PRELIMINARY RESULTS REGARDING THE EFFECT OF DENSITY ON RAINBOW TROUT (*Oncorhynchus mykiss* W a l b a u m, 1792) PERFORMANCE IN A BIOSECURE RECIRCULATING SYSTEM

L. DEDIU[a]*, V. CRISTEA[a], A. DOCAN[a], I. GRECU[a], M. MAEREANU[b]

[a]*Faculty of Food Science and Engineering, Department of Aquaculture, Environment Science and Cadastre, University 'Dunarea de Jos' of Galati, 61–63 Garii Street, 800 003 Galati, Romania*
E-mail: *lorena.dediu@ugal.ro*
[b]*S. C. Kaviar House S.R.L., 6 Presei Street, Bucharest, Romania*
E-mail: *office@casacaviar.ro*

Abstract. The main purpose of the present experiment consists in evaluation of growth performances of rainbow trout (*Oncorhynchus mykiss)* in different stocking densities in a recirculating aquaculture system. The influence of stocking density and water quality on the growth of fish was studied during 25 days. The recirculating system has as experimental rearing units 4 tanks with an individual volume of $1m^3$. During experiment, water quality parameters (pH, O_2, T, NH_3–N, NO_2–N, NO_3–N) were recorded on a daily basis and, every 2 weeks, the fish were weight individually, and weight gain was recorded. Rainbow trout growth performances were evaluated through analysis of various technological indicators: weight gain (W), food conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER).

Keywords: rainbow trout, recirculating aquaculture, stress density.

AIMS AND BACKGROUND

In the last decade we witness an increasing concern on the animal welfare both in terrestrial and aquatic environment. In contradiction with terrestrial farm animals, where there are regulations regarding the minimum spatial area maintained for each animal in order to assure the basic behavioural needs, there are currently no regulations regarding the densities at which fish can be farmed[1].

Ellis[2] reviewed 43 studies and examined the effects of density on production and physiological parameters of rainbow trout. According to the above-mentioned author, increasing stocking density is not necessarily correlated with prolonged crowding stress in rainbow trout. However, it seems that physiological stress can have negative impacts on the performance of cultured fish including decreased

---

* For correspondence.

growth and food conversion[1,2]. Poor technological performance found in higher densities may indicate a reduce welfare status although the magnitude of the effects has tended to be dependent upon study-specific conditions. This is the main reason for wide discrepancies regard to maximum stocking density recommendations for rainbow trout given by different authors quoted in the review of Ellis[2].

The aim of the present work was to characterise the responsiveness to crowding stress of rainbow trout. In order to evaluate such a response, the present study has focused on tertiary responses such as technological performance and nutrient utilisation.

EXPERIMENTAL

Facilities. The study took place in the pilot recirculating system located in a laboratory of 'Aquaculture, Environmental Science and Cadastre' Department, Lower Danube University. The recirculating aquaculture system (RAS) consists of 4 identical rearing units and water treatment equipments.

Rearing units. The main criteria taken into account in designing basins were represented by the production costs, space used, water quality and technology management. The present recirculating aquaculture system was provided with 4 fibreglass octagonal units ($1m^3$ each) whose geometry and hydraulic meet technological requirement in terms of solids rapid removal. Waste water from rearing units is removed through a drain that, by its constructive feature and positioning, works like a trap for solids (sediments, suspended solids).

Water treatment module. Conditioning module was designed to provide an appropriate physical and chemical quality of technological water in order to meet the physiological requirements of the trout. The capacity of the water treatment equipments was correlated with the total volume and flow rate of the waste water filtered in order to achieve a proper removal of the most important parameters that determines the quality of recirculating water, namely the total amount of ammonia nitrogen (TAN), and the oxygen requirements of cultured species. Recirculating flow was 6 m^3/h, ensuring changing of the entire volume of water from a basin every 40 min. Our recirculating system includes the following equipments: a drum filter for sediments and suspended solids removal, a sand filter and an activated carbon filter for suspended and dissolved solids removal, a denitrification filter, a biological trickling filter, a sterilisation UV unit and aerators. The particularity of the above described system consists in a fully automated system for monitoring and control of the water quality parameters. This system, integrated in the classical configuration of RAS, includes a series of transducers and sensors placed strategically within the system in order to have a real-time image of the dynamics of the critical parameters with impact on the physiology of the fish and indirectly on the technological performance.

Experimental design. Rainbow trouts (134 exemplars with mean initial weight ± SEM, 501.96 ± 8.93 g) from a private farm (Horia, county Tulcea) were randomly distributed into the 4 fibreglass tanks of RAS under two different stocking densities: 12 kg/m³(SD$_1$), respectively 20 kg/m³ (SD$_2$). The statistics regarding biomass structure of the experimental cohorts are synthesised in Table 1. The fish were distributed in such a manner to create homogenous groups with similar class frequencies and exemplar number. Statistical testes confirmed normal distribution of the cohorts (K–S test, $p>0.05$).

Table 1. Inital biometric and statistical data of the experimented fish groups

Biomass structure	Experimental variant			
	B$_1$ (SD$_1$)	B$_2$ (SD$_2$)	B$_3$ (SD$_2$)	B$_4$ (SD$_1$)
Total biomass (g)	20268	13285	13393	20317
Number of exemplars	41	28	24	41
Mean weight (g/exemplar)	494.34*	474.46*	558.04*	495.54*
Std. deviation	83.19	53.51	46.51	111.89
Coefficient of variation	0.16	0.10	0.08	0.22

* Unsignificant differences (ANOVA, $p=0.18$).

The ratio used for fish feeding in this experiment was 1.5 % per body weight per day. The fish were fed with Aller Aqua pellets with 45% protein content, 15% crude fat, 21% carbohydrates. Fish welfare (swimming behaviour, feed intake) was evaluate and registered every day. After 3 experimental weeks 10 trout exemplars from each variant were weighed, based on which the following parameters were calculated: weight gain (W) = final weight (W$_t$) – initial weight (W$_0$) (g); food conversion ratio (FCR) = total feed (F)/total weight gain (W) (g/g); specific growth rate (SGR) = $100 \times$ (ln W$_t$ – ln W$_0$)/t (% BW/day); relative growth rate (RGR) = (W$_t$ – W$_0$)/t/BW) (g/ kg/day); protein efficiency ratio (PER) = total weight gain (W)/ amount of protein fed (g); relative weight gain (RWG%) = (W$_t$ – W$_0$) × 100/W$_t$.

Statistical data processing. Statistical analysis was performed using the SPSS 15.0 for Windows. Distribution normality was verified using the Kolmogorov–Smirnov (K-S) test. Statistical differences between variables were tested using *t*-test (α = 0.05). The coefficient of variation (CV) was calculated as the ratio of the standard deviation to the mean in order to have a measure of dispersion.

RESULTS AND DISCUSSION

In general it is considered that poorer technological performance of the fish maintained in crowding conditions is induced not only by the stress density, but also by the specific environment of lower quality determined by higher waste production rate.

In our recirculating system the water quality parameters (mean±SD) including water temperature (21.9±4.8°C), dissolved oxygen (6.5±1.43 mg/l), pH (6.9±1.02), ammonium (1.24±0.30 mg/l), nitrate (38.52±11.8 mg/l) and nitrite (0.006±0.004 mg/l) were fairly constant during the period of experiments and not significantly affected by stocking density (ANOVA, $p > 0.05$). Regarding the dynamics of water quality parameters we did not observe major modification or peaks during the day or after feeding.

Fish survival was slightly higher in SD_1 compared with SD_2 (0.74%), but this difference was not a significant evidence of influence of this indicator on the examined densities.

The data on growth and technological performance during the trial are summarised in Table 2. Final mean weight values of the groups held in different densities were 640.90±105.38 and 630±183.81 g for SD_1 (20 kg/m³), respectively 620.80±167.15 and 626.00± 161.00 g for SD_2 (12 kg/m³). Individual gain for the experimental period was similar for 3 variants (B_1, B_2 and B_3) while for B_4 the exemplars registered a slower accumulation although the differences regarding initial mean weight were not statistically significant.

Table 2. Biometric parameters and technological indices of the fish sampled at the end of the trial

Parameter	B_1 (25 kg/m³)	B_2 (12 kg/m³)	B_3 (12 kg/m³)	B_4 (25 kg/m³)
Mean final weight (g/exemplar)	640.90	620.80	626.00	630.00
Individual weight gain (g/exemplar)	146.56	146.34	147.68	122.82
Total weight gain (g)	1465.60	1463.40	1476.80	1228.20
Specific growth rate (% BW/day)	1.04	1.08	1.08	0.87
Daily growth rate – (g/kg/day)	5.86	5.85	5.91	4.91
Food conversion ratio	1.26	1.22	1.21	1.55
Total protein/tank (g)	834.20	800.65	807.17	855.87
Protein efficiency ratio	1.76	1.83	1.83	1.44

The same pattern of slower growth rate in the last tank with higher stocking density can be observed when analysing SGR (%BW/day). Thus the average SGR values for lower stocking density (SD_1) was 1.08% while for higher density was 0.95%. The statistical analysis performed on the data plotted for individuals tagged in the beginning of the experiment revealed insignificant differences among variants (ANOVA, $p>0.05$).

Regarding efficiency of feed and protein utilisation there were observed slightly differences between the two densities (Table 2). The poorer performance was registered for the last experimental tank where FCR was 1.55 and PER – 1.44.

The results in terms of growth of rainbow trout in our experiment are comparable with other findings regarding technological performance although the water temperature was maintained beyond the optimum range for the studied species.

From this perspective the study emphasises the plasticity of rainbow trout and its potential for warm water aquaculture.

Regarding the influence of stocking density on the behaviour and growth of *O. mykiss* species our results highlights some aspects that are, up to some extent, confirmed by many authors. While many studies revealed an inverse correlation between growth and stocking density[5], some findings confirm our data of lack of rearing density influence when water quality is maintained within acceptable limits for salmonid culture in all variants[6].

Maintaining cohorts in crowding conditions generate some stress that is generated by the behavioural factor which lies in the dominance hierarchy that is established in populations of fish. This could be manifested in the simple limitation of consumed food by smaller fish, because most of the feed would be eaten by the larger, more dominant fish. For our experiment this could explain the poorer performance in B4 tank where the variability was higher even from the beginning of the experiment. As could be seen in Fig. 1 the heterogeneity of all groups grew over the experimental period.
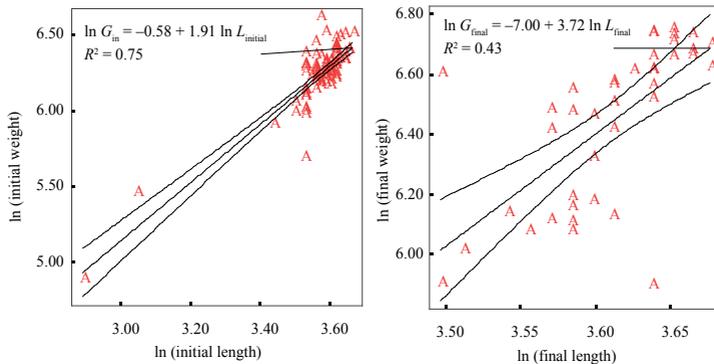
Fig. 1. Length–weight relationship in rainbow trout at the beginning and the end of the experiment

Although there were no important differences between the two tested densities the length–weight regressions emphasise a weaker condition of the fish from higher densities and a deepening of differences among individuals and so of the variability.

CONCLUSIONS

Although there were no differences in the mean weights between density treatments, there appeared to be greater size variation in the 25 kg/m³ treatment at the end of the experiment. Increased size heterogeneity (often expressed as the coefficient of variation (CV) has been suggested as an indicator of the social environment

within fish populations, where an increase over time may indicate inter-individual competition within the fish group[7]. Size variation suggests that inter-individual competition increased possibly as a result of the formation of dominance hierarchies, where a hierarchy can be defined as comprising of a group of dominant individuals at the top of the hierarchy, followed by a number of subdominants and, thereafter, a number of subordinates with low-rank positions[8].

In conclusion, it could be sustained that technological performance of rainbow trout in the rearing conditions tested in this study does not seem to be affected by density in the limits of 25 kg/m^3 (and high temperature, above 21°C) if the individual size distribution could be managed at the beginning and during the trial.

REFERENCES

1. ANON: Summary of the Law Relating to Farm Animal Welfare. MAFF, London, 1996. In: B. P. NORTH, J. F. TURNBULL, T. ELLIS , M. J. PORTER, H. MIGAUD, J. BRON, N. R. BROMAGE: The Impact of Stocking Density on the Welfare of Rainbow Trout (*Oncorhynchus mykiss*). Aquaculture, **255**, 466 (2006).
2. T. ELLIS, B. NORTH, A. P.SCOTT, N. R. BROMAGE, M. PORTER, D. GADD: The Relationships between Stocking Density and Welfare in Farmed Rainbow Trout. J. of Fish Biology, **61**, 493 (2002).
3. B. A.BARTON, G. K. IWAMA: Physiological Changes in Fish from Stress in Aquaculture with Emphasis on the Response and Effects of Corticosteroids. Annual Review of Fish Diseases, **1**, 3 (1991).
4. L. S. WEIL, T. P. BARRY, J. A. MALISON: Fast Growth in Rainbow Trout Is Correlated with a Rapid Decrease in Post-stress Cortisol Concentrations. Aquaculture, **193**, 373 (2001).
5. S. E. PAPOUTSOGLOU, E. PAPAPARASKEVA-PAPOUTSOGLOU, M. N. ALEXIS: Effect of Density on Growth Rate and Production of Rainbow Trout *(Salmo gairdneri* R i c h.) over a Full Rearing Period. Aquaculture, **66**, 9 (1987).
6. J. BLACKBURN, W. C. CLARKE: Lack of Density Effect on Growth and Smolt Quality in Zero-age Coho Salmon. Aquaculture Engineering, **9**, 121 (1990).
7. M. JOBLING: Simple Indices for the Assessment of the Influences of Social Environment on Growth Performance, Exemplified by Studies on Arctic Charr. Aquaculture International, **3**, 60 (1995)
8. P. E. K. SYMONS: The Possible Role of Social and Territorial Behaviour of Atlantic Salmon Parr in the Production of Smolts. Technical Report of Fisheries Research Board of Canada, **206**, 1 (1970).