of 105 kg m^{-3}. Consequently, HIGH group fish displayed a preference index of only 3.4, while NORMAL fish had more space available to them and the preference index of 12.3 was considerably higher (Table 1). A third distinct group difference was that only the HIGH group fish swam at median densities above 30 kg m^{-3}, predominantly during periods 3 and 4. Half of the HIGH fish swam at densities above 57 kg m^{-3} at day during period 4. This packing deeper in the cage (preference index of 3.7) coincided with colder surface waters in general but also with a possible period of severe hypoxia within the HIGH cages. Primarily within the HIGH cages, hypoxia increased during the autumn up to early October, the point at which high quality DO measurements were available (Johansson et al. 2006). The very homogenous temperatures throughout the water column in October and the first half of November, with only a 2 °C decrease within 2-15 m depth (Fig. 3), indicate oxygen solubility and supply should also have been even. Thus, hypoxia may have become more severe in late October (period 3) and November (period 4) resulting from increased biomass (Fig. 1) and thus oxygen demand.

Production parameters

Feeding was not *ad libitum* during the first three weeks, primarily due to technical problems in the HIGH group which reduced the quantity of feed supplied. This led to NORMAL fish being on average 12% heavier compared to HIGH fish by 2 September. Thereafter, the feed intakes of both groups were 0.8% body weight per day in periods 1 to 2 and continued at the same level for the NORMAL group throughout (Table 2). However, the HIGH group fish ate

15 and 25% less than the fish in the NORMAL group during periods 3 and 4, respectively. As a result, the SGRs of the HIGH group were 29% and 60% lower than the NORMAL group in periods 3 and 4, respectively (Table 2), the latter result being statistically significant. The SGRs of the NORMAL group varied from 0.7 to 0.9 during the 4 periods. After the initial period of poor feeding in the HIGH group, there was a trend indicating compensatory growth with HIGH group fish growing 22% better than NORMAL fish in period 2.

There were a clear trend towards a poorer FCR in the HIGH than the NORMAL group in period 4, mainly due to one replicate cage in the HIGH group (Table 2). Both live body weight and fork length were equal at the start, but differed on September 2 ($F>13.0$, $P<0.001$). This difference persisted ($F>12.3$, $P<0.001$) with a parallel increase during period 1 and 2. Subsequently, the groups diverged during period 3 and 4 concurrently as SGR was reduced in the HIGH group. Condition factors were identical at the start (Table 3), but were significantly lower in the HIGH compared to the NORMAL group after three weeks ($F=122.4$, $P<0.001$), and this difference was sustained throughout all following periods ($F>7.4$, $P<0.007$). Condition generally increased during the autumn with converging values between groups (compensation of HIGH group; reduced effect size), but with the HIGH group displaying a clear decrease during period 4.

The prevalence of eye cataracts was more severe in the HIGH compared to the NORMAL group from the start of the experiment onwards ($\chi^2>7.04$, $P<0.008$: Table 3). For the 4 sub-samplings from the 14th of August to the 14th of October, the effect size remained stable, with the HIGH group fish having 1.3-1.5 times the number of cataracts than the NORMAL group fish. However, for the last six weeks of the experiment, corresponding to when HIGH group fish swam at densities above 57 kg m^{-3} during the day, the effect size increased markedly with HIGH group fish having 1.7-1.9 times the number of cataracts than the NORMAL group fish.

At the final sample, fish with cataracts on two eyes were on average 6% smaller in size than both those with no cataracts or only one cataract (F=6.49, P<0.002).

Fin erosion and body lesions were minimal throughout the experimental period apart from the last period (Table 3). In period 4, HIGH group fish developed significantly (χ^2>19.69, P<0.001) more fin erosions (27%) and body lesions (4%) compared to <0.4% incidence for both these parameters on NORMAL group fish (Table 3). Some fish had developed bleeding fin erosions, in particular at the rostral end of the dorsal and caudal fins. Cumulative mortality throughout the experiment did not differ greatly between the NORMAL (0.16%) and the HIGH (0.24%) group. The incidence of sexual maturation in both HIGH and NORMAL groups was < 1%.

Discussion

Atlantic salmon stocked at HIGH compared to NORMAL density in industrial-scale sea-cages preferred specific depths within sea-cages which resulted in observed fish densities up to 17 times the stocking density, with median values varying between 1.1-3.7 times the stocking densities. Specific environmental conditions within the sea-cages invoked behavioural trade-offs in both swimming density and depth, diurnally due to daily changes in light intensities, and seasonally due to changes in temperature depth profiles and hours of daylight. During periods when limited volume was available for fish to swim at depths where favourable environmental conditions existed, fish were forced into sub-optimal cage environments and their welfare compromised. This was particularly the case for the HIGH density group. When strong thermal stratification existed in the sea-cages, a proportion of the salmon in the HIGH density group at a stocking density of 20 kg m^{-3} were unable to realise their preferred swimming depth and we contend that welfare was therefore breached at this level. Production parameters were negatively affected when stocking density exceeded 27 kg m^{-3} in a relative homogenous environment with possibly hypoxic conditions. We therefore hypothesize that high stocking densities have significant detrimental effects, particularly when they are exacerbated by sea-cage environments that drive crowding above median values of 33 kg m^{-3} of observed fish density. These findings are of major relevance when stocking densities in sea-cages are to be considered.

Environmental drivers of swimming depths and densities

Swimming depth and density of Atlantic salmon in sea-cages is dependent upon multiple trade-offs between several environmental parameters adjusted by internal motivations and states (e.g. review by Oppedal et al., 2010). Using a novel application of a regression tree analysis, Johansson et al. (2006) described the behavioural preferences of salmon in detail during three sub-periods with accurate oxygen measurements. Generally, salmon are attracted towards the dark surface at night and avoid the strong light at the surface during the day (e.g. Fernö et al., 1995; Dempster et al., 2008; Korsøen et al., 2009). In this experiment, this pattern was adjusted by avoidance of the high temperatures in mid-water (>17 °C) during early and late September and avoidance of the coldest surface layer in both October and November (<8 °C). Preferences for depths in sea-cages where the warmest available water less than 15 °C occurs have been demonstrated for salmon (e.g. Oppedal et al., 2001; 2007; Dempster et al., 2008; 2009; Korsøen et al., 2009). Within this study, both warm temperature preference and avoidance of depths with temperatures that were too warm or too cold were displayed at both the group (Johansson et al., 2006) and individual level (Johansson et al., 2009). During October and November, when a homogenous, favourable temperature environment extended through most of the cage, fish were distributed more evenly throughout the water column. Avoidance of high surface light intensities may be a reaction to increased light-induced predation risk (Fernö et al., 1995). Optimising temperature is of great physiological significance for poikilotherm fish; thermoregulation may improve metabolic processes such as circulation, food intake, digestion, growth, bioenergetical re-acclimation processes and scope for activity (e.g. Brett, 1971; Biette and Geen, 1980; Claireaux et al., 1995; 2000).

Critical stocking density based on behavioural wants

A large proportion of salmon in the HIGH group (stocking density of ca. 20 kg m^{-3}) were unable to avoid the high water temperature (>17 °C) in the mid-water at night during early September. Within the favoured environment (darkness and cooler temperature) close to the surface, the HIGH group always displayed the highest absolute density in kg m^{-3}. However, the NORMAL group always had the highest relative density, evident from the 4× higher preference index and 3× higher median observed density relative to stocking density in the NORMAL compared to the HIGH group. These results strongly suggest that the lower stocking density in the NORMAL group allowed a greater proportion of the caged population to swim within this preferred, yet highly spatially restricted depth interval. From a welfare perspective, where the degree of fulfilment of the preferences of fish is a measure (e.g. Dawkins, 2004; Volpato, 2009) we argue that welfare was breached at the stocking density of 20 kg m^{-3} in the HIGH group during early September. However, during the late autumn a larger volume of favourable water in the sea-cages below the pycnocline was available to the salmon, and lower fish densities and preference indices were measured. Thus, the severity of stocking density on competition for space depends on the degree of heterogeneity in the environmental conditions, with increased severity where heterogeneity limits the volume of the favoured conditions.

Stocking densities should therefore be set based on the characteristics of each location, to account for the influence of environmental variability. Thermally homogenous waters throughout sea-cages make available more space and can hold a higher biomass of fish while still supplying proper welfare conditions compared to conditions in which the water column is thermally stratified. However, this generalisation is only valid as long as the homogenous water quality is within acceptable limits. Theoretical examples from Norwegian waters may elucidate the practical use of these findings. In a southern fjord where waters are often thermally stratified, low stocking density and deep nets will provide the salmon with an opportunity to avoid the extreme low and high temperatures at the surface in winter and summer, respectively, by allowing them to access favourable conditions by swimming deep in cages. At a typical mid-Norwegian, coastal farm, the water column is typically more thermally homogenous with temperatures seldom reaching upper or lower extremes for salmon. Stocking densities in such locations may therefore be higher without breaching welfare limits.

Critical stocking density based on fundamental production parameters

When the stocking density in the HIGH group exceed 26.5 kg m^{-3} following the sample on 14 October, the feed intake, growth rate and feed utilisation declined and a greater number of cataracts developed. The study revealed fish with cataracts on both eyes were of smaller size than fish with only one or no cataracts. Following the November 5 sample when the stocking density had reached 30 kg m^{-3}, added negative effects included reduced condition factor, a further increase in the development of cataracts, rapid development of fin erosions and body lesions. These findings clearly demonstrate that salmon welfare was breached beyond an upper stocking density level of 25-30 kg m^{-3} under the environmental conditions experienced in this study. Similarly, negative effects of stocking densities were seen above 22 kg m^{-3} at a commercial salmon farm in Scotland (Turnbull et al., 2005). Within existing welfare measures, it is unacceptable to produce farmed animals in conditions where they suffer from injuries (e.g. FAWC, 1996) or growth reductions (Huntingford and Kadri, 2008). Poor growth has specifically been highlighted as a key welfare indicator (EFSA, 2008).

We stress that the specific causes for the negative effects on salmon observed at high densities in this study cannot be pinpointed as our data is correlative; however, several explanations are possible. Hypoxic conditions may have occurred and been detrimental to salmon, as indicated by the oxygen measurements for the last days (5-7 October) of valid oxygen measurements within this study presented by Johansson et al. (2006). Adequate DO levels are a key requirement to ensure fish welfare and development (Kindschi and Koby, 1994; Van Raaij et al., 1996; Ellis et al., 2002). A lack of energy from aerobic metabolism for fish exposed to hypoxia may lead to down-regulation of energy-demanding processes such as feed uptake, growth and immune function (Wu, 2002). Within tank studies, metabolites of NH_3 or CO_2 have caused negative effects at high stocking densities, however in intensive production periods within sea-cages with high biomasses, high values of these metabolites appear absent (Johansson et al., 2007). Behavioural aggression could cause negative effects at high stocking density. Based on a seawater tank study, Cubitt et al. (2008) found that social hierarchies are present in large and densely populated rearing units of fish and suggested that social position is related to brain neurochemistry and thus potentially animal welfare. However, no evidence exists that behavioural aggression occurs in salmon held at high numbers and densities in commercial sized sea-cages. Social interactions measured between adult Atlantic salmon as aggression rates peaked at densities of 15 kg m^{-3} in small seawater tanks holding 57 individuals, with rates declining at higher densities of 25 and 35 kg m^{-3} with approximately 94 and 131 individuals, respectively (Adams et al., 2007). Increased abrasions due to collisions with other individuals, the net wall, the cage bottom, ropes or a high degree of surface exposure may have also been a component of the cause of the negative effects observed at high stocking density. A recent study on European sea bass, *Dicentrarchus labrax*, indicated that stocking density is a major risk factor for fin erosions (Person Le Ruyet and Le Bayon, 2009). In summary, determining the specific causes of the negative effects at high stocking densities requires further research, however low oxygen levels emerge as a prominent candidate. A holistic welfare assessment should be based on multiple parameters and multivariate analyses (e.g. Turnbull et al., 2005). In addition, such assessments must consider both positive and negative environments and states, as have recently been developed for other farm animals through semantic modelling (e.g. Bracke et al., 2008).

General limits based on observed density measures

Our results further the discussion regarding how to define appropriate measures to determine general stocking density limits for salmon within sea-cages. When 50% (median value) of the salmon swam at a density above 33 kg m^{-3}, commonly accepted welfare measures such as feed intake, growth, and cataracts became elevated and breached acceptable levels. In addition, as the median OFD exceeded 40 kg m^{-3}, fin erosions and lesions became more prevalent and severe. Our data suggest that the median observed fish density is a good new candidate measure to be used in salmon farm welfare assessments with a limit just above 30 kg m^{-3}.

In addition, preferences of fish for specific water depths based on the matrix of environmental variables can greatly inform welfare assessments. The degree to which fish can exhibit their natural preferences within stratified waters may be measured using a preference index. To do this, the index should also incorporate the degree of stratification. As an example, optimal temperatures in September were limited to just 3 m out of the 14 m available, while optimal temperatures occurred across 12 m out of 14 m during October and November. Finally, the absence of observed negative effects on production parameters in the HIGH density group of fish during September may have been hidden in a good oxygen environment (Johansson et al. 2006), despite the exposure to high temperatures. Thus, all density measures must be seen in conjunction with the environment and the prevailing levels of temperature and oxygen.

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Table 1. Preference index and relative median fish density to stocking density derived from echo-sounder data during each sub-period from September to November in NORMAL and HIGH density stocked Atlantic salmon groups during day and night. Deviations given are standard deviation. At all times, significant differences were seen between groups, and time of day, except period 2. Significant differences between groups within period are denoted by letters a-d from lowest to highest values based on Student Newman-Keuls post-hoc test. The n observations within each cage, per period, changes with daylight hours and range from 30 to 9 at day and 21 to 45 at night through autumn.

Parameter	Period	NORMAL density		HIGH density	
		Day	Night	Day	Night
Preference index	1	5.5±1.5^{b}	12.3±6.1^{c}	3.4±0.7^{a}	3.3±1.0^{a}
	2	4.2±2.6^{b}	5.2±2.6^{b}	2.3±0.6^{a}	1.6±0.5^{a}
	3	2.6±3.3^{d}	1.4±0.7^{b}	1.9±0.5^{c}	0.9±0.3^{a}
	4	1.1±0.5^{a}	1.5±0.6^{b}	3.7±0.5^{c}	1.4±0.6^{b}
Median OFD/ stocking density	1	2.2	3.7	1.7	1.4
	2	2.1	1.9	1.4	1.4
	3	1.3	1.3	1.4	1.1
	4	1.1	1.3	1.9	1.2

Table 2. Feed intake (feeding as % of biomass), specific growth rate and feed conversion rate during 3-week sub-periods 1 to 4 given as mean ± standard deviation. Atlantic salmon were grown in triplicate cages at NORMAL or HIGH stocking density. Significant differences between groups ($P<0.05$) are denoted by *.

Parameter	Period	NORMAL	HIGH
Feed intake	1	0.8±0.1	0.8±0.1
	2	0.8±0.0	0.8±0.1
	3	0.7±0.0*	0.6±0.1*
	4	0.8±0.0*	0.6±0.0*
SGR	1	0.7±0.2	0.7±0.2
	2	0.9±0.2	1.1±0.2
	3	0.7±0.2	0.5±0.1
	4	0.9±0.2*	0.4±0.2*
FCR	1	1.2±0.3	1.0±0.3
	2	0.9±0.2	0.8±0.2
	3	1.0±0.3	1.2±0.2
	4	0.8±0.2	2.3±1.8

Feed intake, period 3, $T=3.38$, $P=0.03$; period 4, $T=5.20$, $p<0.001$: SGR, period 4, $Z=1.96$, $P<0.05$

Table 3. Condition factor, incidence of cataracts (% of eyes), fin erosions (% of fish) and body lesions (% of fish) at 3-weekly samples. All numbers given as mean ± standard deviation. Effect size is the relative difference between the HIGH and NORMAL groups of any given parameter (calculated as HIGH/NORMAL). Atlantic salmon were grown in triplicate cages at NORMAL or HIGH stocking density. Significant differences between groups (P<0.05) are denoted by *.

Parameter	Time	NORMAL	HIGH	Effect size
Condition factor	14 Aug.	1.10±0.10	1.10±0.10	1.00
	02 Sep.	1.22±0.11*	1.14±0.14*	0.93
	23 Sep.	1.24±0.11*	1.18±0.11*	0.95
	14 Oct.	1.31±0.13*	1.26±0.13*	0.96
	05 Nov.	1.35±0.14*	1.32±0.22*	0.98
	26 Nov.	1.40±0.17*	1.26±0.16*	0.90
Cataracts	14 Aug.	13±4*	17±5*	1.3
	02 Sep.	18±2*	25±2*	1.4
	23 Sep.	30±5*	46±8*	1.5
	14 Oct.	34±11*	45±4*	1.3
	05 Nov.	30±12*	58±14*	1.9
	26 Nov.	41±3*	70±12*	1.7
Fin erosion	14 Aug.	<1	<1	-
	02 Sep.	<1	<1	-
	23 Sep.	<1	<1	-
	14 Oct.	<1	<1	-
	05 Nov.	<1	<1	-
	26 Nov.	0.4±0.7*	27±31*	68
Body lesions	14 Aug.	<1	<1	-
	02 Sep.	<1	<1	-
	23 Sep.	<1	<1	-
	14 Oct.	<1	<1	-
	05 Nov.	<1	<1	-
	26 Nov.	0.2±0.3*	4±6*	20

Figure 1. Experimental setup of HIGH and NORMAL stocking density of Atlantic salmon in triplicate sea-cages. Intensive sub-periods with behavioural observations are indicated between vertical lines and numbered 1 (10-12 September), 2 (1-3 October), 3 (22-24 October) and 4 (12-14 November). Sample dates are noted by symbol with average ± standard deviation between replicate cages.

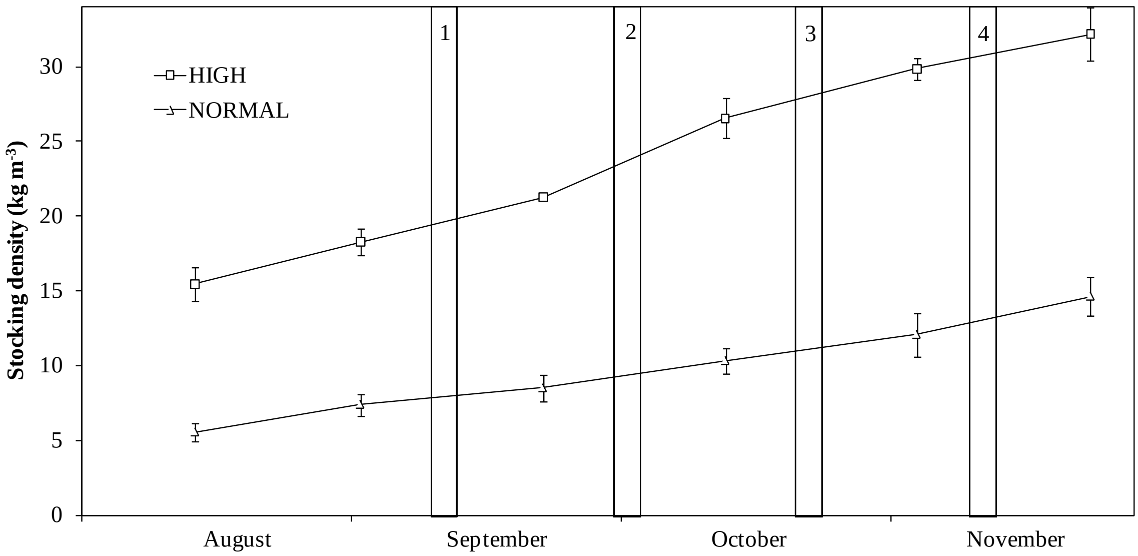
Figure 2. Water temperatures from August to December 2002 from 0 to 15 m with sub-periods of intensive behavioural observations marked as P1-P4. The colour scale represents temperatures from 4 to 20 °C.

Figure 3. Observed fish densities (kg m^{-3}) during 3-day sub-periods (Period 1-4) of intensive behavioural observations based on averages of the triplicate cages of the NORMAL (N) and HIGH (H) stocking density groups. The black and white bars below each period plot denote night and day, respectively. Vertical axis represent depth from 0 to 15 m. Colour scale indicates observed fish densities from 0 to 160 kg m^{-3}.

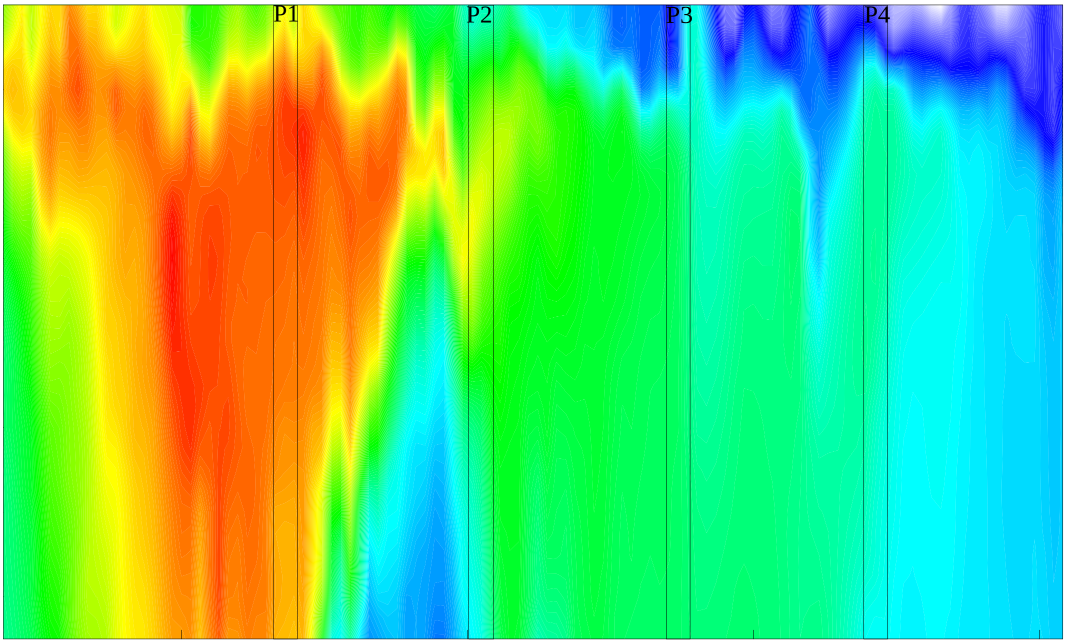
Figure 4. Maximum observed fish density (OFD_{max}) given as mean±s.d. in kg m^{-3} for triplicate sea-cages of Atlantic salmon held at HIGH (H) or NORMAL (N) stocking density at day and night during sub-periods from August to November. Significant differences between groups within period are denoted by letters a-d from lowest to highest values based on Student Newman-Keuls post-hoc test.

Figure 5. Median observed fish density (OFD) given as mean±s.d. in kg m^{-3} for triplicate sea-cages of Atlantic salmon held at HIGH (H) or NORMAL (N) stocking density at day and night during sub-periods from August to November. Significant differences between groups within period are denoted by letters a-d from lowest to highest values based on Student Newman-Keuls post-hoc test.

Oppedal et al. Figure 1



Oppedal et al. Figure 2



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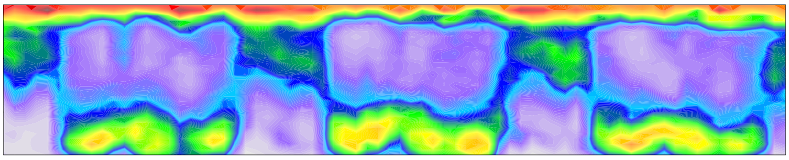
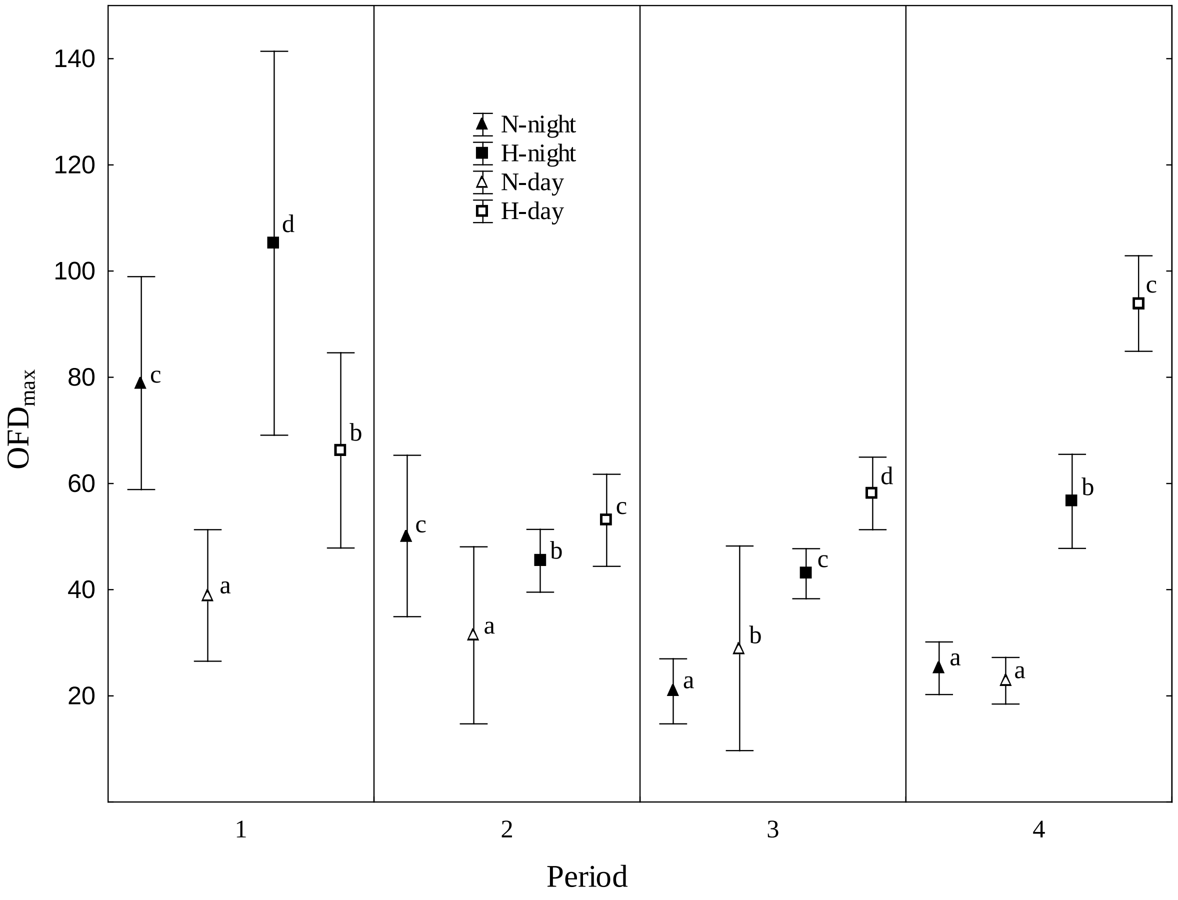


Oppedal et al. Figure 4.



Oppedal et al. Figure 5