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Effects of biodensity on the growth, stress physiology, and welfare of Arctic charr (*Salvelinus alpinus*) in freshwater☆

Andrew Sevier[a], Richard Smith[a], Tillmann Benfey[c], Roy Danzmann[b], Nicholas Bernier[b], Richard Moccia[a,*]

[a] Aquaculture Centre, Department of Animal Biosciences, University of Guelph, 50 Stone Road, Guelph, Ontario, Canada
[b] Department of Integrative Biology, University of Guelph, 50 Stone Road, Guelph, Ontario, Canada
[c] Department of Biology, University of New Brunswick, P.O. Box 4400, Fredericton, New Brunswick, Canada

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ABSTRACT

Biodensity is a major factor affecting the production and welfare of farmed fishes. Arctic charr (*Salvelinus alpinus*) (average mass 176.9 ± 3.9 g) were held at biodensities of 30, 60, 90, 120, and 150 kg/m^3 (4 replicates per treatment) during a 91 day study which examined key growth, stress physiology, and welfare parameters. During experimentation fish were fed to near satiety, and a random subsample of 20 fish (5 per replicate tank) were collected from each treatment every 21 days. Biodensity was found to have no significant effect on mortality rates or physical fin damage. Growth rates were lower in charr reared at the highest biodensities (120, and 150 kg/m^3), while feed efficiency was negatively affected at both the highest (120, and 150 kg/m^3) and lowest (30 kg/m^3) biodensities. Plasma cortisol indicated that Arctic charr are more stressed at lower biodensities, but was not correlated with growth or feed efficiency measures. The results support an optimal biodensity range for charr culture between 60 and 90 kg/m^3 to optimize production and welfare.

1. Introduction

Arctic charr (*Salvelinus alpinus*) is a coldwater salmonid species that exhibits high growth performance at low temperatures ranging from 0.5–14 °C. Preliminary studies on the growth of Arctic charr show a greater tolerance for higher biodensities when compared with other salmonid species such as rainbow trout (*Oncorhynchus mykiss*) (Wallace et al., 1988; Jørgensen et al., 1993). This unique characteristic provides producers with the potential opportunity to increase their production output by maximizing the biomass of fish per cubic metre of water, otherwise known as biodensity. However, extremes in biodensity, both high and low, have been found to have negative impacts on the growth performance and 'welfare' of stocked fish (Wallace et al., 1988; Turnbull et al., 2008). The most detrimental effects of biodensity often occur through agonistic social interactions and reduced water quality, and these effects appear to have upper and lower biodensity thresholds (MacIntyre et al., 2008). For example, some fish species, specifically salmonids, exhibit increased schooling behaviour and reduced instances of agonistic (stressful) behaviour with increasing densities (Christiansen et al., 1989; Jørgensen and Jobling, 1993). Physiological responses to stress can be quantified and evaluated using validated parameters such as plasma cortisol (Braithwaite and Huntingford, 2013; Moccia, 2013). Using assessments such as these, researchers can easily and effectively evaluate the welfare of fish stocks with respect to the stress levels of the animals. The current study investigates the effects of biodensity on the growth performance, stress physiology and welfare of Arctic charr in order to determine an optimum density for their culture.

2. Materials and methods

2.1. Animals and environmental parameters

This study complied with the ARRIVE guidelines and was carried out in accordance with the criteria set out by the Canadian Council of Animal Care (Braithwaite and Huntingford, 2013; CCAC, 2005), under University of Guelph, Animal Utilization Protocol #3594.

Fraser strain Arctic charr (176.9 ± 3.9 g) were obtained from station stock at the Alma Aquaculture Research Station (AARS), an accredited research facility operated by the University of Guelph located in Elora, Ontario. The photoperiod was maintained with a 12 h light: 12 h dark cycle from 0600 to 1800 h, EST, with a 90 min, gradual phase-in and phase-out period of lighting intensity to simulate normal

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* Corresponding author at: Department of Animal Biosciences, University of Guelph, 50 Stone Road, Guelph, Ontario, Canada N1G 2W1.
E-mail address: rmoccia@uoguelph.ca (R. Moccia).

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dawn and dusk periods, and to avoid the stressful 'light shock' response that occurs when going from dark to instant-on, full light intensity.

2.2. Experimental design

Experimental trials were conducted over a period of 91 days between April and July 2017. Fiberglass treatment tanks (n = 20; dimensions of 1.000 × 1.000 × 0.276 m, mean volume of 0.288m^3) were randomly assigned one of five treatment densities (i.e. 30, 60, 90, 120 or 150 kg/m^3) by a randomized block design for a total of four blocks/replicates per treatment. Initial densities were established using a 2-phase process: firstly, fish were randomly distributed among all 20 fiberglass tanks up to a density of 150 kg/m^3; secondly, individual charr were removed at random from the tanks assigned 120 kg/m^3 and lower in order to obtain the desired treatment densities. Densities were determined by bulk weight of total tank biomass and were adjusted by the random removal of fish (by subtraction of weight) with each sampling.

The nature of this investigation posed a unique consideration with regard to the experimental acclimation period. Since biodensity was both an integral aspect of general husbandry as well as being the experimental variable, a traditional acclimation period with the fish initially held at a common density was not desirable, since the required adjustment in biodensity at the start of the experimental period, would obviously induce an additional and unequal stressor variable in the experimental tanks immediately before the collection of the initial samples. Therefore, in this study, the seven day acclimation period (designated T_a as referred to in the Sampling regimen), with the densities already adjusted prior to the first sample collection, was intended to serve as an acclimation to the general husbandry conditions that would be employed throughout the duration of the study. This seven day, pre-sampling acclimation period was intended to be long enough to reduce any potential effects associated with the experiment husbandry conditions, while remaining short enough to avoid significant treatment (i.e. biodensity) effects before the first, base-line sampling period (designated T_0; refer to Sampling regimen). To confirm this, a full suite of density effect parameters were monitored during this acclimation period. Initial tank biomasses (at T_a) were established by bulk weight of fish added to each replicate tank. Despite being fed a maintenance ration (0.85% biomass daily), a small amount of growth was observed in the charr following the 7-day acclimation period. As such, biodensities were readjusted at T_0 by random removal of fish until the correct biomass (to achieve the required biodensity) was attained. These adjustments were repeated with each sampling date and are visualized in greater detail in Fig. 1 below.

2.3. Density maintenance

Treatment densities were maintained within non-overlapping limits throughout the study by regular, random removal of fish from each tank. A sampling period of 21 days was established to accommodate the average growth of the fish over the course of the experiment. Within each sampling period, the total biomass for each replicate tank was determined and individual fish were then removed at random (by weight) in order to attain the prescribed treatment density required for each replicate tank. Dissolved oxygen concentrations were measured and water inflow rates were adjusted following the density maintenance of each sampling period in order to maintain adequate water quality. Water quality parameters for the duration of the study are reported in the following Table 1:

2.4. Feeding regimen

The fish were fed non-pigmented, Skretting Orient LP 18 aquafeed in a pellet size appropriate for their size. During the acclimation period, fish were fed a ration of 0.85% body weight/day through the use of commercial belt feeders. This was used as a 'maintenance' ration based on Skretting Canada feed energetic charts for salmonids adjusted for 8.5 °C. Fish were not fed for a 24 h period immediately following the 7-day acclimation period, or on the days prior to each sampling event.

Once the experimental period and sampling had begun, subsequent feedings were conducted via 2 days of hand feedings (Monday, Thursday) and 5 days of automated, belt feeding. Ration amount was determined by the hand feeding amounts consumed in which fish were fed to near-satiation in the morning (9:00 h) and afternoon (14:00 h) over a period of 2 h; total feed given (g) was determined and recorded for analyses of growth performance. Hand feedings were performed by the same individual throughout the entire duration of the study in order to maintain consistency with the determination of the feeding endpoints. When not being hand fed, fish were fed via automated belt feeders at a ration equal to 85% of the average 'near satiety' levels determined from the two previous hand feeding sessions. This was done to ensure that all of the feed allocated to each replicate tank was entirely consumed, allowing for the accurate determination of feed consumption and efficiency.

2.5. Sampling regimen

Tissue sampling and data collection were separated into 5 sampling periods (T_0 –T_4). Sample periods were separated by a period of 21 days with the exception of the 7-day acclimation period from T_a-T_0. A subsample of 5 fish was selected at random from each replicate tank (20 per treatment density), immediately removed and placed into a water bath containing a euthanizing dose (600 ppm) of MS-222 within 1 min of removing the tank cover. The fish were exposed to the anaesthetic bath for a period of 3 min prior to measurement and the sampling of blood and tissues before being photographed for fin analyses; specific tissue sampling methods are outlined in detail below. Individual wet mass and fork length data were recorded and whole blood was sampled via caudal puncture and used for the determination of plasma glucose and cortisol. The mass of the charr removed for sampling was taken into account for calculations of density, feed efficiency, and growth.

2.6. Data analyses

2.6.1. Mortality, feeding & growth analyses

Analyses of mortality, feeding and growth were performed on data obtained from each tank (4 replicate tanks per treatment density). Mortalities for each tank were monitored daily and recorded throughout the duration of the study (T_a – T_4). The total tank biomass of fish for each replicate, at the time of sampling, was used for the determination of feed efficiency and growth. Following sampling and the subsequent density maintenance, the adjusted biomasses were then used to determine growth throughout the next 21-day period. Feed input was determined for each sampling period from data obtained after each scheduled hand-feeding session. Feed efficiency, FE (gain:feed) for each replicate tank was determined using measurements of total tank biomass and feed input between each sampling period:

$$\boldsymbol{FE} = \frac{Wt_f - Wt_i}{Feed\ Input}$$

where Wt_f is final tank biomass (kg), Wt_i is the initial tank biomass (kg) and Feed Input is the amount of feed consumed by the fish between sampling periods.

Thermal-unit growth coefficients (TGCs) were calculated for each replicate tank based on the mass gained between samples, water temperature, and the number of days between samples:

$$\boldsymbol{TGC} = \frac{\boldsymbol{Wt_f}^{\frac{1}{3}} - \boldsymbol{Wt_i}^{\frac{1}{3}}}{\boldsymbol{T} \times \boldsymbol{t}} \times \mathbf{100}$$

where Wt_f is final tank biomass (kg), Wt_i is the initial tank biomass (kg), T is the average water temperature (°C) and t is the time between

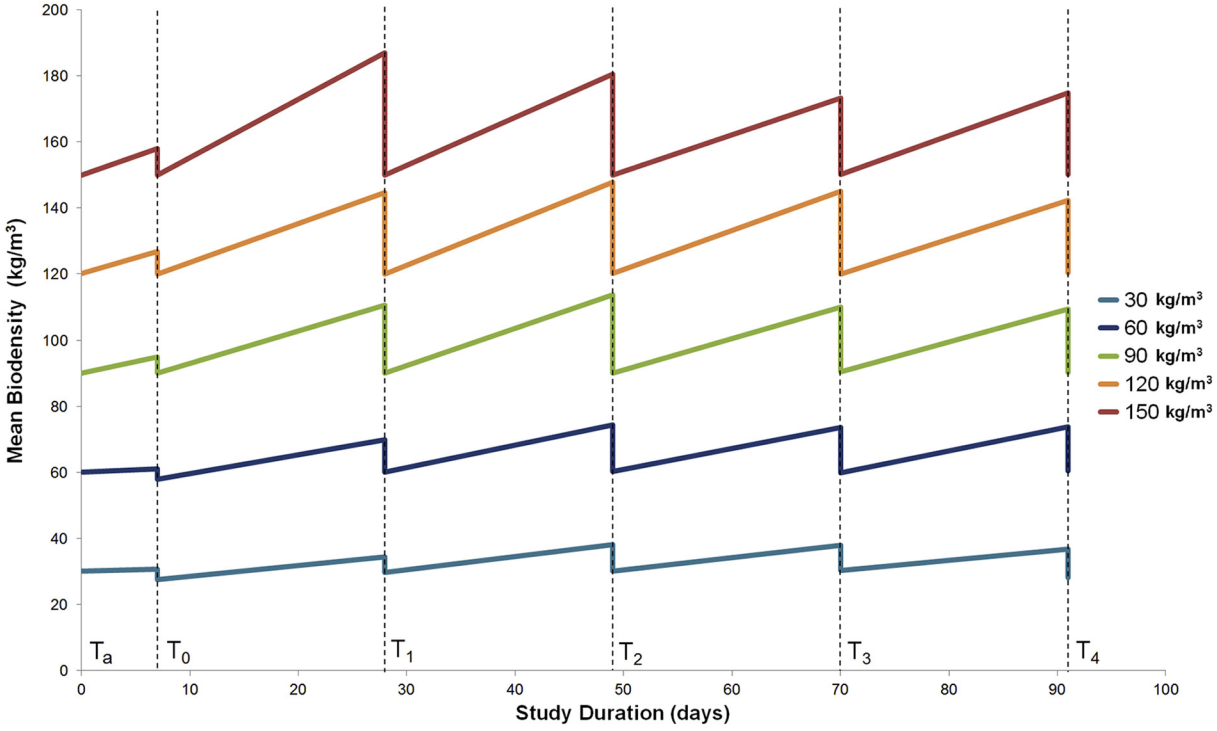


Fig. 1. – Change in mean tank biodensity (kg/m^3) for each treatment density recorded at each sample period throughout the duration of the study.

Table 1
- Mean (± SEM, where applicable) physical/chemical water quality parameters at the Alma Aquaculture Research Station (AARS) over the duration of the study (April – July 2017).

Variable	Measured Value(s)
Physical Parameters	
Water Temperature[a]	8.5 ± 0.18 °C
pH	8.21
Conductivity	506 µmhos/cm
Total Dissolved Solids (TDS)	311 mg/L
Turbidity	0.13 NTU
Alkalinity, Total	242 mg/L
Anions and Nutrients (mg/L)	
[Nitrate]	0.482
[Nitrite]	< 0.01
[Ammonia]	< 0.02
[Chloride]	7.72
[Fluoride]	0.199
[Sulfate]	31.6
[Dissolved Orthophosphate] (P)	< 0.003
Organic/Inorganic Carbon	
Total Organic Carbon (TOC)	1.4 mg/L
Lighting	
Average Intensity[a]	331.8 ± 7.6 lx
Photoperiod	12 h light:dark

[a] Mean ± SE.

sampling periods.

Fulton's Condition Factor (CF) for individual fish was calculated for each sampling period using the equation:

$$CF = \frac{Wt_{Wet}}{L^3} * 100$$

where Wt_{Wet} is wet mass (g) and L = fork length (cm).

2.6.2. Fin damage analyses

Dorsal and caudal fin damage was determined using a validated 6 point macroscopic key (ranging from 0 = no damage, through 5 = heavily damaged) outlined by Hoyle et al. (2007) for use in rainbow trout, a closely related salmonid species with nearly identical fun anatomy. Fins were assigned a score based on the degree of damage observed in the whole body photographs (fins extended) for each fish sampled at the end of each 21-day period.

2.6.3. Physiological analyses

Plasma glucose was measured on individual blood samples using whole blood via a 'OneTouch Verio' handheld glucometers (Lifescan Inc., California, USA). Samples of whole blood (1.0–1.5 mL) were collected via caudal puncture using syringes with 23 gauge needles pretreated with the anticoagulant sodium EDTA (0.5 M). Immediately following sampling, the needles were removed from the syringes and a small droplet of whole blood was deposited onto a fresh test strip and glucose reported in real time in units of mmol/L (mM). The remaining whole blood was then carefully expelled into labeled Eppendorf tubes and placed on ice until all samples were obtained. The blood samples were then centrifuged at 14,000 rpm (12,700 x g) for 5 min. The resultant plasma was decanted into fresh Eppendorf tubes and placed on ice and stored at −25 °C for the analysis of plasma cortisol.

Plasma cortisol was determined using a radioimmunoassay (RIA) developed for cortisol in rainbow trout outlined by Bernier et al. (2008). Frozen plasma samples were assessed in triplicate by a 3-parameter sigmoidal curve regression equation of a standard curve. The following modifications were made to assess plasma cortisol in Arctic charr: diluted mouse monoclonal anti-cortisol antibody (cat # XM210, Abcam, Cambridge, UK) was used in place of rabbit antibody; antibody concentration was adjusted such that 40% of radiolabeled cortisol was bound; the lower detection limit was 65 pg/mL; cortisol was measured in unextracted plasma that was diluted 5 times in assay buffer in order to fall within the 20–80% range of the standard curve.

2.7. Statistical analyses

Data were analyzed via randomized block design using the GLIMMIX procedure in SAS, University Edition 9.4 for Windows 7 (SAS Institute Inc., Cary, NC, USA). This was followed by a Least Significant

Difference (LSD) post-hoc test for multiple comparisons in order to determine differences among treatment densities within a sampling period, and to assess trends over time within a given treatment.

Measurements were obtained from the subsamples of 5 fish per treatment tank ($n = 4$), within each sample period and were used to calculate the standard error of the mean (SEM). Replicate tank means were first assessed for differences in order to confirm that there were no tank effects present, before data was pooled for further analyses.

Dorsal and caudal fin damage scores were assessed using the Odds Ratios command to determine differences in mean values within sample periods.

Potential correlations were analyzed using the Pearson Correlation procedure. Sample data for each treatment density, from each sample period ($T_0 – T_4$), were assessed; the correlation coefficients (r) and their associated *P*-values are presented in table format.

3. Results

3.1. Biodensity maintenance

Densities for each tank were determined using measures of total tank biomass and tank water volume recorded at each sample period. Fig. 1 depicts the fluctuation in mean tank biodensity over time, and confirms that treatment biodensities did not overlap each other for the duration of the study:

Although the nominal treatment biodensities were set at 30, 60, 90, 120, and 150 kg/m^3, it is apparent (see Fig. 1) that the charr grew between each sample period throughout the course of the study. This resulted in average biodensities in the treatment groups of 32.4, 65.1, 99.0, 130.7, and 162.4 kg/m^3 respectively. For the purposes of the analyses and discussion we will continue to refer to the nominal treatment biodensities defined above.

3.2. Mortality and fin damage

Table 2 presents total treatment mortalities, and the results of the analyses of fin damage (caudal and dorsal) for each sample period and treatment density:

Mortalities were negligible across all replicate tanks with total recorded levels of 0.17% ($n = 5$ mortalities / 2943 charr stocked at T_a).

There was no effect on caudal fin damage throughout the study duration ($T_0 – T_4$), across all density treatment groups. Biodensity was found to have small, mixed effects on dorsal fin damage at T_3, though these effects were not persistent outside of this one sample period. Both caudal and dorsal scores were unaffected by biodensity by the end of the study (T_4).

3.3. Feeding & growth performance

Table 3 presents the results of the analyses of mean body mass (g):

Biodensity was found to have no effect on the whole body mass (g) of Arctic charr, irrespective of sample period. The charr were stocked at an initial mean body mass of 177.85 ± 10.06 g. During the acclimation period ($T_a – T_0$) there was no significant increase in mass in any of the treatment densities, thus there was no difference in body mass across all treatment densities. There was a net increase in mass between T_0 and T_4 but there were no significant differences in the mean body mass between the different biodensities at each sampling time.

Table 4 presents the results of the analyses of fork length (cm):

Changes in fork length followed a similar pattern as wet body mass. The charr were stocked at an initial, mean fork length of 24.75 ± 0.37 cm. During the acclimation period ($T_a – T_0$) there was no significant increase in any of the biodensities so that, like total wet mass, at T_0, there was no difference in fork length across all treatment densities. As with wet body mass there was a net increase in fork length between T_0 and T_4 but, again like wet mass, there were no significant differences in the mean fork length between the different biodensities at each sampling time.

The results of the analyses of condition factor (CF) and eviscerated carcass mass (g) are shown in Table 5:

There were some subtle and transient (but statistically significant) effects of density on condition factor (CF). The mean CF of the AARS station stock (original population allocation) at T_a was 1.15 ± 0.03. During acclimation ($T_a – T_0$), CF decreased in charr stocked at 60 kg/m^3 but in no other density. Thus, at T_0, the charr stocked at 60 kg/m^3 were found to have a slightly lower CF than the charr stocked at 120 kg/m^3.

Table 2

- Total experimental mortalities and mean (± SEM) caudal and dorsal fin damage for each treatment density at each sampling period; similar characters indicate the mean values are statistically similar ($\alpha = 0.05$) within a sample period (across a row).

		Parameter	Nominal Biodensity (kg/m^3)					Biodensity Effects
			30	60	90	120	150	*p-value*
Original Population Average		**Fin Damage**						
		Caudal	1.40 ± 0.25					–
		Dorsal	1.87 ± 0.26					–
Sample Period	T_0	Mortality	0	0	0	0	0	–
		Fin Damage						
		Caudal	0.85 ± 0.15^a	0.75 ± 0.14^a	0.75 ± 0.20^a	0.85 ± 0.21^a	1.25 ± 0.19^a	0.2251
		Dorsal	1.05 ± 0.23^A	1.10 ± 0.025^A	0.90 ± 0.20^A	1.20 ± 0.33^A	1.20 ± 0.25^A	0.9739
	T_1	Mortality	0	0	0	0	0	–
		Fin Damage						
		Caudal	0.60 ± 0.15^a	0.85 ± 0.13^a	0.60 ± 0.21^a	1.05 ± 0.18^a	0.70 ± 0.19^a	0.1606
		Dorsal	0.70 ± 0.18^A	0.90 ± 0.23^A	1.05 ± 0.25^A	1.35 ± 0.26^A	0.90 ± 0.22^A	0.4441
	T_2	Mortality	0	0	0	0	1	–
		Fin Damage						
		Caudal	1.25 ± 0.18^a	0.95 ± 0.15^a	1.20 ± 0.17^a	0.95 ± 0.15^a	0.80 ± 0.14^a	0.2217
		Dorsal	0.90 ± 0.20^A	0.75 ± 0.22^A	1.15 ± 0.26^A	0.85 ± 0.22^A	1.20 ± 0.24^A	0.6608
	T_3	Mortality	0	1	1	1	1	–
		Fin Damage						
		Caudal	1.35 ± 0.20^a	1.80 ± 0.20^a	1.70 ± 0.25^a	1.65 ± 0.17^a	1.10 ± 0.14^a	0.1148
		Dorsal	0.55 ± 0.18^B	1.75 ± 0.32^A	1.05 ± 0.22^{AB}	1.40 ± 0.22^A	1.25 ± 0.24^A	0.4296
	T_4	Mortality	0	0	0	0	0	–
		Fin Damage						
		Caudal	1.45 ± 0.15^a	1.50 ± 0.20^a	1.40 ± 0.20^a	1.25 ± 0.16^a	1.15 ± 0.13^a	0.6092
		Dorsal	1.00 ± 0.25^A	0.95 ± 0.20^{A*}	1.30 ± 0.29^A	1.50 ± 0.27^A	1.50 ± 0.29^A	0.5043

Table 3
- Treatment mean (± SEM) body mass (g) for each treatment density at each sampling period. Similar superscript characters indicate statistical similarities (α = 0.05) within each sample period (across a row). Similar subscript characters indicate statistical similarities (α = 0.05) within each treatment density over time (down a column).

	Original Population Average	177.85 ± 10.06					
	Sample Period	Nominal Biodensity (kg/m^3)					Biodensity Effects
		30	60	90	120	150	*p-value*
Body mass (g)	T_0	$_{d}177.85 \pm 8.09^{a}$	$_{E}171.69 \pm 5.75^{a}$	$_{d}179.2 \pm 6.25^{a}$	$_{D}183.85 \pm 4.28^{a}$	$_{d}180.79 \pm 5.76^{a}$	0.651
	T_1	$_{c}236.15 \pm 14.35^{A}$	$_{D}214.85 \pm 8.14^{A}$	$_{c}238.18 \pm 12.78^{A}$	$_{C}247.75 \pm 9.20^{A}$	$_{c}232.19 \pm 11.06^{A}$	0.4523
	T_2	$_{c}282.86 \pm 21.16^{A}$	$_{C}270.53 \pm 14.31^{A}$	$_{b}282.85 \pm 13.50^{A}$	$_{C}274.77 \pm 13.09^{A}$	$_{c}262.81 \pm 14.42^{A}$	0.9199
	T_3	$_{b}348.17 \pm 25.05^{A}$	$_{B}342.68 \pm 24.78^{A}$	$_{a}373.72 \pm 23.25^{A}$	$_{B}331.09 \pm 10.68^{A}$	$_{b}326.23 \pm 9.94^{A}$	0.758
	T_4	$_{a}488.93 \pm 30.23^{A}$	$_{A}417.29 \pm 28.89^{A}$	$_{a}435.91 \pm 25.44^{A}$	$_{A}415.02 \pm 18.63^{A}$	$_{a}397.38 \pm 14.75^{A}$	0.4073
	Temporal effects p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

Table 4
- Treatment mean (± SEM) fork length (cm) for each treatment density at each sampling period. Similar superscript characters indicate statistical similarities (α = 0.05) within each sample period (across a row). Similar subscript characters indicate statistical similarities (α = 0.05) within each treatment density over time (down a column).

	Original Population Average	24.75 ± 0.37					
	Sample Period	Nominal Biodensity (kg/m^3)					Biodensity Effects
		30	60	90	120	150	*p-value*
Fork length (cm)	T_0	$_{D}25.13 \pm 0.33^{a}$	$_{c}25.18 \pm 0.28^{a}$	$_{D}25.21 \pm 0.30^{a}$	$_{d}25.26 \pm 0.22^{a}$	$_{D}25.22 \pm 0.24^{a}$	0.9961
	T_1	$_{C}26.69 \pm 0.42^{a}$	$_{c}26.39 \pm 0.28^{a}$	$_{C}26.80 \pm 0.44^{a}$	$_{c}27.40 \pm 0.32^{a}$	$_{C}26.58 \pm 0.39^{a}$	0.4462
	T_2	$_{C}27.80 \pm 0.50^{a}$	$_{b}27.91 \pm 0.41^{a}$	$_{B}28.63 \pm 0.72^{a}$	$_{c}28.17 \pm 0.46^{a}$	$_{B}27.80 \pm 0.38^{a}$	0.7996
	T_3	$_{B}29.11 \pm 0.58^{a}$	$_{a}29.48 \pm 0.60^{a}$	$_{A}30.25 \pm 0.51^{a}$	$_{b}29.66 \pm 0.30^{a}$	$_{A}29.93 \pm 0.25^{a}$	0.7712
	T_4	$_{A}31.96 \pm 0.53^{a}$	$_{a}30.57 \pm 0.53^{a}$	$_{A}31.19 \pm 0.46^{a}$	$_{a}30.98 \pm 0.46^{a}$	$_{A}30.87 \pm 0.36^{a}$	0.3767
	Temporal effects p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

Between T_0 and T_1 there was an increase in the CF of charr stocked at 30, 90, and 150 kg/m^3 such that, at T_1, the charr stocked at 150 kg/m^3 had a higher CF than the charr stocked at 60 kg/m^3. At T_2 the CF was similar in all densities. At T_3 the CF of charr stocked at 30 and 90 kg/m^3 was greater than the CF of charr stocked at 150 kg/m^3 but by T_4 there was again no difference in CF between the stocking biodensities. Meanwhile, biodensity was found to have no effect on mean eviscerated carcass mass (g), irrespective of the sample period.

Fig. 2 shows the results of the analyses of feeding efficiency (FE); panel A depicts the effects of biodensity at each sample period; panel B depicts the effects of each treatment density over the course of the study (T_a – T_4):

Between sample periods T_1 and T_4, FE was consistently the highest (or equal to the highest) in charr stocked at 60 kg/m^3. Meanwhile, the lowest (or equal to the lowest) values of FE were consistently observed in the 30 and 150 kg/m^3 densities.

Fig. 3 shows the results of the analyses of TGC (depicted and annotated in the same manner as FE):

Biodensity showed mixed effects on mean TGC at T_1; charr stocked at 30 and 150 kg/m^3 were found to have equally the highest TGC, but not different from the 90 kg/m^3 treatment. Mean TGCs were lowest in the 60 and 120 kg/m^3 densities during this interval. For the remainder of the study (T_2 – T_4), the highest (or equal highest) values were observed in the 30 kg/m^3 treatment while the 150 kg/m^3 density exhibited the lowest (or equal lowest) values during this interval.

3.4. Stress physiology

Fig. 4 shows the results of the analyses of plasma cortisol (depicted and annotated in the same manner as TGC):

Between sample periods T_0 and T_4, plasma cortisol was consistently the highest (or equal highest) in charr stocked at 30 kg/m^3 and consistently lowest (or equal lowest) cortisol concentrations were consistently seen in charr stocked at 150 kg/m^3.

Fig. 5 shows the results of the analyses of plasma glucose (depicted and annotated in the same manner as plasma cortisol:

Biodensity showed mixed effects on mean plasma glucose at T_0; the values seen in the 60, 120, and 150 kg/m^3 densities were similar to those seen in the 30 kg/m^3 (highest) and 90 kg/m^3 (lowest) densities. For the remainder of the study (T_1 – T_4), biodensity was found to have no effect on circulating plasma glucose.

3.5. Welfare parameters and correlates

Correlation analyses were performed comparing concentrations of plasma cortisol to either growth, physiology, or other welfare-related parameters. The results of these analyses are presented in matrix tables depicting the correlations performed for each treatment density at each sample period. Significant correlations are indicated in bold, and by the addition of lines of best fit to estimate slope. An example can be seen in Table 6 where correlations between plasma cortisol and TGC are assessed:

There were no correlations, at any sampling period, for fish stocked at 30, 60, and 90 kg/m^3. TGC and plasma cortisol were positively correlated fish stocked at 120 kg/m^3 sampled at T_1 and in fish stocked at 150 kg/m^3 sampled at T_2. However, these correlations were not observed at any other sample periods.

No correlations were observed between cortisol and FE for any treatment densities irrespective of sample period.

There were also no consistent correlations between fin damage (caudal or dorsal) and plasma cortisol. There were positive correlations in the 30 kg/m^3 charr at T_1 and in the 90 kg/m^3 treatment at T_0 for the caudal and dorsal fins respectively. However, these did not persist outside their respective sample periods and were not present by the end of the study (T_4).

And finally, there were no consistent correlations between plasma

Table 5
- Treatment mean (± SEM) condition factor (CF) and eviscerated carcass mass (g) for each treatment density at each sampling period. Similar superscript characters indicate statistical similarities (α = 0.05) within each sample period (across a row). Similar subscript characters indicate statistical similarities (α = 0.05) within each treatment density over time (down a column).

		Parameter	Nominal Biodensity (kg/m^3)					Biodensity Effects
			30	60	90	120	150	*p-value*
Original Population Average		Condition factor (CF)	1.15 ± 0.03					–
		Eviscerated carcass mass (g)	157.63 ± 8.14					–
Sample Period	T_0	Condition factor (CF)	$_{c}1.11 \pm 0.02^{AB}$	$_{D}1.07 \pm 0.01^{B}$	$_{c}1.11 \pm 0.02^{AB}$	$_{C}1.15 \pm 0.04^{A}$	$_{c}1.12 \pm 0.02^{AB}$	0.2072
		Eviscerated carcass mass (g)	$_{d}157.92 \pm 7.32^{A}$	$_{E}154.34 \pm 5.44^{A}$	$_{c}158.52 \pm 5.79^{A}$	$_{D}161.87 \pm 3.97^{A}$	$_{d}158.75 \pm 5.18^{A}$	0.8693
	T_1	Condition factor (CF)	$_{b}1.22 \pm 0.02^{AB}$	$_{CD}1.16 \pm 0.02^{B}$	$_{b}1.21 \pm 0.02^{AB}$	$_{C}1.18 \pm 0.02^{AB}$	$_{b}1.23 \pm 0.02^{A}$	0.1800
		Eviscerated carcass mass (g)	$_{c}206.15 \pm 12.29^{A}$	$_{D}190.06 \pm 7.02^{A}$	$_{b}210.01 \pm 11.63^{A}$	$_{C}219.18 \pm 8.11^{A}$	$_{c}203.37 \pm 9.54^{A}$	0.4641
	T_2	Condition factor (CF)	$_{b}1.26 \pm 0.05^{A}$	$_{BC}1.23 \pm 0.04^{A}$	$_{b}1.21 \pm 0.04^{A}$	$_{BC}1.21 \pm 0.01^{A}$	$_{b}1.20 \pm 0.03^{A}$	0.7459
		Eviscerated carcass mass (g)	$_{c}244.41 \pm 18.15^{A}$	$_{C}233.75 \pm 11.98^{A}$	$_{b}247.07 \pm 12.28^{A}$	$_{C}242.24 \pm 11.72^{A}$	$_{c}232.37 \pm 12.45^{A}$	0.9346
	T_3	Condition factor (CF)	$_{b}1.42 \pm 0.12^{A}$	$_{B}1.28 \pm 0.03^{AB}$	$_{a}1.31 \pm 0.03^{A}$	$_{B}1.26 \pm 0.02^{AB}$	$_{b}1.21 \pm 0.02^{B}$	0.1812
		Eviscerated carcass mass (g)	$_{b}303.71 \pm 21.97^{A}$	$_{B}300.17 \pm 22.15^{A}$	$_{a}328.96 \pm 20.46^{A}$	$_{B}292.40 \pm 9.46^{A}$	$_{b}287.77 \pm 8.86^{A}$	0.7427
	T_4	Condition factor (CF)	$_{a}1.45 \pm 0.04^{A}$	$_{A}1.41 \pm 0.05^{A}$	$_{a}1.41 \pm 0.03^{A}$	$_{A}1.38 \pm 0.03^{A}$	$_{a}1.34 \pm 0.02^{A}$	0.4060
		Eviscerated carcass mass (g)	$_{a}428.84 \pm 25.81^{A}$	$_{A}365.47 \pm 25.09^{A}$	$_{a}382.43 \pm 21.97^{A}$	$_{A}366.47 \pm 16.72^{A}$	$_{a}350.14 \pm 12.97^{A}$	0.3762
Temporal Effects	*p-value*	Condition factor (CF)	0.0005	0.0002	0.0002	< 0.0001	0.0002	
		Eviscerated carcass mass (g)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	

glucose and cortisol. Plasma glucose and cortisol were negatively correlated in fish stocked at 60 kg/m^3 at sample T_0 (the acclimation period) but there were no correlations of plasma glucose and cortisol in fish held at a density of 60 kg/m^3 thereafter. The 150 kg/m^3 treatment exhibited a positive correlation at T_2. However, these correlations were not observed in any of the other densities at any sampling time.

4. Discussion

This study investigated the effects of biodensity on the growth performance and stress physiology of Arctic charr, with an emphasis on key welfare parameters. This was done in order to determine a desirable biodensity at which there is optimal production performance (growth, feed efficiency, etc.) without negatively affecting the welfare of these animals. Somewhat surprisingly, the wide range of biodensities observed here, was found to have little effect on mortality, whole body mass, fork length, and plasma glucose, though there were significant effects on their growth (TGC), feed efficiency, condition factor, and plasma cortisol.

4.1. Mortality, feeding & growth performance

Studies on the effects of biodensity on Arctic charr aquaculture have been performed with densities ranging as low as 15 kg/m^3 to those in excess of 120 kg/m^3 (Wallace et al., 1988; Jørgensen and Jobling, 1993; Jørgensen et al., 1993). The suggested biodensity for Arctic charr culture is anywhere between 40 and 120 kg/m^3 for optimal growth and reduced conspecific aggression (Johnston, 2002). Though there have been studies examining the potential of an 'optimal' biodensity for Arctic charr, the emphasis of these studies was usually on production traits and did not include any assessment of the welfare of these animals (Wallace et al., 1988; Jørgensen and Jobling, 1993; Jørgensen et al., 1993; Johnston, 2002). As such, the present study provides a natural extension to these earlier studies by investigating the effects of biodensity on the growth performance and stress physiology, as well as the welfare of Arctic charr in order to determine an optimum density for their culture.

The nominal mortalities observed across all treatment densities is indicative of the biodensity tolerance of Arctic charr and demonstrates that this species can be cultured at densities ranging from 30 to 150 kg/m^3 with minimal losses. These very low rates of mortality are consistent with other studies examining the effect of biodensity on Arctic charr, with densities ranging from 20 to 150 kg/m^3 (Christiansen et al., 1991; Jørgensen and Jobling, 1993; Summerfelt et al., 2004).

The whole body mass, length, and CF of Arctic charr were unaffected by biodensities up to 150 kg/m^3; i.e. at densities beyond the optimal range for feeding and growth. In fact these data suggest that a high stocking density, such as 150 kg/m^3, if anything promote proportioned growth in non-somatic tissues.

Though growth performance is often reported as units of specific growth rate (SGR), the present study measured growth rate in terms of thermal-unit growth coefficient (TGC) due to its capacity to compare growth across different temperature ranges (Iwama and Tautz, 1981; Jobling, 1994; Lyytikäinen and Jobling, 1998. Studies examining the effects of biodensity on rainbow trout have shown very poor growth rates and feed conversion efficiency when fish were stocked at high densities. However in Arctic charr, several studies have observed the poorest growth in fish held at densities as low as 5–10 kg/m^3 (Wallace et al., 1988; Baker and Ayles, 1990). Wallace et al. (1988) proposed an optimal range between 130 and 170 kg/m^3 with growth in juvenile fish being positively correlated to biodensity, whereas Baker and Ayles (1990) argue that this correlation is reversed when densities exceed 40–50 kg/m^3. In a study by Christiansen et al. (1991), growth in Arctic charr was negatively affected at densities exceeding 110 kg/m^3 and optimized when fish were held at densities of 23–70 kg/m^3. The perturbation to growth and feed intake of the current study were observed

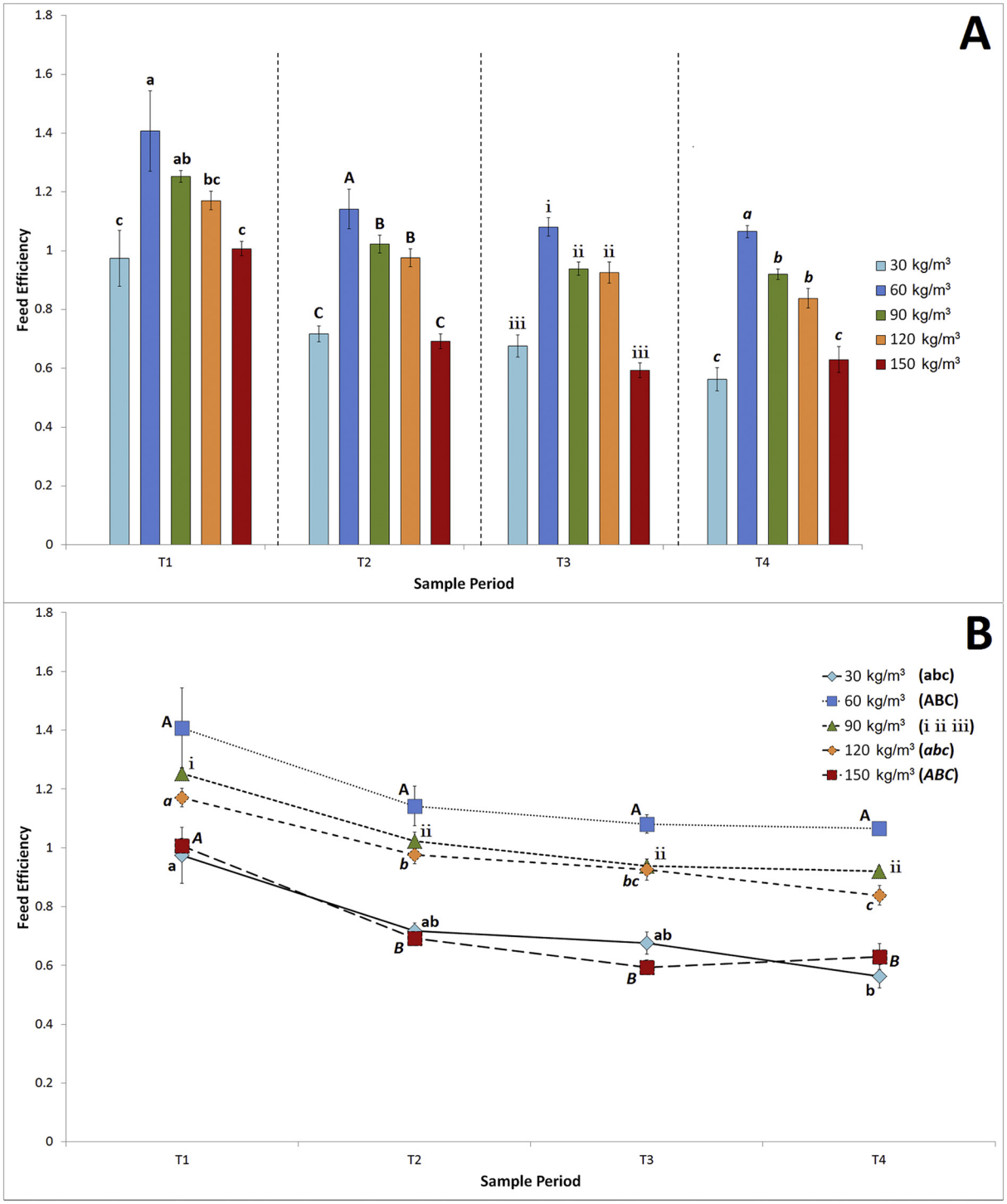


Fig. 2. – Panel A – mean (± SEM) feed efficiency (FE) for each treatment density compared within each sampling period; similar characters indicate the mean values are statistically similar (α = 0.05) within a sample period; asterisks (*) indicate the effect of acclimation on means at T_0 and differences in means from the station stock sample from $T_{1–4}$. Panel B – mean FE compared within each density over the study duration; similar characters indicate the mean values are statistically similar (α = 0.05) within treatment densities, over time.

at a slightly higher biodensity than those reported by Christiansen et al. (1991). However, it should be noted that the slight disparity between the upper limits of biodensity reported by Christiansen et al. (1991) could also be explained by the use of younger fish in the previous study. The present results are also supported by results published by Jørgensen et al. (1993) and Brännäs and Linnér (2000) in which growth rate of Arctic charr was found to increase with increasing density from 15 to 120 kg/m^3.

The results of the FE analyses indicate that medium-to-high biodensities may be optimal for feeding Arctic charr in freshwater production systems, based on the efficiency of feed conversion. The feed efficiency of fish held at 60 kg/m^3 (1.07 ± 0.02) was found to be slightly higher than those reported in other studies (0.69–0.98) examining the growth performance of Arctic charr (Tabachek, 1988; Yang and Dick, 1994). Thus, it is evident that densities below 60 kg/m^3 and those in excess of 120 kg/m^3 result in perturbations of feeding and

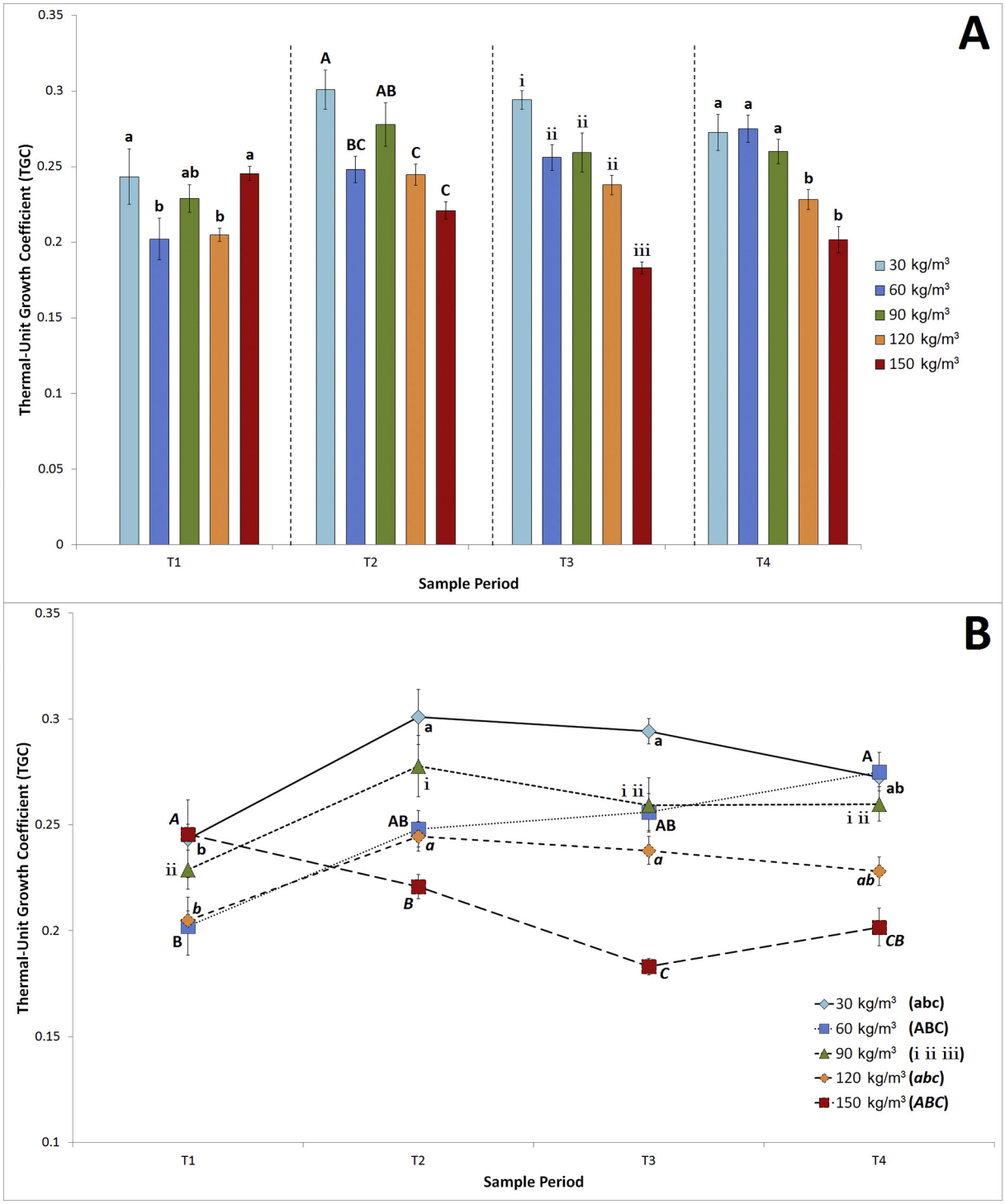


Fig. 3. – Panel A – mean (± SEM) thermal-unity growth coefficient (TGC) for each treatment density compared within each sampling period; similar characters indicate the mean values are statistically similar (α = 0.05) within a sample period; asterisks (*) indicate the effect of acclimation on means at T_0 and differences in means from the station stock sample from $T_{1–4}$. Panel B – mean TGC compared within each density over the study duration; similar characters indicate the mean values are statistically similar (α = 0.05) within treatment densities, over time.

growth performance, and suggest that these parameters may be optimized within this range of densities. Overall, optimal feeding efficiency and growth of Arctic charr was achieved at nominal stocking biodensities of 30–90 kg/m^3 (actuals of 32.4–99.0 kg/m^3) whereas higher densities may even promote enhanced animal welfare, as defined by plasma cortisol.

4.2. Stress physiology

The majority of plasma cortisol levels observed in this study are consistent with those of non-stressed salmonids which are below 10 ng/mL (Pickering and Pottinger, 1989; Øverli et al., 1999a, b), though some treatments did exceed this threshold over the course of the study. The levels observed in the present study are similar to those published in a study by Laursen et al. (2013) investigating the effects of stocking

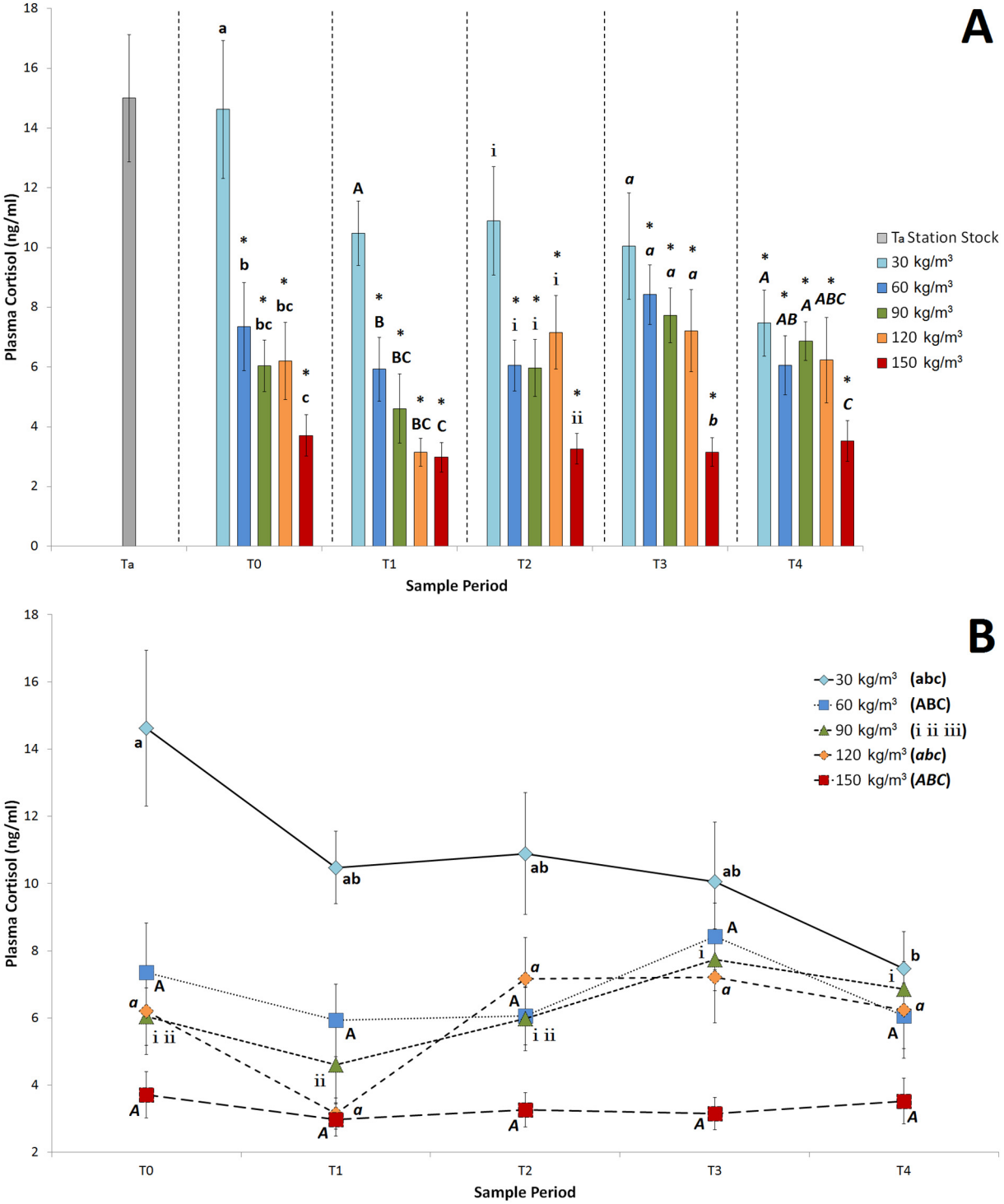


Fig. 4. – Panel A – mean (± SEM) plasma cortisol for each treatment density compared within each sampling period; similar characters indicate the mean values are statistically similar (α = 0.05) within a sample period; asterisks (*) indicate the effect of acclimation on means at T_0 and differences in means from the station stock sample from $T_{1–4}$. Panel B – mean plasma cortisol compared within each density over the study duration; similar characters indicate the mean values are statistically similar (α = 0.05) within treatment densities, over time.

density in rainbow trout, which found no difference in mean plasma cortisol in farmed rainbow trout reared at densities ranging from 25 to 140 kg/m^3. However, Laursen et al. (2013) did find evidence of increased serotonergic brain activity with higher densities which has been associated with reduced growth and feed consumption in several salmonids (Øverli et al., 1999a; Chaouloff, 2000). In a similar study involving rainbow trout, Procarione et al. (1999) examined the effect of biodensity on the physiological stress response following an acute stress challenge. Fish were exposed to air for a period of 1 min and the results indicate that biodensity was found to have no effect on the levels of serum cortisol. However, both cortisol and glucose levels were higher in fish held at low densities after a period of 24 h while no such difference was observed in the higher densities. A decline in mean plasma cortisol (ng/mL) was observed in all densities, following the acclimation period, with the exception of fish stocked at 30 kg/m^3 which displayed levels comparable to those displayed by fish sampled at T_a. It should be noted

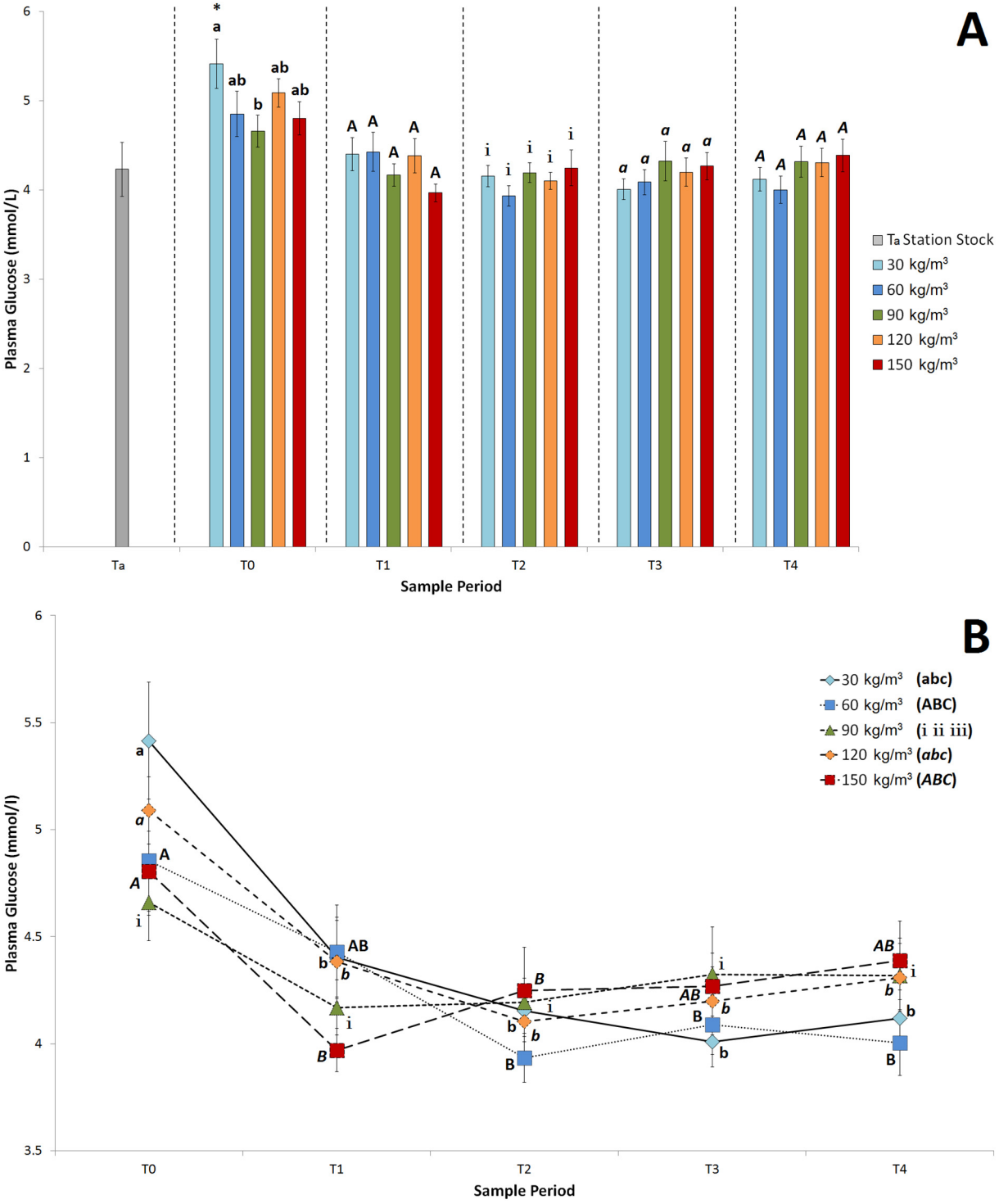
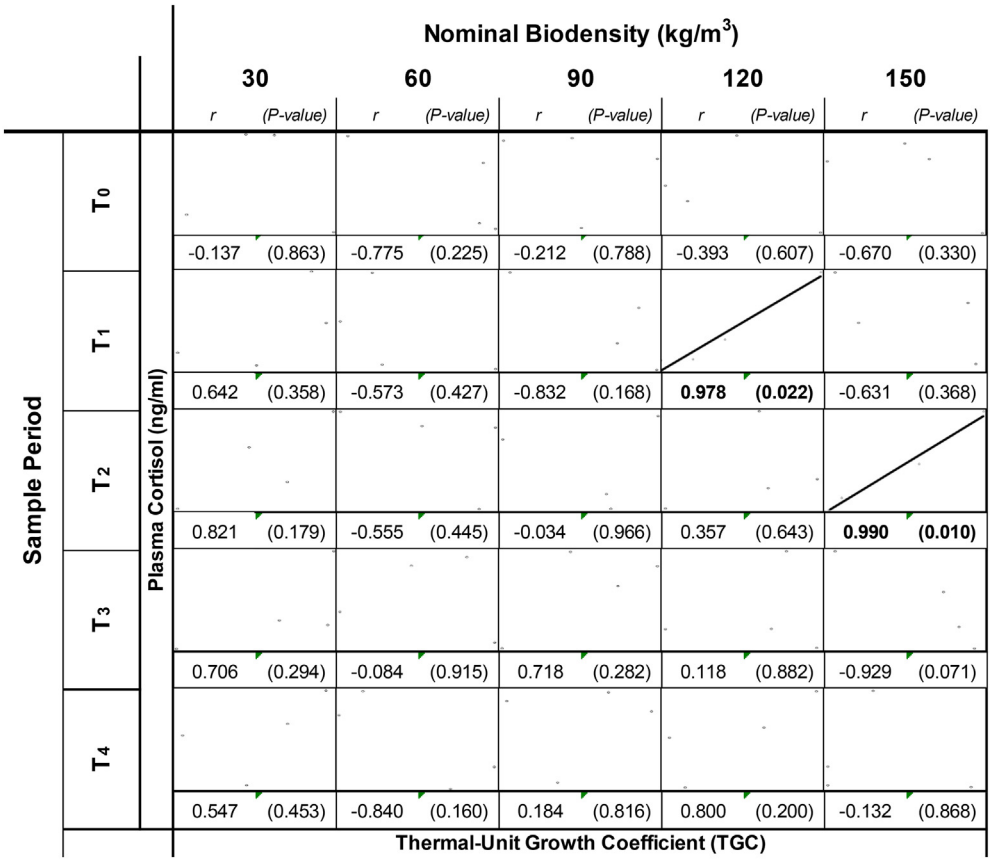


Fig. 5. – Panel A – mean (± SEM) plasma glucose for each treatment density compared within each sampling period; similar characters indicate the mean values are statistically similar (α = 0.05) within a sample period; asterisks (*) indicate the effect of acclimation on means at T_0 and differences in means from the station stock sample from $T_{1–4}$.Panel B – mean plasma glucose compared within each density over the study duration; similar characters indicate the mean values are statistically similar (α = 0.05) within treatment densities, over time.

that the fish sampled for T_a were exposed to more rigorous handling and husbandry as initial biodensities were established and fish were assigned to treatment tanks. Due to this fact the concentration of cortisol within these individuals might be expected to be higher than those sampled for T_0 onward, during which both the time and degree of handling were standardized and greatly reduced. Because fish assigned to the 30 kg/m^3 treatment were found to have levels of circulating cortisol at T_0 similar to those observed in T_a, it is evident that fish stocked at this density are as stressed as fish that have undergone extensive handling. Furthermore, the 30 kg/m^3 density was the only treatment to exhibit a decline in mean plasma cortisol over time. This could suggest a period of prolonged acclimation in fish stocked at 30 kg/m^3, compared to fish stocked from 60 to 150 kg/m^3. In the study by Procarione et al. (1999), serum cortisol levels in rainbow trout were higher in fish stocked at low densities after a period of 24 h, which could indicate the initial development of social hierarchies.

Table 6
- Results of the Pearson Correlations reported as scatter plots of mean TGC (x-axis) vs mean plasma cortisol (ng/mL; y-axis); significant correlations are indicated with trend lines and bolded values. Correlation results indicated as r, and *P*-values (in parentheses).



Interestingly, the cortisol levels of all treatment densities were found to decline over the course of the 24 day study which is believed to represent a prolonged period of acclimation to the density conditions of the culture tank.

Ultimately, the results of the plasma cortisol analyses indicate that higher biodensities result in reduced stress in Arctic charr. Due to the fact that cortisol levels tended to decrease as charr were stocked at higher densities and that levels were consistently lowest in the 150 kg/m^3 treatment indicates that charr may have the capacity to be reared at densities even greater than those seen in the current study. In other words, the results of this study indicate that we have not found the upper limit for biodensity with regards to the stress (plasma cortisol) associated with overcrowding. However, it is likely that culture at such extremes would be limited by the water quality conditions associated with high density culture. The present results are opposite to the stress associated with high biodensity that is experienced by rainbow trout, which is species-specific and thought to be lower than that of Arctic charr (Wallace et al., 1988; Jørgensen and Jobling, 1993). In previous publications, Arctic charr have been shown to exhibit the opposite effect in that stress is elevated when fish are stocked at low densities (Wallace et al., 1988; Jørgensen and Jobling, 1993; Jørgensen et al., 1993) as a result of increased social interactions (Vijayan and Leatherland, 1990; Christiansen et al., 1991).

The average concentrations of plasma glucose (mmol/L) observed in this study were within the range of those published for Arctic charr (Frøiland et al., 2012; Jørgensen et al., 2013) and rainbow trout (Wells and Pankhurst, 1999; Polakof et al., 2010), in the ranges of 3.6–10.2 mM and 3.8–10.1 mM, respectively. Acclimation resulted in no change in plasma glucose across all densities with the exception of the 30 kg/m^3 treatment which showed an increase over this period. Increased stress (expressed as elevated cortisol) can result in an increase in circulating levels of glucose (Silbergeld, 1974; Barton et al., 1987; Mendl, 1999). Thus, it is plausible that the elevated levels of circulating glucose observed in the 30 kg/m^3 treatment at T_0 may be attributed to the elevated plasma cortisol also observed within the same sample period. However, based on the results of the Pearson correlation of plasma cortisol vs glucose, this was not the case in the current study as no consistent correlations were observed. However, the concentrations of plasma cortisol observed in the present study are either only slightly above, or at resting baseline levels for salmonids (Barton and Iwama, 1991). As such, the plasma cortisol levels in this study may have been high enough to initiate a gluconeogenic response in the liver.

4.3. Welfare parameters and correlates

4.3.1. Fin damage and cortisol

It is interesting to note that biodensity was ultimately found to have no effect on either caudal or dorsal fin damage by the end of the study (T_4) given the territoriality and agonistic behaviour exhibited by most salmonids (Christiansen et al., 1989; Winberg and Nilsson, 1993; Alanärä and Brännäs, 1996; MacIntyre et al., 2008). It has been shown that fin degradation can result in noxious stimuli, skin lesions (as a result of the presence of an open integument), and hinder the animals balance or locomotion (Webster, 2001; Fraser, 2008; Braithwaite and Huntingford, 2013).

Previous studies have argued that fin damage would be correlated with increased levels of plasma cortisol as a response to the erosion of the fin and the exposure of the nociceptors (Sneddon et al., 2003; Braithwaite and Huntingford, 2013). The lack of a consistent correlation between levels of plasma cortisol and fin damage (caudal and

dorsal), could indicate that fin damage may not be as stressful to Arctic charr as with other salmonid species.

4.3.2. Growth, feed efficiency, and cortisol

Increased stress (using cortisol as an indicator) has been associated with perturbations in growth and feed efficiency in salmonids (Jobling, 1985; Barton et al., 1987; Andersen et al., 1991; Jørgensen et al., 1993; Gregory and Wood, 1999; Madison et al., 2015). When examining the results for growth (TGC), feed efficiency, and plasma cortisol, it appears that the higher densities exhibit the lowest growth and feed efficiency, while also consistently having the lowest recorded levels of plasma cortisol. Conversely, the lowest densities were found to have the highest rates of growth with reduced feed efficiency and consistently produced the highest levels of plasma cortisol, indicating higher levels of stress. Thus, one could make the assumption that growth and feed efficiency may be correlated with levels of plasma cortisol. However, no consistent correlations were observed between stress levels (cortisol) and growth/feed efficiency. This may indicate that Arctic charr are quite resilient to stress such that it may not affect their overall growth, which is critical for the welfare of these animals.

5. Conclusions

The purpose of this study was to provide an estimation of the optimal biodensity for Arctic charr aquaculture, with an emphasis on production performance and welfare. High intensity aquaculture operations rely upon high biodensities which may compromise the welfare of the animals being farmed (Moccia, 2013; Browman et al., 2018). Thus, there are two drivers at play in determining an optimal biodensity with respect to the *economics* of fish growth and production performance, and the *ethics* associated with welfare consideration. Ultimately, the determination of an optimal biodensity for Arctic charr aquaculture must be the result of the careful consideration of these two approaches.

As the results have shown, biodensity had no effect on mortality, plasma glucose or fin damage (caudal and dorsal). However the combination of reduced growth rates (i.e. lower TGC's; 0.20–0.21) observed at the high densities (120, and 150 kg/m^3) and reduced feed efficiency (0.56–0.83) at the extremes (30, 120, and 150 kg/m^3) suggest a density between 60 and 90 kg/m^3 for optimal economic gains (TGC: 0.23–0.26; FE: 0.92–1.06). The morphometric data presented here also provide additional considerations for the choice of stocking density in terms of the potential commercial productivity for Arctic charr.

Alternatively, the results of the welfare analyses show evidence of reduced stress (as assessed by circulating levels of plasma cortisol) at higher biodensities, with fish stocked at 150 kg/m^3 exhibiting the lowest levels of all treatments.

The two approaches provide slightly different results on which to base a suggestion of an optimal biodensity. The economics approach suggests stocking at densities > 30 kg/m^3 and < 90 kg/m^3 for the benefit of producing product of similar quality while improving feed efficiency and growth. However, stocking at higher densities (150 kg/m^3) resulted in reduced stress which is associated with the welfare status of farmed animals (Turnbull et al., 2008). Therefore, using a combination of an economics and ethics based approach, the suggested biodensity for Arctic charr aquaculture is somewhere in the range of 60–90 kg/m^3 (actuals of 65.1–99.0 kg/m^3). Within this range, Arctic charr exhibited optimal feed efficiency and growth while maintaining cortisol levels below the 10 ng/mL which is a nominal threshold of cortisol implied for stressed salmonids (Pickering and Pottinger, 1989).

Future studies could expand on the results observed here. The present investigation was performed on juvenile Arctic charr (average mass of 177.2 g) and was run for a period of 91 days after which the average mass was 478.9 g, which is significantly less than the average market weight (900 g – 1.4 kg) (Ojima et al., 2009; Eriksson et al., 2010; Gunnarsson et al., 2014). Therefore it must be conceded that this leaves a large gap in the development of these animals towards market weight in which it is possible the effects of biodensity could vary from those reported here. Thus we suggest that future studies should begin with fingerling fish and continue throughout the entire life cycle of Arctic charr in aquaculture systems.

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