Document mentioned under Op 3.1: Protocols for on-growth of Arctic char

Rearing density of Arctic char

Aquaculture conditions and technology are important factors to achieve good production in fish farming industry. For the project ARCTAQUA, Akvatik and Nord in collaboration with the main Arctic char (*Salvelinus alpinus*) producer in Norway, Sigerfjord Fisk AS conducted one experiment to optimize the rearing densities during the culture of the fish. For Sigerfjord, it is essential to gather knowledge about rearing parameters. Among them, finding the optimal stocking density is very important because usually fish farms make a compromise between maximum occupation of available tank space, welfare and growth parameters.

Arctic char maintains low social and agonistic interactions at high stocking densities, and the associated shoaling behaviour allows the fish to preserve its energy and use it to achieve better growth rates (1). To test the hypothesis that there is no difference in growth, fish welfare and water quality associated with the fish stocked at low, medium and high densities, Arctic char was reared for 73 days (from 1 June 2020 to 12 August 2020). The three groups will be referred to as high, medium and low groups. At start, the stocking densities for the duplicate tanks of the high group were 59.2 kg/m^3 and 54.5 kg/m^3; the corresponding values for the medium group were 42.7 and 49.5 kg/m^3, and the values of the low density group were 36.6 and 36.8 kg/m^3 (Table 1). At termination of the experiment in August, the stocking densities increased to 74.2 and 83.3 kg/m^3 for the high group, 47.5 and 71.5 kg/m^3 for medium, and 47.3 and 56.0 kg/m^3 for low (Table 1).

Table 1. Overview of stocking densities for all three experimental groups, at start and end of the experiment. Values are in kg/m^3 for mean ± SD.

Date	High density	Medium density	Low density
Start, 1 June	56.9 ± 2.4	46.1 ± 3.4	36.7 ± 0.1
End, 12 august	78.8 ± 4.6	59.5 ± 12.0	51.7 ± 4.4

For rapid and homogeneous growth, the fish has to be held at stocking densities of 50–120 kg/m^3 in deeper tanks, but the advice is to maintain optimum water quality (2). Hence, in the present study, we have analysed the water quality parameters too. The freshwater flow rate in the tanks (4.5 m diameter and 22 m^3 volume) were in the range 100-120 l/min, being lowest (100 l/min) in the high group. During the experimental period, the high group was fed 649.7 g of feed compared to 557.0 and 538.9 g of the medium and low groups, respectively (Figure 1).

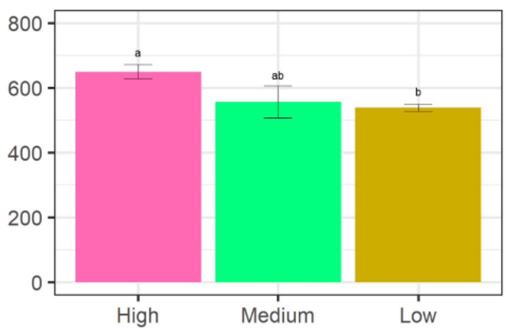

Figure 1. Total feed consumption of the 3 study groups. Y-axis labels are total feed consumption and the values are in kg.

Generalised additive model-based analyses were employed to find differences in fish and feed performance and water quality associated with the study groups. Recorded days were fitted using local regression for temperature, oxygen and total gas pressure. Chi-square test and the associated post-hoc tests were used to analyse the welfare scores. R packages such as gam, emmeans and ggplot2 helped to perform the exploratory and statistical analyses.

Table 2 provides an overview of the fish and feed performance of the different study groups. The initial fish weight of the medium and low groups were comparable. Initial and final weights of the high density group were significantly different compared to those of the other two groups. Previous studies have reported the positive correlation between growth rate and high stocking density of Arctic char (1), and Jobling, Tveiten and Hatlen (3) have stated that “poor and heterogeneous rates of growth during grow-out may be the result of rearing the fish at sub-optimal stocking densities”. At the end of the feeding trial, the weight gain and specific growth rate of the high group were apparently higher, but we did not find any significant differences. The medium density group had poorer feed conversion ratio, though not significantly different from the other groups. We noted that the feed consumption of the low group was significantly lower compared to the high group. During late summer – i.e. the week before the experimental animals were transferred to net pens in sea – Sigerfjord Fisk AS experienced fish mortality in all the study groups. However, the survivability of the 3 study groups were similar.

Table 2. Overview of fish performance during the experimental period of 111 days.

Parameter	High density	Medium density	Low density
Initial weight, g*	108.35 ± 11.25[a]	73.70 ± 3.80[b]	73.85 ± 3.75[b]
Weight, g**	128.00 ± 5.00[a]	85.00 ± 7.00[b]	79.00 ± 10.00[b]
Final weight, g	179.65 ± 24.15[a]	110.40 ± 22.90[b]	110.70 ± 4.20[b]
Weight gain (37 days)[§]	39.83 ± 13.41	28.54 ± 16.36	41.72 ± 12.62
Weight gain (111 days)[§§]	65.27 ± 5.13	48.59 ± 23.41	50.00 ± 1.93
SGR (37 days)[§]	0.89 ± 0.26	0.66 ± 0.35	0.93 ± 0.24
SGR (111 days)[§§]	0.45 ± 0.03	0.35 ± 0.14	0.37 ± 0.01
FCR (37 days)	1.23 ± 0,23	1.73 ± 0.60	1.37 ± 0.34
Survival (%)	99.47 ± 0.26	99.44 ± 0.18	99.43 ± 0.24

*24 April 2021, ** 07 July 2021; § for the period 07 July 2021 to 12 August 2021, §§ for the period 24 April 2021 to 12 August 2021; Weight gain (%) = (Mean final weight in a tank – Mean initial weight in a tank)/Mean initial weight in a tank) x 100; Specific growth rate (SGR, %/day) = 100 x (ln(Mean final weight in a tank)- ln (Mean initial weight in a tank)/Number of feeding days); Feed conversion ratio, FCR = Apparent feed intake in dry basis/(Final biomass - Initial biomass + Dead fish biomass); Survival (%) = (Number of fish at the end of the experiment/Number of fish at the start of the experiment)*100 Data are mean ± sem, and based on the mean values from the duplicate tanks.

Growth rate of Arctic char is also dependent on temperature. Dissolved oxygen and carbon dioxide has biological significance, and the water solubility of these gases is subject to factors such as temperature, salinity and partial pressure gradients (4). The fish grows well at temperatures < 10°C, but the upper limit of 0+ Arctic char that is intended for long-term rearing is 21.5°C (5). Figure 2 shows that the water temperature in the tanks of the three treatment groups followed almost similar paths, and it did not go beyond 16°C. Sæther, Siikavuopio and Jobling (6) has stated that it is possible to obtain good growth when the rearing water temperature is in the range 10-18°C. Figure 3 indicates that the total gas pressure of the study groups did not vary much. Analyses of temperature and total gas pressure data indicated that day is a significant factor and not the groups. As for the dissolved oxygen and carbon dioxide values, the medium density group was associated with significantly lower oxygen and higher carbon dioxide compared to the other two groups (Figures 2 and 3). In addition, the dissolved oxygen in the low group was significantly lower compared to the high group. Date was a significant factor in the carbon dioxide model also. The findings concerning the high oxygen content in the high density group could be indicating the less oxygen consumption during the inactive state adopted by the fish, supported by the lower flow rate for this group.

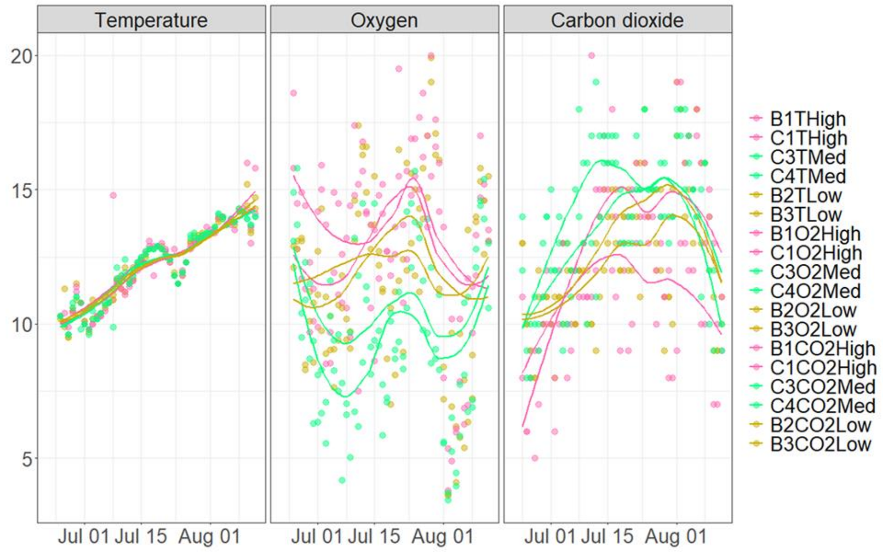

Figure 2. Temperature, oxygen and carbon dioxide in the rearing system. The "loess" smoothing method was employed to generate the curves. Y-axis units are °C for temperature (T), mg/l for oxygen (O_2) and carbon dioxide (CO_2). The suffixes High, Med and Low in the legend indicates the high, medium and low density groups, while B1-B3 and C1, C3, C4 indicate individual tank numbers.

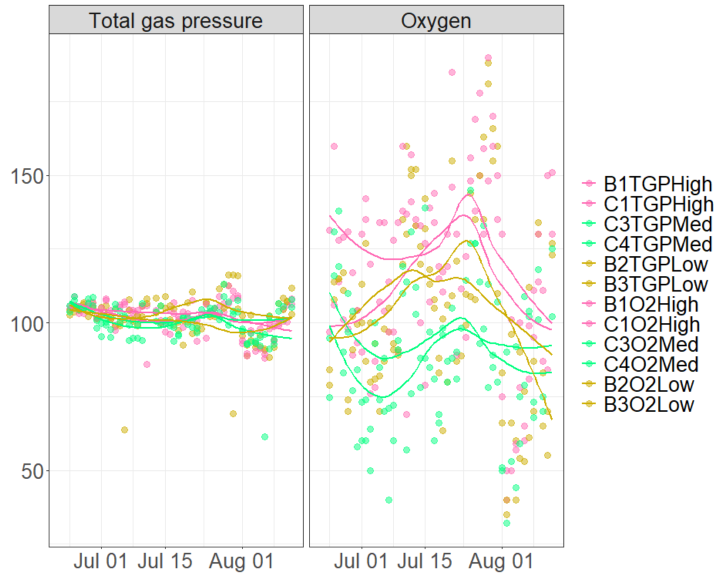

Figure 3. Total gas pressure and oxygen in the rearing system. The "loess" smoothing method was employed to generate the curves. Y-axis unit is %. The suffixes High, Med and Low in the legend indicates the high, medium and low groups, while B1-B3 and C1, C3, C4 indicate individual tank numbers.

Along with the depletion of oxygen content, we find higher values of the nitrogenous wastes associated with the medium density groups. Our analyses detected significant differences for pH and ammonium-nitrogen (Table 3). Both the parameters were significantly higher in the rearing water of the low group compared to the high group. We also found a significantly higher concentration of CO_2 linked to the medium group (Figure 2, table 3). The low group which had a greater pH value (6.4 vs 6.0) also had a CO_2 concentration lower than the medium group. Increase in CO_2 is known to reduce the pH due to an increase in H^+ (7). In all the groups, pH was < 7. Maximum productivity of Arctic char is said to be in the range 6.5-8.5 (6). Only for the low density group, the pH level was near the recommended range. When the rearing water pH is in the range 7-9, CO_2 will be in the bicarbonate form and when pH is >11 carbonate ions will be more in water (6). Since pH in none of the tanks went < 6, we assume that we avoided the risk of free CO_2 acccumulation in the tanks. The recommended CO_2 level for intensive rearing of Arctic char is 10–15 mg/l (8, 9). The levels of both the high and low density group was in this range, but in some instances the values for one of the tanks in the medium group went outside this range (Figure 2). In addition, the water temperature was similar between all tanks and groups, with an average of 12.2°C from 24 June to 12 August, increasing during the experiment (Figure 2).

Recommended safe levels of ammonia and total ammonia levels for Arctic char in freshwater should be < 0.015 mg/L and < 1.0 mg /L, respectively (9, 10). In our study, the ammonium-N in the high density group was significantly lower compared to the low density group. It is known that ammonia toxicity increases with pH and alkalinity, and the low density group had higher ammonium-N, pH and alkalinity. The form, $NH4^+$ is less toxic and high values are associated with acidic solution. Ammonia and ammonium ion in rearing tanks can come from fish and feed decomposition (10). We observed high values of NO_2-N and ammonium (though not significantly different) in the medium density group. If these parameters are not kept in check, it will affect fish welfare (6). The recommended nitrite level for Atlantic salmon is 0.5 mg/L and 0.1 mg/L in the case of seawater and freshwater, respectively (11). In recirculating aquaculture systems, the biofilter helps to keep the nitrite levels in rearing water of Arctic char below 0.2 mg/l (12). It is also known that nitrite absorption can be reduced by increasing the chloride level (13).

Table 3. Water quality parameters in the tanks of the experimental fish at 25 June.

Parameter	High density	Medium density	Low density
pH	6.02 ± 0.00^{a}	6.17 ± 0.18^{ab}	6.43 ± 0.08^{b}
CO_2, mg/L *	12.05 ± 0.85^{b}	13.85 ± 0.45^{a}	12.61 ± 0.33^{b}
Alkalinity, mg/L	10.28 ± 4.03	11.20 ± 2.70	17.52 ± 3.83
Nitrite-N, NO_2-N (mg/L)	0.003 ± 0.003	0.008 ± 0.001	0.003 ± 0.003
Ammonium-N, $NH4^+$-N, (mg/L)	0.21 ± 0.02^{a}	0.33 ± 0.05^{ab}	0.33 ± 0.02^{b}
Ammonium ion, $NH4^+$ (mg/L)	0,28 ± 0,03	0,43 ± 0,07	0,43 ± 0,03

* CO_2 is shown as the mean of daily measurements from 24 July to 12 August.

In the present study, welfare scores such as scale loss, fin damage, gill operculum damage and weight loss were also assessed. However, we found significant differences (in all comparisons) only for scale loss and weight loss (Figure 5). In the case of scale loss, the difference was due to the score 0, and for weight loss it was because of the score 1. High stocking density can inflict stress, abrasion injuries and fin damage to fishes (6, 14). Fish in the high group might not have encountered such undesirable conditions because the number of fish with 0 score was more in this group. Scale loss is listed as one of the signs of stress and bad environment during culture (15). Weight loss in the medium group could be due to reduced feeding adopted because of low oxygen content. We did not find any evidence of a remarkable weight loss in the case of the high density group.

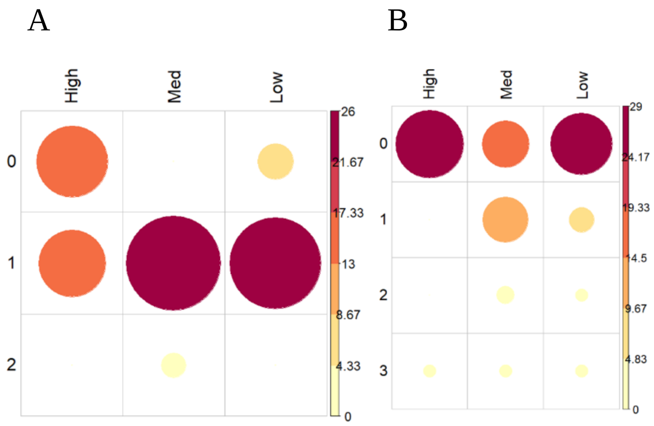

Figure 5. Corrplot showing the differences in the scale loss and weight loss. A) Scale loss, B) Weight loss. The scores 0, 1 and 2 (shown on the left) were employed to understand the parameters of interest. The scale bar on the right side indicates the counts of each score in a particular group. Based on chi-square test and the associated post-hoc, the significant difference is due to the count zero and one for the scale loss and weight loss, respectively.

Conclusion

For this experiment it is concluded that Arctic char can be reared at a stocking density of 78.8 kg/m^3, without compromising growth, welfare scores and water quality parameters.

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