The influence of stocking density on the growth, body composition and energy budget of Atlantic salmon *Salmo salar* L. in recirculating aquaculture systems

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Abstract Atlantic salmon *Salmo salar* were reared at four stocking densities—high density D_1 (final density ~39 kg/m^3), medium densities D_2 (~29 kg/m^3) and D_3 (~19 kg/m^3), and low density D_4 (~12 kg/m^3)—for 40 days to investigate the effect of stocking density on their growth performance, body composition and energy budgets. Stocking density did not significantly affect specific growth rate in terms of weight (SGR_w) but did affect specific growth rate in terms of energy (SGR_e). Stocking density significantly influenced the ration level (RL_w and RL_e), feed conversion ratio (FCR_w and FCR_e) and apparent digestibility rate (ADR). Ration level and FCR_w tended to increase with increasing density. Fish at the highest density D_1 and lowest density D_4 showed lower FCR_e and higher ADR than at medium densities. Stocking density significantly affected protein and energy contents of the body but did not affect its moisture, lipid, or ash contents. The expenditure of energy for metabolism in the low-density and high-density groups was lower than that in the medium-density groups. Stocking density affected energy utilization from the feces but had no effect on excretion rate. The greater energy allocation to growth at high density and low density may be attributed to reduced metabolic rate and increased apparent digestibility rate. These findings provide information that will assist selection of suitable stocking densities in the Atlantic-salmon-farming industry.

Keywords: stocking density; Atlantic salmon; growth; body composition; energy budget; recirculating aquaculture system

1 INTRODUCTION

Aquaculture is one of the fastest growing global animal producing sectors and contributes approximately 50% of all fish consumed (FAO, 2010). Stocking density, a potential source of chronic stress that may affect the physiology and behavior of farmed fish, is widely recognized as a critical husbandry factor in intensive aquaculture (Wedemeyer, 1997; Ellis et al., 2002; Ashley, 2007). Therefore, stocking density must be taken into account in planning and monitoring of performance in fish industry, and in setting production limits by authorities (Ellis et al., 2002;

Turnbull et al., 2005; North et al., 2006; Oppedal et al., 2011). For these reasons, stocking density has attracted particular attention in fish farming.

Several studies have demonstrated that inappropriate stocking densities may impair the growth, feed conversion ratio (FCR), feed intake, apparent digestibility rate (ADR), as well as welfare parameters in fish (Lambert and Dutil, 2001; Kristiansen et al., 2004; Schram et al., 2006; Merino et al., 2007; Sirakov, 2007; Sirakov and Ivanchev, 2008; Tolussi et al., 2010). However, owing to the diversity of physiological stress responses in fish, these effects appear to be species-specific (Barton, 2002). The causative mechanisms by which stocking density affects growth and feed utilization are still unclear, making it difficult to establish threshold guidelines for rearing density (Larsen et al., 2012).

The growth of fish is determined by the amount of energy available for growth (Zhang and Tang, 2002; Lupatsch et al., 2010). Determination of fish energy budgets in relation to growth performance might help us to understand the mechanisms whereby inappropriate stocking densities impair growth and feed utilization. To date, studies of the effect of stocking density on growth and energy expenditure have provided inconsistent results. Lefrancois et al. (2001) found that density did not affect the routine metabolic rate (RMR) of rainbow trout *Oncorhynchus mykiss*, but Larsen et al. (2012) showed that high stocking density groups had higher RMR, which resulted in a lower specific growth rate (SGR). Moreover, in European sea bass (*Dicentrarchus labrax*), low-density groups required more energy for maintenance and growth than did high-density groups (Lupatsch et al. 2010).

The Atlantic salmon *Salmo salar* is one of the most important aquaculture species among salmonids. Recirculating aquaculture systems (RAS) used in the culture of Atlantic salmon provide an important model for the global aquaculture industry in terms of conservation of resources, low environmental impact, and product safety (Timmons and Ebeling, 2007; Martins et al., 2010). Although high-stocking density is the main feature of RAS, little is known about its influence on fish growth. Most studies of the effect of stocking density on Atlantic salmon have focused on the apparent growth performance and health of fish in sea-cages (Soderberg et al., 1993; EFSA, 2008; CIWF, 2009; Hosfeld et al., 2009). Clearly, there is a need for more information on the causative mechanisms by which stocking density affects growth of Atlantic salmon in RAS.

The present study attempts to understand the relationships among stocking density, growth and energy partitioning of Atlantic salmon based on mortality, growth performance, feed intake, digestibility rate and energy budgets of salmon reared at four different stocking densities. These data on the growth characteristics and energy strategy of Atlantic salmon will enhance the management of RAS-cultured salmon in terms of finding the optimal stocking density.

2 MATERIALS AND METHODS

2.1 Holding facilities

The experiment was conducted in 12 RASs each consisting of a rearing tank, a whirl-separator for solids removal, biofilter, sump, foam separator, and UV-sterilizer (Fig. 1). The rearing tanks were 100 cm in diameter and 50 cm deep, and contained 348±6 L of water. The volume of the whirl-separator was approximately 60 L, which was large enough to collect most of the feces. Total water flow to the rearing tanks through standpipes covered with 1.0 cm screen was 800 L h^{-1}, replacing 100 % of the volume of the seawater in the system daily. Recommended values for water quality parameters in Norwegian land-based salmonid farming facilities are: O2 >80% at the outlet; CO2 <15 mg L−1); and total ammonia nitrogen <2 mg L−1 (Anon, 2004). In the present work, the water quality of all rearing tanks was monitored periodically and maintained at 15.5±0.5 ℃, pH 8.0±0.07, NH_4^+-N <0.7 mg/L, NO_2^--N <0.6 mg/L, chemical oxygen demand (COD) <1.5 mg/L, O_2 90%–100% saturation, CO_2 <15 mg/L and salinity 22–23. The ammonia, nitrite and COD were measured every 2 days as per APHA (1998). The oxygen saturation was measured daily with an oxygen meter (YSI Inc. Yellow Springs, OH) and adjusted by bubbling from an oxygen cylinder. The CO_2 level was monitored every 2 days using a portable analyzer (OxyGuard® CO2 Portable, WMT Inc., Baton Rouge, LA) and modified by adjusting the aeration of the biofilter and sump.

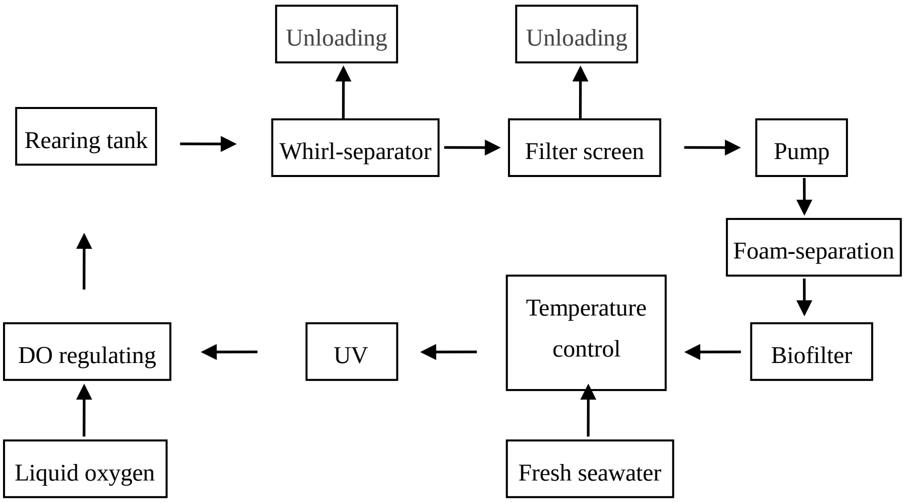

Fig. 1 Schematic diagram of experimental systems

2.2 Fish and feeding

Atlantic salmon post-smolts, with an average body mass of 95.09±3.28 g and approximately 8 months old, were obtained from Shandong Oriental Ocean Sci-Tech Co., Ltd., Yantai, China. The fish were acclimated in rearing tanks for 14 days. Stocking densities were selected according to previous studies in sea cages (CIWF, 2009). Salmon were randomly distributed in 12 tanks at four initial densities, nominally indicated as high density D_1 (22.17±0.34 kg/m^3), medium density D_2 (16.34±0.09 kg/m^3) and D_3 (11.04±0.10 kg/m^3), and low density D_4 (6.61±0.08 kg/m^3), i.e., three tanks at each stocking density. The experiment lasted for 40 days. All fish were exposed to continuous artificial light and fed twice daily (8:00 and 20:00) to apparent satiation, as indicated by the occurrence of uneaten pellets. The feed was commercial Atlantic salmon diet (produced in China; 48.66% crude protein, 17.43% crude fat, 11.91% ash, 21.33 KJ/g gross energy content). Celite was added as a source of acid insoluble ash (AIA) for determination of digestibility. The feed supplied to each tank was weighed before feeding (to 0.001 g).

2.3 Uneaten feed and feces

Uneaten pellets were collected from the whirl-separator of each tank 30 min after feeding and the quantity of uneaten pellets was used to adjust the quantity of feed supplied. Uneaten feed was easily distinguished from fecal pellets by differences in coloration and firmness. Feces were also collected from the whirl-separator before and after feeding for 1.5 h. The feces were oven-dried at

75 ℃, weighed and homogenized for analysis of nitrogen content and energy. Total fecal production F_w, (g) was calculated using the following formula (Goddard and McLean, 2001):

$F_w = F_{collected} \times (\mathrm{Feed}_{AIA}/\mathrm{Feces}_{AIA})$,

where $F_{collected}$ is the weight of feces collected (g), Feed_{AIA} is the total AIA of feed intake (g), and Feces_{AIA} is the total AIA in collected feces (g).

2.4 Sampling

Before sampling, fish were starved for 48 h to allow elimination of feces. Then, all the fish were killed with MS222 (300 ppm), weighed and oven-dried for subsequent analysis.

2.5 Chemical analysis

Moisture contents of fish and feed were calculated by oven-drying to constant weight at 70 ℃ (Fang et al., 2010). Lipid, nitrogen, ash and gross energy contents were determined using a BUCHI Extraction SystemB-811 (BUCHI, Switzerland), Vario ELIII Elemental Analyzer (Elementar, Germany), Muffle furnace (SXL-1030, China) and PARR1281 Calorimeter (PARR Instrument Company, USA), respectively. The AIA contents of feeds and feces were determined by the method of Atkinson et al. (1984). Each measurement was made in triplicate. Protein content was calculated by multiplying nitrogen content by 6.25.

2.6 Energy determination and budget

The energy budget was calculated using the following equation (Carefoot, 1987):

$C = G + F + U + R$,

where C is energy consumed; G is energy for growth; F is energy loss to feces; U is the energy of loss as ammonia excretion; and R is the energy of loss as respiration. C, G, and F in the budget equation were calculated as follows:

$C = C_w \times \mathrm{IF}_e$,

$G = W_t \times E_t – W_0 \times E_0$,

$F = F_w \times \mathrm{FE}_e$,

where C_w and F_w are food intake and fecal production, respectively, in terms of weight (g); IF_e and FE_e are the energy contents of the feed and feces, respectively (KJ/g); W_t and W_0 are the final and initial wet body weights, respectively, of fish (g); and E_t and E_0 are final and initial energy contents respectively, of the fish (KJ/g). Based on the nitrogen budget equation of Elliott (1976) ($U= (C_N−G_N−F_N)\times 24.83$, where C_N, F_N and G_N are the nitrogen consumed from food, lost in feces and deposited in the animal body, respectively), we assumed that the is the energy content of excreted ammonia was 24.83 KJ/g.

R was calculated as the difference between energy consumption and the energy allocated to excretion, feces, and growth:

$R = C − (U + F + G)$.

2.7 Data analysis of growth performance

Specific growth rate in terms of weight and energy content (SGR_w and SGR_e, %/day), ration level in terms of weight and energy (RL_w, % body weight/day; RL_e, % body energy content/day), feed conversion ratio (FCR) in terms of weight and energy (FCR_w and FCR_e) and apparent digestibility rate (ADR, %) were calculated as follows (Sun et al., 2006):

$SGR_w = 100 \times (\ln W_t − \ln W_0)/t$,

$SGR_e = 100 \times (\ln E_t − \ln E_0)/t$,

$RL_w = 100 \times C_w/((W_t + W_0)/2 \times t)$,

$RL_e = 100 \times C_e/((E_t + E_0)/2 \times t)$,

$FCR_w = C_w/(W_t − W_0)$,

$FCR_e = C_e/(E_t − E_0)$,

$ADR = 100 \times (C_w − F_w)/ C_w$,

where W_t and W_0 are final and initial wet body weight (g) of fish, respectively, t is the feeding duration (days), E_t and E_0 are final and initial energy contents of the fish body, respectively, C_w and C_e are feed intakes in terms of weight (g) and energy (KJ), respectively, and F_w is total fecal production (g).

Data were analyzed using the statistical package (SPSS 13.0 for Windows). Effects of density were analyzed using one-way ANOVA. Differences between treatment groups were analyzed using Duncan's multiple comparisons test and considered statistically significant if $P<0.05$.

3 RESULTS

3.1 Water quality and mortality

Ranges in the mean values of water quality parameters among the four density groups were: ammonia 0.64±0.06 mg/L to 0.69±0.08 mg/L; nitrite 0.42±0.06 mg/L to 0.44±0.06 mg/L; COD 1.39±0.17 mg/L to 1.48±0.12 mg/L; oxygen saturation 92.41±0.74% to 95.47±1.26%; and CO_2 12.03±0.38 mg/L to 13.58±0.67 mg/L (Table 1). There were no significant differences in these water quality parameters among the groups.

During the growth trial, mortality was zero for all experimental groups. The density selected in this experiment had no significant effect on the mortality of salmon with average body masses of 95.09±3.28 g to 166.50±28.05 g.

Table 1 Water quality parameters for the rearing tanks stocked at four densities (D_1–D_4).

Parameter	D_1	D_2	D_3	D_4
NH_4^+-N (mg/L)	0.69±0.08	0.67±0.07	0.66±0.05	0.64±0.06
NO_2^--N (mg/L)	0.44±0.06	0.42±0.06	0.42±0.05	0.42±0.06
COD (mg/L)	1.48±0.12	1.45±0.13	1.43±0.14	1.39±0.17
O_2 (% saturation)	95.47±1.26	93.22±1.08	94.05±0.76	92.41±0.74
CO_2 (mg/L)	13.58±0.67	12.82±0.42	12.93±0.45	12.03±0.38

Values are means ± S.E. D_1, high density; D_2 and D_3, medium density; D_4, low density (see Materials and Methods section for actual density values)..

3.2 Growth performance

There were no significant differences in the initial body weights among the four groups ($P>0.05$, Table 2). After 40 days of culture, the weights of salmon in the density groups D_1, D_2, D_3 and D_4, increased from 95.27±3.23 g to 167.99±16.91 g, from 95.15±3.22 g to 166.85±27.86 g, from 95.01±3.58 g to 166.12±24.42 g, and from 94.50±3.27 g to 165.03±22.61 g, respectively (means ± S.E.). Stocking densities did not significantly affect the final mean weights of the fish ($P>0.05$, Table 2). The final stocking densities of salmon in density groups D_1, D_2, D_3 and D_4 increased from 22.17±0.34 kg/m^3 to 39.11±0.64 kg/m^3, from 16.34±0.09 kg/m^3 to 28.67±0.36 kg/m^3, from 11.04±0.10 kg/m^3 to 19.31±0.28 kg/m^3, and from 6.61±0.08 kg/m^3 to 11.54±0.16 kg/m^3, respectively.

The SGR_w of salmon in the density groups D_1, D_2, D_3 and D_4 were 1.41±0.02%/d, 1.40±0.02%/d, 1.40±0.01%/d, and 1.39±0.03%/d, respectively and were not significantly different among the groups ($P>0.05$, Table 2). Corresponding SGR_e values of the salmon were 1.50±0.03%/d, 1.42±0.04%/d, 1.40±0.03%/d, and 1.43±0.03%/d. There was a significant effect of stocking density on SGR_e (ANOVA, $P<0.05$, Table 2). SGR_e of D_1 was significantly higher than the other density groups ($P<0.05$, Table 2).

Table 2 Growth parameters of salmon reared at different stocking densities.

Groups	Initial weights (g)	Final weights (g)	Initial density (kg/m^3)	Final density (kg/m^3)	SGR_w (%/d)	SGR_e (%/d)
D_1	95.27±3.23	167.99±16.91	22.17±0.34	39.11±0.64	1.41±0.02	1.50±0.03 ^{a}
D_2	95.15±3.22	166.85±27.86	16.34±0.09	28.67±0.36	1.40±0.02	1.42±0.04 ^{b}
D_3	95.01±3.58	166.12±24.42	11.04±0.10	19.31±0.28	1.40±0.01	1.40±0.03 ^{b}
D_4	94.50±3.27	165.03±22.61	6.61±0.08	11.54±0.16	1.39±0.03	1.43±0.03 ^{b}

Values are means ± S.E Means with different letter superscripts in the same column are significantly different ($P<0.05$). D_1, high density; D_2 and D_3, medium density; D_4, low density (see Materials and Methods section for actual density values).

3.3 Ration level, feed conversion ratio and apparent digestibility rate

The RL_w of salmon ranged from 0.98±0.003 to 1.07±0.005%/day among the four groups. There was a significant effect of stocking density on RL_w ($P<0.05$, Fig. 2). Simple regression analysis indicated a linear relationship between RL_w and final stocking density ($y = -0.003x + 0.9515$, where $y = RL_w$ and x= stocking density; $R^2 = 0.9585$).

The RL_e of salmon in different densities groups ranged from 3.64±0.03 to 3.93±0.03%/day and showed a similar trend to that of RL_w ($P<0.05$, Fig. 2).

The FCR_w values in the four density groups D_1, D_2, D_3, and D_4 were 0.77±0.006, 0.76±0.006, 0.75±0.004, and 0.72±0.004, respectively. The corresponding FCR_e values were 2.70±0.04, 2.79±0.05, 2.81±0.03, and 2.61±0.03. One-way ANOVA showed that stocking densities had a significant effect on both the FCR_w and FCR_e values of salmon ($P<0.05$, Fig. 3.). There was linear relationship between FCR_w and final stocking density ($y = 0.0017x + 0.7072$, $y = FCR_w$, and x = stocking density; $R^2 = 0.914$)

The ADR values in the groups D_1, D_2, D_3, and D_4 were 77.07±0.17%, 76.28±0.34%, 76.03±0.19%, and 76.91±0.22%, respectively. Stocking densities had a significant effect on the ADR of salmon ($P<0.05$, Fig. 4)

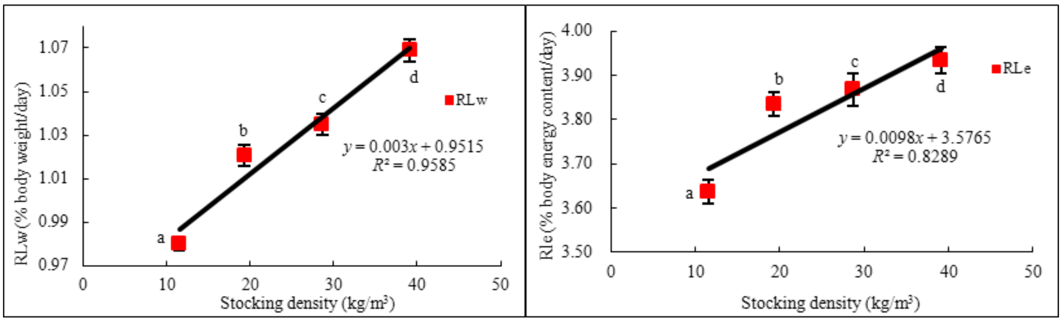

Fig. 2 Ration level in terms of weight (RL_w) and energy (RL_e) of salmon reared at four different stocking densities (plotted against final densities, which were 11.54, 19.31, 28.67, and 39.11 kg m^{-3}). Mean values labeled with different letters are significantly different (Duncan's multiple comparisons, $P<0.05$).

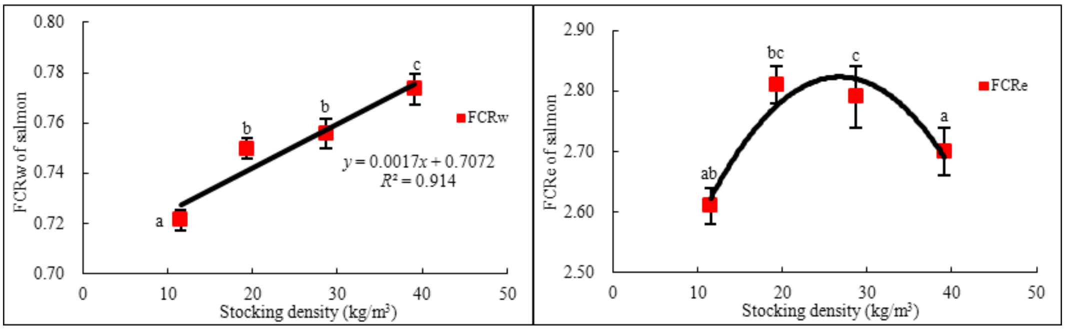

Fig. 3 Feed conversion ratio in terms of weight (FCR_w) and energy (FCR_e) of salmon reared at four different stocking densities (plotted against final densities). Mean values labeled with different letters are significantly different

(Duncan's multiple comparisons, *P*<0.05).

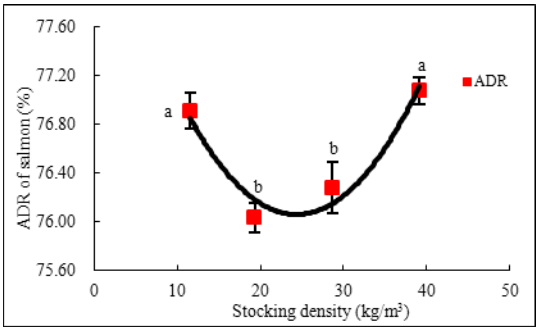

Fig. 4 Apparent digestibility rate (ADR) of salmon reared at four different stocking densities. (plotted against final densities). Mean values labeled with different letters are significantly different (Duncan's multiple comparisons, *P*<0.05).

3.4 Body composition

The moisture contents of the fish ranged from 72.42±1.00% to 73.18±1.11% among the groups and lipid contents ranged from 7.51±0.29% to 7.78±0.38%. The crude protein contents were 16.44±0.37%, 16.19±0.34%, 16.01±0.29% and 16.02±0.33% in density groups D_1, D_2, D_3, and D_4, respectively, and their ash contents ranged from 2.38±0.07% to 2.43±0.06%. The energy contents in terms of wet body weight were 5.86±0.07 KJ/g, 5.71±0.08 KJ/g, 5.68±0.06 KJ/g and 5.76±0.07 KJ/g in the D_1, D_2, D_3, and D_4 density groups, respectively. One-way ANOVA analysis showed that stocking density had a significant effect on protein and energy contents (*P*<0.05) but did not affect moisture, lipid and ash contents (*P*>0.05, Table 3).

Table 3 Moisture, lipid, protein, ash, and energy contents of salmon at the end of the experiment

Groups	Moisture (%)	Lipid (%)	Protein (%)	Ash (%)	Energy content (KJ/g)
D_1	72.42±1.00	7.78±0.38	16.44±0.37^{a}	2.43±0.06	5.86±0.07^{a}
D_2	73.02±0.59	7.51±0.29	16.19±0.34^{ab}	2.41±0.07	5.71±0.08^{bc}
D_3	73.18±1.11	7.53±0.16	16.01±0.29^{b}	2.39±0.07	5.68±0.06^{c}
D_4	72.96±1.18	7.73±0.30	16.02±0.33^{b}	2.38±0.07	5.76±0.07^{b}

Values (means ±S.E) are relative to wet weight). Mean initial values were: moisture 72.99±0.92 %; lipid 6.90±0.27 %; protein 17.15±0.26 %; ash 2.43±0.09 %; and energy 5.67±0.06 KJ g^{-1}. The energy contents of feces (dry weight) were 9.93±0.08 KJ/g, 10.08±0.06 KJ/g, 10.07±0.02 KJ/g and 9.58±0.14 KJ/g in density groups D_1, D_2, D_3, and D_4, respectively. Mean values labeled with different letters are significantly different (Duncan's multiple comparisons, $P<0.05$)..

3.5 Energy budget

The energy contents of the feces (dry weight) were 9.93±0.08 KJ/g, 10.08±0.06 KJ/g, 10.07±0.02 KJ/g and 9.58±0.14 KJ/g in density groups D_1, D_2, D_3, and D_4, respectively. The energy content of feces in density group D_4 was significantly lower than that in the other groups ($P<0.05$).

According to the energy budget equation (Carefoot, 1987), the energy available for growth (G) was 37.00±0.97%, 35.81±1.09%, 35.56±0.56% and 38.34±1.11% of the total energy consumed (C) in groups D_1, D_2, D_3, and D_4, respectively. Energy lost in the feces (F) was 10.67±0.08%, 11.29±0.09%, 11.31±0.02%, and 10.35±0.12% of total energy consumed in the D_1, D_2, D_3, and D_4 density groups, respectively. G and F were significantly dependent on stocking density ($P<0.05$, Table 4).

Energy for the excretion (U) ranged from 4.41% to 4.47% and was not significantly affected by stocking density ($P>0.05$, Table 4). Energy consumed in metabolism (R) was 47.92±1.01%, 48.48±1.06%, 48.48±0.39%, and 46.90±0.95% of total energy consumed (C) in groups D_1, D_2, D_3 and D_4, respectively. The total energy consumed in metabolism in group D_4 was significantly lower than that in groups D_2 and D_3 ($P<0.05$). However, stocking density did not affect the expenditure of energy for metabolism among all the groups ($P>0.05$, Table 4).

Table 4 Energy partitioning in salmon reared at different stocking densities.

Groups	C (%)	G (%)	F (%)	U (%)	R (%)
D_1	100	37.00±0.97 ^{a}	10.67±0.08 ^{a}	4.40±0.13	47.92±1.01 ^{ab}
D_2	100	35.81±1.09 ^{ab}	11.29±0.05 ^{b}	4.42±0.11	48.48±1.06 ^{b}
D_3	100	35.56±0.56 ^{b}	11.31±0.04 ^{b}	4.64±0.09	48.48±0.39 ^{b}
D_4	100	38.34±1.11 ^{c}	10.35±0.12 ^{c}	4.41±0.10	46.90±0.95 ^{a}

Values are means ±S.E. C, total consumed; G, growth; F, feces; U, urine; R, respiration. Mean values labeled with different letters are significantly different (Duncan's multiple comparisons, $P<0.05$).

4 DISCUSSION

In the salmonid fish farming industry, space and water availability are always limiting factors. Therefore, it is necessary to optimize the use of water resources by maintaining fish at the highest possible stocking density. Such conditions may cause aggressive behavior and adversely affect the growth potential of salmonids (Leatherland, 1993; Wedermeyer, 1997). However, other opinions suggest that such effects on the performance of fish largely occur in response to a decline in the water quality at high densities (Ellis et al., 2002; Hosfeld et al., 2009). To minimize the influence of water pollution in our experiments, water quality was optimized and adjusted according to previous studies (Anon, 2004; Hosfeld et al., 2009) (Table 1).

Mortality is an important indicator of fish adaptation to the environment. In several studies, high stocking density resulted in injury or death of fish (Ellis et al., 2002; Ashley, 2007; EFSA, 2008; CIWF, 2009). However, all selected stocking densities in our study had no significant effect on mortality of Atlantic salmon in RAS. Similar results were also reported in farmed fish under conditions of good water quality, e.g., rainbow trout, sea bass, red porgy (*Pagrus pagrus*), African catfish (*Clarias gariepinus*), and Nile tilapia (*Oreochromis niloticus*) (Siddiqui et al., 1989; Hengsawat et al., 1997; North et al., 2006; Sammouth et al., 2008; Laiz-Carrión et al., 2012).

Most studies of salmonid growth have indicated an adverse effect of increasing density by reducing growth (Leatherland, 1993; Soderberg et al., 1993; Ellis et al., 2002; Larsen et al., 2012). However, in the present study, our selected stocking densities did not significantly affect the SGR_w of Atlantic salmon (Table 2). These observations are consistent with other density trials in rainbow trout (Kebus et al., 1992; Bagley et al., 1994; North et al., 2006) and Atlantic salmon (Hosfeld et al., 2009) in which water quality parameters were maintained at adequate levels. Interestingly, our findings suggest that SGR_e of Atlantic salmon was affected by stocking density. The maximum SGR_e occurred in fish fed to satiation at the highest density (D_1), which may be attributed to the high food consumption and body energy content compared with the other treatments.

The present study showed that the ration levels (RL_w and RL_e) of salmon increased significantly with increasing densities (Fig 2), which is consistent with reports on Arctic charr *Salvelinus alpinus* (Jorgensen et al., 1993) and African catfish (Hecht and Uys, 1997). However, this contrasts with traditional opinion that high stocking density has a negative effect on food intake of fish, e.g., in Atlantic cod (Lambert and Dutil, 2001) and rainbow trout (Leatherland, 1993; Boujard et al., 2002; Ellis et al., 2002). When salmon are transferred into a novel environment, resumption of feeding may be impaired (Øverli et al., 2002). Under our experimental conditions in the RAS, salmon displayed a more rapid resumption of feeding and higher motivation for feeding with increasing stocking density (data not shown), which might explain why high stocking density increased ration level.

FCR_w increased significantly with increasing stocking density in this study (Fig. 3). This is supported by a majority of studies that show negative impacts on feed conversion at high stocking density (Ellis et al., 2002; Abou et al., 2007; Larsen et al., 2012). FCR_e of Atlantic salmon was also related largely to stocking density but exhibited a different trend to that of FCR_w. The lower FCR_e at high density D_1 (final density ~39 kg/m^3) and low density D_4 (~12 kg/m^3) might be related to the increased body energy content. The present study is the first to report that stocking density has a significant effect on apparent digestibility rate (ADR, Fig 4). The ADR of Atlantic salmon in density groups D_1 and D_4 was higher than that in D_2 (~29 kg/m^3) and D_3 (~19 kg/m^3), which also corresponds to the results of FCR_e.

The energy budgets of seven marine fishes were examined and classified into three patterns of energy allocation (Tang et al., 2003). In this study, the proportions of food energy allocated to growth and metabolism for Atlantic salmon fed to satiation were 35.56–38.34% and 46.90–48.48%, respectively, which are close to values observed in *Sebastes schlegeli* and *Chaeturichthys stigmatias* (Tang et al., 2003). The energy partitioning of Atlantic salmon therefore exhibits a pattern of low metabolism and high growth within the density range of the present experiments.

Our observations on the dependence of energy budgets on stocking density in Atlantic salmon (95.09±3.28 g, 8 months old) differed from those reported in rainbow trout (261±5 g; 100 g, 8 months old) and European sea bass (72 g) (Lefrancois et al., 2001; Lupatsch et al., 2010; Larsen et al., 2012). The energy consumed in metabolism (R) in the medium density groups D_2 (~29 kg/m^3) and D_3 (~19 kg/m^3) was higher than in groups D_1 and D_4. Previous studies indicated that social interactions might alter with stocking density and affect metabolism (Lupatsch et al., 2010; Larsen et al., 2012). In addition, rates of aggression in Atlantic salmon peaked at 15 kg/m^3 in seawater tanks (Adams et al., 2007). More intense social interactions might result in increased metabolic demands in the medium density groups of Atlantic salmon. The partitioning of energy into growth (G) among the four stocking densities showed the opposite trend to that of energy directed into metabolism. The large amount of energy directed toward growth in D_1 and D_4 might be attributable to their reduced metabolic rate and increased apparent digestibility rate.

In conclusion, under the present experiment conditions, stocking densities did not significantly affect the SGR_w of salmon. RL and FCR_w were increased with increasing densities. According to the energy budget equation, salmon required less energy for metabolism and utilized more energy for growth in the low (~12 kg/m^3) and high (~39 kg/m^3) density groups than in the medium density groups (~29 kg/m^3 and ~19 kg/m^3). In relation to growth performance and economic returns, we deduce that Atlantic salmon should grow well at high stocking densities (e.g. ~40 kg/m^3) provided the water quality is maintained at a suitable level. This work provides useful information for the selection of the appropriate stocking density and for maintenance of health in the salmon breeding industry.

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