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Short-Term Stress Response of Juvenile Rainbow Trout Subjected to Two Different Rearing Densities

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Abstract

Juvenile rainbow trout *Oncorhynchus mykiss* were subjected to one of four treatments in a two-by-two experimental design: 1) fed at a density of 1.8 g/m^3, 2) Fasted at 1.8 g/m^3, 3) fed at 30.1 g/m^3, and 4) fasted at 30.1 g/m^3. Blood glucose and hematocrit were measured at 4, 6, 48, 168, and 336 hours after placement in one of the two rearing densities, with relative fin lengths and organosomatic indices recorded at 336 hours. Glucose levels over time were not significantly different among the density and feeding treatments. Hematocrit levels over time were also not significantly different. Total lengths, weight, hepatosomatic index, viscerosomatic index, and any of the relative fin lengths were not significantly different between the high and low densities. However, the hepatosomatic index was significantly greater in the fed fish compared to those fasted. The splenosomatic index was significantly greater in the higher density treatment. These results likely indicate no short-term stress response to the higher rearing density used in this short-term experiment and no interaction between starvation and density-related stressors.

Keywords

Rainbow Trout, Density, Feeding, Glucose, Hematocrit

1. Introduction

Density is the total weight of fish per rearing unit volume during fish rearing [1]. Higher rearing densities maximize fish production, which is desirable, but once carrying capacity is exceeded, fish growth slows or stops [2]. Fish subjected to higher densities over longer time periods can also experience increased stress, impaired fish health, decreased post-stocking survival in natural environments,

and exhibit undesirable behaviors [3]-[9].

Changes in rearing density usually occur slowly over time, with rearing unit biomass gradually increasing as fish grow. Prior studies have focused on longer-term density-related stress responses. Physiological stress responses in fish increase during periods of intense crowding associated with netting and movement during culture operations [10] [11] [12] [13]. However, fish may become habituated over time from repeated chronic handling stressors [14], and the short-term stress responses of fish subjected to different rearing densities have not been evaluated.

Starvation may also increase salmonid stress hormone levels [15]. Sumpter *et al.* [16] reported that cortisol levels in rainbow trout *Oncorhynchus mykiss* deprived of food initially increased but quickly returned to basal levels. Starvation also produces oxidative and other stress responses [17] [18] [19]. The effects of multiple stress inducers can lead to deleterious effects, including mortality [20]. Thus, rearing density and the presence or absence of food may interact to produce stress responses in fish.

The objective of this study was to document the stress response of fed and unfed juvenile rainbow trout subjected to a sudden change in rearing density using blood glucose, hematocrit, and other secondary stress indicators [10] [13] [21].

2. Methods

This experiment occurred at McNenny State Fish Hatchery, Spearfish, South Dakota, USA, over the course of two weeks, using de-gassed and aerated well water (11˚C; total hardness 360 mg/L $CaCO_3$; alkalinity as $CaCO_3$, 210 mg/L; pH 7.6, total dissolved solids 390 mg/L). Twelve 190-L semi-square tanks were used, with three tanks for each of the four treatments (n = 3). The experimental design was a 2 × 2 factorial, with two rearing densities (1.8 g/m^3 or 30.1 g/m^3) and two feeding regimes (fed or fasted). Thus, the treatments were: 1) Fed at a density of 1.8 g/m^3, 2) Fasted at 1.8 g/m^3, 3) Fed at 30.1 g/m^3, and 4) Fasted at 30.1 g/m^3 (**Table 1**). For the tanks that were fed, feed amounts used the hatchery constant method [22], with a projected feed conversion rate of 1.1 and a projected growth rate of 0.075 cm/day. These projected rates were at, or slightly above, satiation,

Table 1. Study design with 2 × 2 factorial of if fish were fed or unfed, or with low or high density.

Treatment	Fed		Density	
	Yes	No	Low (1.8 g/m^3)	High (30.1 g/m^3)
1	x		x	
2		x	x	
3	x			x
4		x		x

based on prior hatchery experience. Feeding occurred every hour from 0900 to 1700 using 1.5-mm pellets (Protect FW, Skretting USA, Tooele, Utah, USA) in vibratory feeders (Pentair AES 0.5-L Vibratory Feeder, Pentair Aquatic Eco-Systems Inc., Apopka, Florida, USA).

Trout were euthanized in 200 mg/L tricaine methanesulfonate (MS-222; Tricaine-S, Syndel, Ferndale, Washington, USA), and blood was collected via caudal fin severance. Glucose (mg/dL) was obtained using a blood glucose monitor (Accu-Chek Aviva Plus; Roche Diabetic Care, Indianapolis, Indiana, USA). Heparinized microhematocrit capillary tubes (Fisher Scientific, Pittsburg, Pennsylvania, USA) were used to measure hematocrit. The tubes were centrifuged for 10 minutes at 11,500 revolutions per minute (RPM), after which red blood and total blood volume in the tube were measured, and the volume percentage of red blood cells (hematocrit) was calculated.

At the start of the experiment, glucose and hematocrit were sampled from ten rainbow trout from a common pool. An additional ten fish from the pool were euthanized, measured (total length) to the nearest 0.01 mm, and weighed to the nearest 0.01 g. Their dorsal, pectoral, and pelvic fin lengths were recorded to the nearest 0.01 mm, and viscera, spleen, and livers were weighed to the nearest 0.001 g. Immediately after this initial data collection, 300 g (approximately 25 fish) from the common pool were placed into each of the six tanks to achieve an initial density of 1.8 g/m^3. Then 5000 g (approximately 418 fish) were placed into another six tanks to achieve an initial density of 30.1 g/m^3.

At 4 hours, 6 hours, 48 hours (2 days), 168 hours (7 days), and 336 hours (14 days) after the start of the experiment, one fish from each tank was removed to obtain blood glucose and hematocrit. In addition, at the end of the two-week experiment (336 hours), total length, weight, dorsal, pectoral, and pelvic fin lengths, and viscera, spleen, and liver weights were obtained from one fish per tank.

The following formulas were used [23] [24]:

$$\text{Hematocrit}(\%) = 100 \times \text{red} / (\text{whole blood})$$

$$\text{Viscerosomatic Index}(\text{VSI}) = 100 \times (\text{viscera weight}(\text{mg})) / (\text{total fish weight}(\text{mg}))$$

$$\text{Splenosomatic Index}(\text{SSI}) = 100 \times (\text{spleen weight}(\text{mg})) / (\text{total fish weight}(\text{mg}))$$

$$\text{Hepatosomatic Index}(\text{HSI}) = 100 \times (\text{liver weight}(\text{mg})) / (\text{total fish weight}(\text{mg}))$$

$$\text{Relative Fin Lengths}(\%) = 100 \times \frac{\text{fin length}(\text{mm})}{\text{fish length}(\text{mm})}$$

SPSS statistical analysis computer program (Version 24.0; IBM; Armonk, New York, USA) was used for data analysis, with significance predetermined at $p <$

0.05. Hematocrit and glucose data were analyzed using Repeated Measures Analysis and a Bonferroni post-hoc test. Glucose and hematocrit at each sampling point were analyzed using two-way Analysis of Variance (ANOVA). Two-way ANOVA was also used for length, weight, organosomatic indices, and relative fin lengths.

3. Results

Individual fish glucose, hematocrit, total length, weight, hepatosomatic index (HSI), viscerosomatic index (VSI), splenosomatic index (SSI), and relative fin lengths (dorsal, pectoral, pelvic) at the beginning of the experiment are listed in Table 2. Glucose levels over time were not significantly different among the density and feeding treatments (Figure 1). Hematocrit levels over time were also not significantly different among the treatments (Figure 2). Total lengths, weight, HSI, VSI, and any of the relative fin lengths were not significantly different between the low and high densities (Table 3). HSI was significantly greater in the fish that were fed compared to the unfed treatment. SSI was significantly greater in the higher density treatment. Dorsal fin relative length was also significantly shorter for the fish that were fed compared to the fish that were fasted.

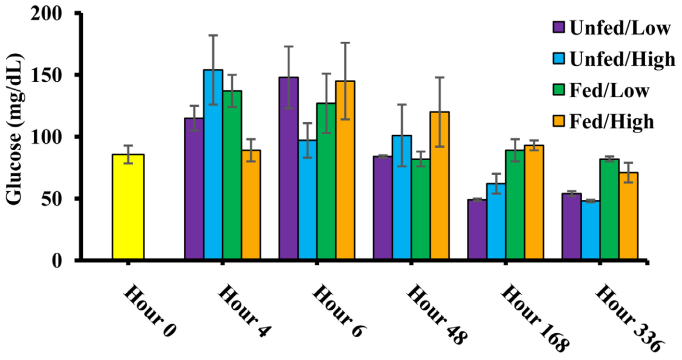

Figure 1. Mean (±SE) glucosevalues over time of rainbow trout at either low (1.8 g/m^3) or high (30.1 g/m^3) densities and either fed or unfed.

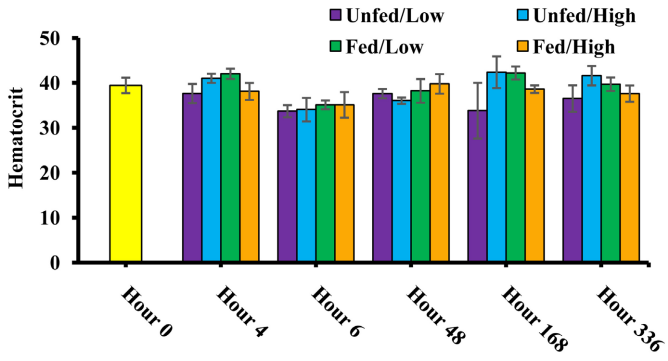

Figure 2. Mean (±SE) hematocrit[a] values over time of rainbow trout at either low (1.8 g/m^3) or high (30.1 g/m^3) densities and either fed or unfed. [a]Hematocrit (%) = 100 × (red blood/whole blood).

Table 2. Individual fish mean (±SE) glucose, hematocrit[a], total length, weight, organosomatic indice [viscerosomatic index (VSI)[b], splenosomatic index (SSI)[c], hepatosomatic index (HSI)[d]], and relative fin lengths (% dorsal, pectoral, pelvic)[e] at the beginning of the experiment (n = 10).

	Mean		
Glucose (mg/dL)	86	±	9
Hematorcrit (%)	39.45	±	1.72
Total length (mm)	101.22	±	2.88
Weight (g)	11.96	±	1.15
VSI (%)	18.40	±	1.94
SSI (%)	0.08	±	0.01
HSI (%)	1.88	±	0.20
Dorsal (%)	9.02	±	0.28
Pectoral (%)	10.21	±	0.25
Pelvic (%)	8.94	±	0.18

[a]Hematocrit (%) = 100 × (red blood/whole blood); [b]HSI = 100 × (liver weight/body weight); [c]VSI = 100 × (visceral weight/body weight); [d]SSI = 100 × (spleen weight/body weight); [e]Dorsal = 100 × (dorsal fin length/fish length); [f]Pectoral = 100 × (pectoral fin length/fish length); [g]Pelvic = 100 × (pelvic fin length/fish length).

Table 3. Individual fish mean (±SE) total length, weight, organosomatic indices [hepatosomatic index (HSI)[a], viscerosomatic index (VSI)[b], splenosomatic index (SSI)[c]], and fin indices (dorsal[d], pectoral[e], pelvic[f]) after 336 hours of rearing at either at one of two densities and either fed or starved (unfed) (n = 3). Overall means with different letters in same row or column differ significantly ($p < 0.05$).

			Density						Overall		
			Low (1.8 g/m^3)			High (30.1 g/m^3)					
Total length (mm)	Fed	No	97	±	2	99	±	6	98	±	3
		Yes	103	±	6	104	±	2	104	±	3
	Overall		100	±	3	102	±	3			
Weight (g)	Fed	No	8.38	±	0.51	9.29	±	0.51	8.83	±	1.17
		Yes	11.76	±	2.15	10.64	±	0.82	11.20	±	1.06
	Overall		10.07	±	1.25	9.96	±	1.22			
HSI (%)	Fed	No	0.95	±	0.05	0.82	±	0.10	0.88	±	0.06 y
		Yes	1.87	±	0.40	1.37	±	0.12	1.62	±	0.54 z
	Overall		1.41	±	0.27	1.09	±	0.14			
VSI (%)	Fed	No	8.94	±	0.33	9.31	±	0.21	9.12	±	0.19 y
		Yes	13.03	±	0.54	12.26	±	1.47	12.65	±	0.72 z
	Overall		10.98	±	0.96	10.79	±	0.94			

Continued

SSI (%)	Fed	No	0.08	±	0.01	0.12	±	0.02	0.10	±	0.01
		Yes	0.06	±	0.00	0.09	±	0.01	0.08	±	0.01
	Overall		0.07	±	0.01 y	0.11	±	0.02 z			
Dorsal (%)	Fed	No	13.72	±	0.54	13.97	±	0.34	13.84	±	0.29 z
		Yes	13.52	±	0.90	10.70	±	0.53	12.11	±	0.78 y
	Overall		13.62	±	0.47	12.33	±	0.78			
Pectoral (%)	Fed	No	10.33	±	0.47	11.04	±	0.32	10.69	±	0.30
		Yes	10.99	±	1.47	11.38	±	0.91	11.19	±	0.78
	Overall		10.66	±	0.70	11.21	±	0.44			
Pelvic (%)	Fed	No	9.61	±	0.26	9.69	±	0.54	9.65	±	0.27
		Yes	10.43	±	0.75	10.52	±	0.40	10.48	±	0.38
	Overall		10.02	±	0.40	10.11	±	0.35			

[a]HSI = 100 × (liver weight/body weight); [b]VSI = 100 × (visceral weight/body weight); [c]SSI = 100 × (spleen weight/body weight); [d]Dorsal = 100 × (dorsal fin length/fish length); [e]Pectoral = 100 × (pectoral fin length/fish length); [f]Pelvic = 100 × (pelvic fin length/fish length).

4. Discussion

The results of this study generally indicate little short-term effects of density on stress in rainbow trout, if the fish are fed or not. Glucose and hematocrit levels typically increase following a stressful event [21], and such a response was not observed in this study. However, SSI did increase, likely indicating either an increase in hematopoietic capacity or antibody production [25]. In contrast, Iguchi *et al.* [5] observed a density-dependent increase in cortisol and a decrease in ayu *Plecoglossus altirelis,* a non-salmonid fish species.

The impacts of rearing density on SSI are uncertain. Wagner *et al.* [26] and Vijayan and Leatherland [27] reported no significant differences in SSI among rearing densities. However, another experiment by Wagner *et al.* [26] indicated a possible relationship between SSI and rearing density. It is possible that all fish in the current study were responding to a general immunological or environmental factor because all of the SSI values were higher than that previously reported for juvenile rainbow trout [25].

Similar to the current study, Papoutsoglou *et al.* [28] also reported no effect of density on blood glucose. However, Leatherland and Cho [29], Trenzado *et al.* [30], Yarahmadi *et al.* [31], and Wedemeyer [32] all observed increases in blood glucose with increasing rearing density. Contrarily, Vijayan and Leatherland [27] observed a decrease in blood glucose with increasing density. The difference in fish species, strains, sizes, densities, water chemistry, and rearing history may explain different results among the studies.

Hematocrit, the volume percentage of red blood cells, tends to increase when

fish are under stress [21]. The lack of influence of density on hematocrit in this study supports the results reported by Leatherland and Cho [29] and Miller *et al.* [33]. In contrast, North *et al.* [34], Trenzado *et al.* [30], Mazur and Iwama [25], and Yarahmadi *et al.* [31] reported increases in hematocrit at higher fish rearing densities. Wagner *et al.* [26] observed inconsistent results with one experiment resulting in a density effect on hematocrit and another indicating no effect. Just as with blood glucose, differences in species, strains, sizes, densities used, and prior rearing experiences may all impact the response of hematocrit to elevated densities [10] [35].

The short-term stress results of blood glucose and hematocrit from this study were similar whether the fish were fed or not. However, feeding did influence HSI and VSI. This is not surprising. HSI is an indicator of nutritional status, indirectly measuring glycogen and carbohydrates, and VSI is an indicator of lipid shortage, being positively correlated with lipid levels [25]. Starvation, as experienced by the fasted fish in this study, will result in decreased HSI and VSI. Wagner *et al.* [26] and Vijayan and Leatherland [27] also reported no effect of rearing density on HSI. However, Leatherland and Cho [30] observed an increase in HSI at lower rearing densities. Similar to this study, Zahedi *et al.* [36] reported no effect of density on VSI.

Relative fin lengths may be long-term indicators of fish stress, and are influenced by numerous behavioral, environmental, or physiological factors [11] [26] [37]. Thus, it is not surprising that no differences, other than between fed and unfed fish and the dorsal fin, among the treatments were observed in this two-week study. Soderberg *et al.* [38] also reported no impact of rearing density on fin condition. The significantly shorter dorsal fin for the fish fed could be due to aggressive nipping commonly observed on the dorsal fin [39], and fish that were fed are aggressively going after the food available or diet [23] [26].

The relatively short duration of this study is obviously a limitation. In addition, the densities used in this study were generally lower than those used in other density experiments with rainbow trout [30] [31] [32] [34] [40] [41] [42] [43]. Lastly, it would have been beneficial to measure cortisol directly as recommended by Martinez-Porchas *et al.* [44] and Sopinka *et al.* [21].

5. Conclusion

In conclusion, blood glucose and hematocrit levels indicated no short-term stress response at either of the densities used in this experiment. The splenosomatic index was elevated in fish in the higher density treatments, however. Future studies should examine the stress response at higher densities, possibly identifying the limits of fish crowding during hatchery rearing.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ewing, R.D. and Ewing, S.K. (1995) Review of the Effects of Rearing Density on Survival to Adulthood for Pacific Salmon. *The Progressive Fish-Culturist*, **57**, 1-25. https://doi.org/10.1577/1548-8640(1995)057<0001:ROTEOR>2.3.CO;2

[2] Piper, R.G., McElwain, I.B., Orme, L.E., McCraren, J.P., Fowler, L.G. and Leonard, J.R. (1982) Fish Hatchery Management. U.S. Fish and Wildlife Service, Washington DC.

[3] Ewing, R.D., Sheahan, J.E., Lewis, M.A. and Palmisano, A.N. (1998) Effects of Rearing Density and Raceway Conformation on Growth, Food Conversion, and Survival of Juvenile Spring Chinook Salmon. *The Progressive Fish-Culturist*, **60**, 167-178. https://doi.org/10.1577/1548-8640(1998)060<0167:EORDAR>2.0.CO;2

[4] Ellis, T., North, B., Scott, A.P., Bromage, N.R., Porter, M. and Gadd, D. (2002) The Relationships between Stocking Density and Welfare in Farmed Rainbow Trout. *Journal of Fish Biology*, **61**, 493-531. https://doi.org/10.1111/j.1095-8649.2002.tb00893.x

[5] Iguchi, K.I., Ogawa, K., Nagae, M. and Ito, F. (2003) The Influence of Rearing Density on Stress Response and Disease Susceptibility of Ayu (*Plecoglossus altevelis*). *Aquaculutre*, **220**, 515-523. https://doi.org/10.1016/S0044-8486(02)00626-9

[6] Weber, E.D. and Fausch, K.D. (2003) Interactions between Hatchery and Wild Salmonids in Streams: Differences in Biology and Evidence for Competition. *Canadian Journal of Fisheries and Aquatic Science*, **60**, 1018-1036. https://doi.org/10.1139/f03-087

[7] Person-Le Ruyet, J., Le Bayon, N. and Gros, S. (2007) How to Assess Fin Damage in Rainbow Trout, *Salmo gairdneri*. *Aquatic Living Resources*, **20**, 191-195. https://doi.org/10.1051/alr:2007031

[8] Hosfeld, C.D., Hammer, J., Handeland, S.O., Fivelstad, S. and Stefansson, S.O. (2009) Effects of Fish Density on Growth and Smoltification in Intensive Production of Atlantic Salmon (*Salmo salar* L.). *Aquaculture*, **294**, 236-241. https://doi.org/10.1016/j.aquaculture.2009.06.003

[9] Barnes, M.E., Wipf, M.M., Domenici, N.R., Kummer, W.M. and Hanten, R.P. (2013) Decreased Hatchery Rearing Density Improves Poststocking Harvest and Return to Spawning of Landlocked Fall Chinook Salmon. *North American Journal of Aquaculture*, **75**, 244-250. https://doi.org/10.1080/15222055.2013.768573

[10] Biron, M. and Benfey, T.J. (1994) Cortisol, Glucose and Hematocrit Changes during Acute Stress, Cohort Sampling, and the Diel Cycle in Diploid and Triploid Brook Trout (*Salvelinus fontinalis*, Mitchill). *Fish Physiology and Biochemistry*, **13**, 153-160. https://doi.org/10.1007/BF00004340

[11] Wagner, E.J. and Driscoll, D.M. (1994) Physiological Stress Responses of Cutthroat Trout to Loading by Fish Pump, Conveyor, or Dip Net. *Journal of Applied Aquaculture*, **4**, 19-27. https://doi.org/10.1300/J028v04n01_02

[12] Iwama, G.K., Morgan, J.D. and Barton, B.A. (1995) Simple Field Methods for Monitoring Stress and General Condition of Fish. *Aquaculture Research*, **26**, 273-282. https://doi.org/10.1111/j.1365-2109.1995.tb00912.x

[13] Benfey, T.J. and Biron, M. (2000) Acute Stress Response in Triploid Rainbow Trout

(*Oncorhynchus mykiss*) and Brook Trout (*Salvelinus fontinalis*). *Aquaculture*, **184**, 167-176. https://doi.org/10.1016/S0044-8486(99)00314-2

[14] Jentoft, S., Aastveit, A.H., Torjesen, P.A. and Andersen, Ø. (2005) Effects of Stress on Growth, Cortisol and Glucose Levels in Non-Domesticated Eurasian Perch (*Perca fluviatilis*) and Domesticated Rainbow Trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology*, *Part A*, **141**, 353-358. https://doi.org/10.1016/j.cbpb.2005.06.006

[15] López-Luna, J., Bermejo-Poza, R., Bravo, F.T. and Vilarroel, M. (2016) Effect of Degree-days Fasting Stress on Rainbow Trout, *Oncorhynchus mykiss*. *Aquaculture*, **462**, 109-114. https://doi.org/10.1016/j.aquaculture.2016.05.017

[16] Sumpter, J.P., Le Bail, P.Y., Pickering, A.D., Pottinger, T.G. and Carragher, J.F. (1991) The Effect of Starvation on Growth and Plasma Growth Hormone Concentrations of Rainbow Trout, *Oncorhynchus mykiss*. *General and Comparative Endocrinology*, **83**, 94-102. https://doi.org/10.1016/0016-6480(91)90109-J

[17] Salem, M., Silverstein, J., Rexroad, C.E. and Yao, J. (2007) Effect of Starvation on Global Gene Expression and Proteolysis in Rainbow Trout (*Oncorhynchus mykiss*). *BMC Genomics*, **8**, Article No. 328. https://doi.org/10.1186/1471-2164-8-328

[18] Furné, M., García-Gallego, M., Hidalgo, M.C., Morales, A.E., Domezain, A., Domezain, J. and Sanz, A. (2009) Oxidative Stress Parameters during Starvation and Refeeding Periods in Adriatic Sturgeon (*Acipenser naccarii*) and Rainbow Trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*, **15**, 587-595. https://doi.org/10.1111/j.1365-2095.2008.00626.x

[19] Bayir, A., Sirkecioglu, A.N., Bayir, M., Haliloglu, H.I., Kocaman, E.M. and Aras, N.M. (2011) Metabolic Responses to Prolonged Starvation, Food Restriction, and Refeeding in the Brown Trout, *Salmo trutta*: Oxidative Stress and Antioxidant Defenses. *Comparative Biochemistry and Physiology Part B*: *Biochemistry and Molecular Biology*, **159**, 191-196. https://doi.org/10.1016/j.cbpb.2011.04.008

[20] Macek, K.J. (1968) Growth and Resistance to Stress in Brook Trout Fed Sublethal Levels of DDT. *Journal of the Fisheries Board of Canada*, **25**, 2443-2451. https://doi.org/10.1139/f68-212

[21] Sopinka, N.M., Donaldson, M.R., O'Connor, C.M., Suski, C.D. and Cooke, S.J. (2016) Stress Indicators in Fish. In: Schreck, C.B., Tort, L., Farrell, A.P. and Brauner, C.J., Eds., *Biology of Stress in Fish*, Elsevier Inc., London, 405-462. https://doi.org/10.1016/B978-0-12-802728-8.00011-4

[22] Butterbaugh, G.L. and Willoughby, H. (1967) A Feeding Guide for Brook, Brown and Rainbow Trout. *The Progressive Fish-Culturst*, **29**, 210-215. https://doi.org/10.1577/1548-8640(1967)29[210:AFGFBB]2.0.CO;2

[23] Kindschi, G.A., Shaw, H.T. and Bruhn, D.S. (1991) Effect of Diet on Performance, Fin Quality, and Dorsal Skin Lesions in Steelhead. *Journal of Applies Aquaculture*, **1**, 113-120. https://doi.org/10.1300/J028v01n01_10

[24] Barton, B.A., Morgan, J.D. and Vijayan, M.M. (2002) Physiological and Condition-Related Indicators of Environmental Stress in Fish. In: Adams, S.M., Ed., *Biological Indicators of Aquatic Ecosystem Stress*, American Fisheries Society, Bethesda, 111-148.

[25] Voorhees, J.M., Barnes, M.E., Chipps, S.R. and Brown, M.L. (2018) Dietary Bioprocessed Soybean Meal Does Not Affect the Growth of Exercised Juvenile Rainbow Trout (*Oncorhynchus mykiss*). *Journal of Animimal Research and Nutrition*, **3**, 1-13.

[26] Wagner, E.J., Jeppsen, T., Arndt, R., Routledge, M.D. and Bradwisch, Q. (2011) Effects

of Rearing Density upon Cutthroat Trout Hematology, Hatchery Performance, Fin Erosion, and General Health and Condition. *The Progressive Fish-Culturist*, **59**, 173-187. https://doi.org/10.1577/1548-8640(1997)059<0173:EORDUC>2.3.CO;2

[27] Vijayan, M.M. and Leatherland, J.F. (1988) Effect of Stocking Density on the Growth and Stress-Response in Brook Charr, *Salvelinus fontinalis*. *Aquaculture*, **75**, 159-170. https://doi.org/10.1016/0044-8486(88)90029-4

[28] Papoutsoglou, S.E., Papaparaskeva-Papoutsoglou, E. and Alexis, M.N. (1987) Effect of Density on Growth Rate and Production of Rainbow Trout (*Salmo gairdneri* Rich.) over a Full Rearing Period. *Aquaculture*, **66**, 9-17. https://doi.org/10.1016/0044-8486(87)90279-1

[29] Leatherland, J.F. and Cho, C.Y. (1985) Effect of Rearing Density on Thyroid and Interrenal Gland Activity and Plasma Hepatic Metabolite Levels in Rainbow Trout, *Salmo gairdneri* Richardson. *Journal of Fish Biology*, **27**, 583-592. https://doi.org/10.1111/j.1095-8649.1985.tb03203.x

[30] Trenzado, C.E., Morales, A.E. and de la Higuera, M. (2006) Physiological Effects of Crowding in Rainbow Trout, *Oncorhynchus mykiss*, Selected for Low and High Stress Responsiveness. *Aquaculture*, **258**, 583-593. https://doi.org/10.1016/j.aquaculture.2006.03.045

[31] Yarahmadi, P., Miandare, H.K., Hoseinifar, S.H., Gheysvandi, N. and Akbarzadeh, A. (2015) The Effects of Stocking Density of Hemato-Immunological and Serum Biochemical Parameters of Rainbow Trout (*Oncorhynchus mykiss*). *Aquaculture International*, **23**, 55-63. https://doi.org/10.1007/s10499-014-9797-z

[32] Wedemeyer, G.A. (1976) Physiological Response of Juvenile Coho Salmon (*Oncorhynchus kisutch*) and Rainbow Trout (*Salmo gairdneri*) to Handling and Crowding Stress in Intensive Fish Culture. *Journal of the Fisheries Research Board of Canada*, **33**, 2699-2702. https://doi.org/10.1139/f76-320

[33] Miller, S.A., Wagner, E.J. and Bosakowski, T. (1995) Performance and Oxygen Consumption of Rainbow Trout Reared at Two Densities in Raceways with Oxygen Supplementation. *The Progressive Fish-Culturist*, **57**, 206-212. https://doi.org/10.1577/1548-8640(1995)057<0206:PAOCOR>2.3.CO;2

[34] North, B.P., Turnbull, J.F., Ellis, T., Porter, M.J., Migaud, H., Bron, J. and Bromage, N.R. (2006) The Imapct of Stocking Density on the Welfare of Rainbow Trout. *Aquaculture*, **255**, 466-479. https://doi.org/10.1016/j.aquaculture.2006.01.004

[35] Mazur, C.F. and Iwama, G.K. (1993) Effect of Handling and Stocking Density on Hematocrit, Plasma Cortisol, and Survival in Wild and Hatchery-Reared Chinook Salmon. *Aquaculture*, **11**, 291-299. https://doi.org/10.1016/0044-8486(93)90390-K

[36] Zahedi, S., Akbarzadeg, A., Mehrzad, J., Noori, A. and Harsij, M. (2019) Effect of Stocking Density on Growth Performance, Plasma Biochemistry and Muscle Gene Expression in Rainbow Trout (*Oncorhynchus mykiss*). *Aquaculture*, **498**, 271-278. https://doi.org/10.1016/j.aquaculture.2018.07.044

[37] Barnes, M.E., Sayler, W.A. and Cordes, R.J. (2011) Baffle Usage in Covered Raceways. *The Progressive Fish-Culturist*, **58**, 286-288. https://doi.org/10.1577/1548-8640(1996)058<0286:BUICR>2.3.CO;2

[38] Soderberg, R.W. and Meade, J.W. (1987) Effects of Rearing Density on Growth, Survival, and Fin Condition of Atlantic Salmon. *The Progressive Fish-Culturist*, **49**, 280-283. https://doi.org/10.1577/1548-8640(1987)49<280:EORDOG>2.0.CO;2

[39] Abott, J.C. and Dill, L.M. (1985) Patterns of Aggressive Attack in Juvenile Steelhead Trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 1702-1706. https://doi.org/10.1139/f85-213

[40] Irwin, S., O'Halloran, J. and FitzGerald, R.D. (1999) Stocking Density, Growth and Growth Variation in Juvenile Turbot, *Scophthalmus maximus* (Rafinesque). *Aquaculture*, **178**, 77-88. https://doi.org/10.1016/S0044-8486(99)00122-2

[41] Pickering, A.D. and Pottinger, T.G. (1987) Crowding Causes Prolonged Leucopenia in Salmonid Fish, Despite Interrenal Acclimation. *Journal of Fish Biology*, **30**, 701-712. https://doi.org/10.1111/j.1095-8649.1987.tb05799.x

[42] Procarione, L.S., Barry, T.P. and Malison, J.A. (1999) Effects of High Rearing Densities and Loading Rates on the Growth and Stress Responses of Juvenile Rainbow Trout. *North American Journal of Aquaculture*, **61**, 91-96. https://doi.org/10.1577/1548-8454(1999)061<0091:EOHRDA>2.0.CO;2

[43] Refstie, T. (1977) Effect of Density on Growth and Survival of Rainbow Trout. *Aquaculture*, **11**, 329-334. https://doi.org/10.1016/0044-8486(77)90082-5

[44] Martínez-Porchas, M., Martínez-Córdova, L.R. and Ramos-Enriquez, R. (2009) Cortisol and Glucose: Reliable Indicators of Fish Stress? *Pan-American Journal of Aquatic Sciences*, **4**, 158-178.