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The effects of stocking density on hemato-immunological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*)

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Abstract In the present study, the effect of stocking density on hematological, biochemical and immunological parameters of rainbow trout (*Oncorhynchus mykiss*) was investigated. Experiments were performed in a 30-day period at two different stocking densities: low density (LD 10 kg/m^3) and high density (HD 45 kg/m^3). Fish held at HD showed significant higher levels of hemoglobin and red blood cells (12.76 mg/dl and 1.8×10^6 mm^3, respectively) compared with those held at LD (11.43 mg/dl and 1.23×10^6 mm^3, respectively; $P < 0.05$). Stocking density had no significant effect on mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration levels. The level of HCT was higher in fish reared at HD, but it was not statistically significant. In the case of biochemical parameters, plasma glucose and cortisol increased significantly in HD compared with LD ($P < 0.05$), but stocking density caused no significant changes in total protein, globulin and albumin levels. Our results also revealed that the levels of plasma cholesterol and triglycerides were significantly lower in LD compared with HD ($P < 0.05$). Results of some immune parameters of *O. mykiss* reared at two different stocking densities revealed that at HD, the level of white blood cells was lower significantly compared with LD ($P < 0.05$). The percentage of lymphocytes, neutrophiles and monocytes showed no significant changes between two groups. In addition, the level of serum lysozyme was significantly higher in animals maintained at HD compared with LD ($P < 0.05$). Overall, our results show that high stocking density caused chronic stress in rainbow trout and consequently altered the levels of some hematological, biochemical and immunological parameters compared with fish kept at low stocking density.

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Introduction

Stress in farmed fish is of considerable significance to both welfare and productivity as it has been linked to reduction in growth, abnormal behavior and immunodepression (Wedemeyer 1996; Ashley 2007). The overall effect of stress may be considered as a change in biological condition beyond the normal resting state that challenges homeostasis and, thus, presents a threat to the fish's health. Stress-induced changes are grouped as primary; secondary, which includes metabolic, hematological, hydromineral and structural; and tertiary or 'whole animal' responses. Many of these responses can be used as quantitative indicators of stress (Barton and Iwama 1991).

Stocking density is an important factor affecting growth in fish. Determination of optimal value for this factor is a prerequisite to warrant the economic viability of fish production. However, commercial production must also take into account other factors such as animal welfare, stress, health, immune system and physiology (Salas-Leiton et al. 2010). Particular attention has been drawn to stocking density as one of the key factors to influence the perceived level of stress in fish (Ellis et al. 2002; North et al. 2006). It has been demonstrated that rearing of fish at inappropriate stocking densities may impair the growth and reduce immune competence due to factors such as social interaction and the deterioration of water quality, which can affect both the feed intake and conversion efficiency of the fish (Ellis et al. 2002; Lupatsch et al. 2010). Although high density (HD) in aquaculture systems may be followed by an increase in fish production, it may have some impacts on aquatic species in terms of physiological, hormonal and immune systems (Salas-Leiton et al. 2010). It has been well known that HD storage alters the levels of some blood hormones and metabolites in which subsequently leads to reduced food intake and growth (Vijayan and Leatherland 1988; Vijayan et al. 1990; Montero et al. 1999).

Poor growth and increased incidence of disease have often been observed in different fish species. In this regard, much research effort has been focused on evaluating the effects of rearing density on fish growth, fish survival and food intake (Soderberg and Meade 1987; Holm et al. 1990; Jørgensen et al. 1993). The effects of stocking density on growth performance, welfare, physiology, immune system and health of farmed fish have been studied in different fish species (North et al. 2006; Sirakov 2008 #214; Sirakov and Ivanchev 2008; Salas-Leiton et al. 2010; Yousefi et al. 2012; Hasanalipour et al. 2013). The evaluation of blood cells, blood biochemistry and hormones has been known to be useful for the diagnosis of diseases and monitoring of the physiological status of fish (Caruso et al. 2005; Carbonara et al. 2010).

Rainbow trout, *Oncorhynchus mykiss*, is one of the most recognized and accepted farmed fish species. Considering the importance of the stocking density in the success of rainbow trout production, the present study aimed to evaluate the effects of high and low stocking densities on some hematological, biochemical and immune parameters in rainbow trout. The results of this research could be very useful and applicable for rainbow trout aquaculturists.

Materials and methods

Fish and rearing conditions

The experiments were carried out on sub-adult rainbow trout (weighting 65 ± 2.2 g) in a period of 30 days after 10-day acclimation to laboratory condition at the disease laboratory at The University of Tehran. Fish reared at 500-L tanks were randomly allocated in six circular tanks assigned to two levels of experimental stocking densities, nominally indicated as low density (LD 10 kg/m^3) as base density and HD (45 kg/m^3) based on previous study (North et al. 2006) at the beginning of the experiment and 1.42-fold at the end of experiment with three replication. The rearing was performed in open water flow-through system and tanks were supplied with same source of water from central pipe. The flow rate was adjusted to maintaining the water quality parameters in all tanks with different density and exchange rate of 1.66 per hours. The tanks were aerated with two air stones connected to central compressor. Dissolved oxygen was measured daily in water outflow and maintained at the same level as in the control group (6.6 mg/l) using an Oxymeter (WTW oxi 330i, Weilheim, Germany). Water temperature was 18 ± 1.2 °C during the experiment with day/night light cycles (12 h dark vs. 12 h light). Unionized ammonia and pH were measured weekly and maintained (<0.009 and 6.9 ± 0.4, respectively) and no significant different observed in all treatment. Both groups of fish were fed with commercial food twice a day, in a rate of 3 % of body weight per day. The approximate composition of the feed used is given in Table 1.

Sampling

After a 30-day rearing period, nine fish from each treatment (three from each tank) were quickly anesthetized using Clove powder (200 mg/l). In total, 1 mL of blood was collected using a hypodermic syringe from the caudal blood vessels. The blood samples were transferred to heparinized tubes for hematological parameters and non-heparinized tubes to obtain serum. The blood was centrifuged at 3,000 rpm for 10 min at 4 °C. The collected plasma was stored at −20 °C for further analyses.

Hematological assay

Red blood cells (RBCs) and white blood cells (WBCs) were counted under a light microscope using a Neubauer hemocytometer after dilution with phosphate-buffered saline (Sarder et al. 2001). Hematocrit was determined by centrifuging whole blood in heparinized microhematocrit capillary tubes at 3,500*g* for 10 min (Brown 1988). Hemoglobin (Hb) concentration was measured using the cyanohemoglobin method. Red cell indices, mean corpuscular volume (MCV µm^3/cell), mean corpuscular hemoglobin (MCH pg/cell) and mean corpuscular hemoglobin concentration (MCHC g/l) were calculated from RBC (Asadi et al. 2012).

Serum biochemical parameters assay

Total protein and albumin levels were measured by a commercial kit according to the manufacturer protocol (Parsazmon Co. Iran). Cholesterol levels were measured by the cholesterol oxidase with commercial Pars Azmon kit on spectrophotometer. Glucose levels

Table 1 Approximate composition of the experimental diet

Approximate analysis (%)	
Dry matter	90
Crude lipid	14
Crude protein	37
Ash	10
Phosphorous	>0.7

were obtained by the colorimetric glucose oxidase procedure with commercial Pars Azmon kit (Benfey and Biron 2000). Triglycerides (TG) were measured using Pars Azmon kit following the manufacturer's instructions. Cortisol levels were measured by Radio Immuno Assay commercial kit (Immunotech, France) following the manufacturer's instructions.

Lysozyme assay

Serum lysozyme activity of rainbow trout was measured using the turbidimetric method as described by Parry, Chandanand Shahani (Parry et al. 1965). In total, 50 µl of plasma was added to 2 ml of a suspension of *Micrococcus lysodeikticus* (0.2 mg/ml) in a 0.05 M sodium phosphate buffer (pH 6.2). The reaction was carried out at room temperature, and absorbance was measured spectrophotometrically at 450 nm. PBS was used as a blank. A unit of lysozyme activity was defined as the sample amount causing a decrease in absorbance of 0.001/min. Lysozyme of sample was calibrated using a standard curve determined with hen's egg white lysozyme (Sigma) in PBS.

Statistical analyses

The results were subjected to independent *t* test to compare the statistical differences between two treatments. SPSS software (version 16) was used for determining significant differences. Significance was set at a probability level of 95 % ($P < 0.05$).

Results

There was no mortality in the period of experiment, and survival rate was 100 %. At the end of experiment, final weight was 92.37 ± 2.36 and no significant difference observed between two different densities.

Hematological parameters

The hematological parameters of *O. mykiss* reared at two different stocking densities are presented in Table 2. Fish held at high stocking density showed significant higher levels of Hb and RBC (12.76 mg/dl and 1.8 × 106 mm^3, respectively) compared with those held at low stocking density (11.43 mg/dl and 1.23 × 106 mm^3, respectively; $P < 0.05$). Stocking density had no significant effect on MCH and MCHC levels. The level of HCT was higher in fish reared at HD, but it was not statistically significant (Table 2).

Table 2 Levels of hematological parameters in fish reared at LD and HD

Stocking density	RBC ($^{a}10^6$/mm^3)	Hb (mg dl^{-1})	HCT (%)	MCV (mm^3 10^{-5})	MCH (pg 10^{-5})	MCHC (%)
LD	1.23 ± 0.25	11.43 ± 0.58	37 ± 4.35	30.56 ± 4.15	96.77 ± 25.06	31.38 ± 4.32
HD	1.8 ± 0.13[a]	12.76 ± 0.98[a]	40.66 ± 1.15	22.65 ± 1.39[a]	70.16 ± 3.9	30.98 ± 0.22

Data in a column assigned with different superscripts denote significant difference ($P < 0.05$)

Biochemical parameters

Changes in some biochemical parameters of *O. mykiss* reared at two different stocking densities are shown in Table 3. Plasma glucose and cortisol increased significantly in HD compared with LD ($P < 0.05$), so that plasma glucose and cortisol levels increased up to 76.2 mg/dl and 14.43 ng/ml, respectively, in fish held at high stocking; these values were nearly twofold that of fish held at low stocking density (37.28 mg/dl and 6.8 ng/ml, respectively). Whereas plasma glucose and cortisol showed a remarkable increase when animals reared at HD, stocking density caused no significant changes in total protein, globulin and albumin levels (Fig. 1). Our results also revealed that the levels of plasma cholesterol and TG were significantly lower in LD compared with HD ($P < 0.05$).

Immune parameters

Results of some immune parameters of *O. mykiss* reared at two different stocking densities are revealed in Table 4. At HD, the level of WBC was significantly lower compared with LD ($P < 0.05$). The percentage of lymphocytes, neutrophiles and monocytes showed no significant changes between two groups. The levels of serum lysozyme were significantly higher in animals maintained at HD compared with LD ($P < 0.05$).

Discussion

The major finding of the present study was that rearing of rainbow trout in high stocking density (45 kg/m^3) caused a crowding stress compared with low stocking density (10 kg/m^3) in water flow-through tanks with maintained water quality parameters such as ammonia, pH, dissolve oxygen and temperature as same level. Our results revealed that the levels of non-specific stress response such as blood RBC and Hb, as well as plasma glucose and cortisol were significantly elevated in fish reared at high stocking density compared with LD (10 kg/m^3). The negative impact of high stocking density on morphological parameter, feed conversion ratio and growth has been reported in salmonid fish such as rainbow trout and brown trout (North et al. 2006; Sirakov and Ivanchev 2008).

A previous study indicated that stocking density had no significant effect on growth of rainbow trout (North et al. 2006), and Papoutsoglou et al. (1987) revealed that stocking density did not affect growth parameters until 60 days of experiment, but at the end of trial, fish reared at the lowest density (group 1) had the highest final body weight. Although it is not the case of our findings, (Schreck 1981) stated that chronic stress may impair the performance such as growth and disease resistance of the fish.

Table 3 Levels of total protein, albumin, triglyceride, globulin, cholesterol, glucose and cortisol reared at LD and HD

	LD	HD
TP (mg dl^{-1})	3.71 ± 0.12^{a}	3.92 ± 0.05^{a}
AL (mg dl^{-1})	1.2 ± 0.2^{a}	1.6 ± 0.2^{a}
GOL (mg dl^{-1})	2.51 ± 0.29^{a}	2.32 ± 0.15^{a}
TG (mg dl^{-1})	274.65 ± 29.35^{a}	204.02 ± 7.83^{b}
CHO (mg dl^{-1})	157.28 ± 7.77^{a}	117.22 ± 4.58^{b}
GULO (mg dl^{-1})	38.27 ± 0.76^{b}	76.2 ± 9.62^{a}
Cortisol (ng dl^{-1})	6.8 ± 0.45^{b}	14.43 ± 0.77^{a}

Data in a row assigned with different superscripts denote significant difference ($P < 0.05$)

Chronic stress caused by HD of fish changed some hematological parameters of rainbow trout. The significant elevation of RBC and Hb levels observed in the present study could be attributed to the increased stress and oxygen demand. RBC and Hb are responsible for more blood oxygen-carrying capacity (Srivastava and Sahai 1987a). Similar results were also achieved in gilthead sea bream (*Sparus aurata*) reared under conditions of HD (Montero et al. 1999).

Fish kept at HD showed remarkable levels of serum cortisol compared with those reared at LD. It has been known that the changes in corticosteroid hormones can be contributed to the first response of animal to stress (Barton and Iwama 1991; Barton 2002). As a gluconeogenic and ionregulatory hormone, cortisol is considered to be a primary indicator of stress response (Tintos et al. 2006). The enhanced levels of cortisol in high stocking density observed in the present study are consistent with similar observations in different fish species, e.g., gilthead sea bream, *S. aurata* (Montero et al. 1999), zebrafish, *Danio rerio*, (Ramsay et al. 2006), Japanese flounder, *Paralichthys olivaceus* (Bolasina et al. 2006) and European sea bass *Dicentrarchus labrax* (Lupatsch et al. 2010). On the contrary, stocking density had no significant effect on cortisol levels in great sturgeon, *Huso huso* juveniles (Rafatnezhad et al. 2008) and Siberian Sturgeon, *Acipenser baerii* (Hasanalipour et al. 2013). Such an inconsistency in cortisol response to high stocking density could be due to the differences in the biology and physiology responses to stress in different fish species.

Following the increase in cortisol as a primary response to stress, some other biochemical parameters like glucose were significantly elevated in response to crowding stress in rainbow trout. It has been known that the levels of plasma cortisol and glucose are usually correlated with each other (Almeida et al. 2005; Monteiro et al. 2005). Cortisol induces the plasma glucose levels by the induction of gluconeogenesis and glycogenolysis for supplying the new energy demand (Iwama et al. 1999; Vinodhini and Narayanan 2009). Opposed to our results, plasma glucose concentrations were not altered in great sturgeon, *H. huso* juveniles (Rafatnezhad et al. 2008) and Siberian Sturgeon, *A. baerii* (Hasanalipour et al. 2013), when kept at high stocking densities.

Our results also revealed that the immune system of rainbow trout has been influenced by stocking density. The total number of white blood cells, as well as the percentage of lymphocytes and neutrophil decreased at high stocking. Moreover, the level of lysozyme as a non-specific defense factor was also depressed significantly in animals reared at high density. The reduction of some immune system parameters such as leukocytes number and lysozyme level has been observed in different fish species such as rainbow trout (Cristea

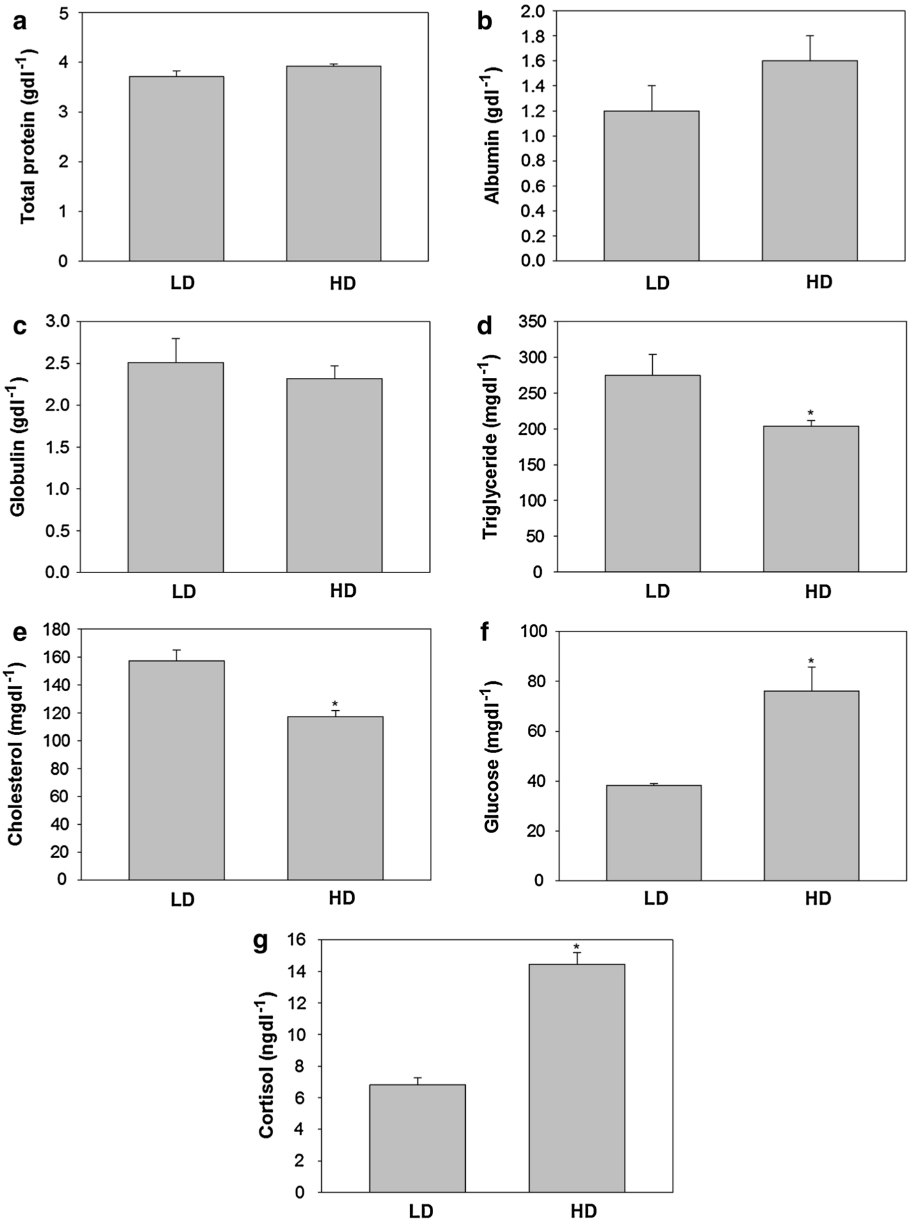

Fig. 1 Levels of total protein (**a**), albumin (**b**), triglyceride (**c**), globulin (**d**), cholesterol (**e**), glucose (**f**) and cortisol (**g**) reared at LD and HD, $*p < 0.05$ *t* test

et al. 2012), Indian freshwater catfish, *Heteropneustes fossilis* (Srivastava and Sahai 1987b) and common carp *Cyprinus carpio* (Yin et al. 1995) when subjected to high stocking density. The general reduction of immune parameters at high stocking density observed in the present study is likely to be a characteristic response to stressful conditions. It has been known that the release of catecholamines such as cortisol has depressive effects on a

Table 4 Levels of some immune parameters in fish reared at LD and HD

Stocking density	Lysozyme (U ml^{-1})	WBC (10^4/mm^3)[a]	Lymphocyte (%)	Monocyte (%)	Neutrophil (%)
LD	369.26 ± 14.33	6.5 ± 0.7	89.11 ± 2.83	2.55 ± 0.69	8.33 ± 1.19
HD	296.93 ± 18.46[a]	4.47 ± 0.66[a]	84.44 ± 3	3.22 ± 0.83	6.55 ± 1.38

[a] $P < 0.05$

number of immune responses in fish (Harris and Bird 2000; Yada and Nakanishi 2002). It has also been reported that the increased levels of cortisol as a result of stress can weaken the immune system in fish (Tripp et al. 1987; Barton and Iwama 1991).

In conclusion, the results of this study indicated that high stocking density caused chronic stress in rainbow trout and consequently altered the levels of some hematological, biochemical and immunological parameters compared with fish kept at low stocking density with maintained water quality parameters such as ammonia, pH, dissolve oxygen and temperature as same level and same feeding, rearing and cleaning condition.

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References

Almeida JS, Meletti PC, Martinez CB (2005) Acute effects of sediments taken from an urban stream on physiological and biochemical parameters of the neotropical fish (*Prochilodus lineatus*). Comp Biochem Physiol C Toxicol Pharmacol 140:356–363

Asadi M, Mirvaghefei A, Nematollahi M et al (2012) Effects of Watercress (*Nasturtium nasturtium*) extract on selected immunological parameters of rainbow trout (*Oncorhynchus mykiss*). Open Vet J 2:32–39

Ashley PJ (2007) Fish welfare: current issues in aquaculture. Appl Anim Behav Sci 104:199–235

Barton BA (2002) Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol 42:517–525

Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annu Rev Fish Dis 1:3–26

Benfey TJ, Biron M (2000) Acute stress response in triploid rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*). Aquaculture 184:167–176

Bolasina S, Tagawa M, Yamashita Y et al (2006) Effect of stocking density on growth, digestive enzyme activity and cortisol level in larvae and juveniles of Japanese flounder (*Paralichthys olivaceus*). Aquaculture 259:432–443

Brown BA (ed) (1988) Routine hematology procedures. In: Hematology: principles and procedures. Leo and Febiger, Philadelphia, PA, pp 7–122

Carbonara P, Corsi I, Focardi S et al (2010) The effects of stress induced by cortisol administration on the repeatability of swimming performance tests in the European sea bass (*Dicentrarchus labrax* L.). Mar Freshw Behav Physiol 43:283–296

Caruso G, Genovese L, Maricchiolo G et al (2005) Haematological, biochemical and immunological parameters as stress indicators in *Dicentrarchus labrax* and *Sparus aurata* farmed in off-shore cages. Aquac Int 13:67–73

Cristea V, Mocanu MC, Antache A et al (2012) Effect of stocking density on leuckocyte reaction of *Oncorhynchus mykiss* (Walbaum, 1792). Anim Sci Biotechnol 45(2):31–36

Ellis T, North B, Scott A et al (2002) The relationships between stocking density and welfare in farmed rainbow trout. J Fish Biol 61:493–531

Harris J, Bird DJ (2000) Modulation of the fish immune system by hormones. Vet Immunol Immunopathol 77:163–176

Hasanalipour A, Eagderi S, Poorbagher H et al (2013) Effects of stocking density on blood cortisol, glucose and cholesterol levels of immature Siberian Sturgeon (*Acipenser baerii* Brandt, 1869). Turk J Fish Aquat Sci 13:01–06

Holm JC, Refstie T, Bø S (1990) The effect of fish density and feeding regimes on individual growth rate and mortality in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 89:225–232

Iwama GK, Vijayan MM, Forsyth RB et al (1999) Heat shock proteins and physiological stress in fish. Am Zool 39:901–909

Jørgensen EH, Christiansen JS, Jobling M (1993) Effects of stocking density on food intake, growth performance and oxygen consumption in arctic charr (*Salvelinus alpinus*). Aquaculture 110:191–204

Lupatsch I, Santos G, Schrama J et al (2010) Effect of stocking density and feeding level on energy expenditure and stress responsiveness in European sea bass (*Dicentrarchus labrax*). Aquaculture 298:245–250

Monteiro SM, Mancera JM, Fontaínhas-Fernandes A et al (2005) Copper induced alterations of biochemical parameters in the gill and plasma of (*Oreochromis niloticus*). Comp Biochem Physiol C Toxicol Pharmacol 141:375–383

Montero D, Izquierdo M, Tort L (1999) High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. Fish Physiol Biochem 20:53–60

North B, Turnbull J, Ellis T et al (2006) The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 255:466–479

Papoutsoglou SE, Papaparaskeva-Papoutsoglou E, Alexis M (1987) Effect of density on growth rate and production of rainbow trout (*Salmo gairdneri* Rich.) over a full rearing period. Aquaculture 66:9–17

Parry RM, Chandan RC, Shahani KM (1965) A rapid and sensitive assay of muramidase. In Proceedings of the society for experimental biology and medicine. Society for Experimental Biology and Medicine (New York, NY). Royal Society of Medicine Publication, pp 384–386

Rafatnezhad S, Falahatkar B, Tolouei Gilani MH (2008) Effects of stocking density on haematological parameters, growth and fin erosion of great sturgeon (*Huso huso*) juveniles. Aquac Res 39:1506–1513

Ramsay JM, Feist GW, Varga ZM et al (2006) Whole-body cortisol is an indicator of crowding stress in adult zebrafish, (*Danio rerio*). Aquaculture 258:565–574

Salas-Leiton E, Anguis V, Martín-Antonio B et al (2010) Effects of stocking density and feed ration on growth and gene expression in the Senegalese sole (*Solea senegalensis*): potential effects on the immune response. Fish Shellfish Immunol 28:296–302

Sarder MRI, Thompson KD, Penman DJ et al (2001) Immune responses of Nile tilapia (*Oreochromis niloticus* L.) clones: I. non-specific responses. Dev Comp Immunol 25:37–46

Schreck C (1981) Stress and compensation in teleostean fishes: response to social and physical factors. In: Pickering AD (ed) Stress and fish. Academic Press, London, pp 295–321

Sirakov I, Ivanchev E (2008) Influence of stocking density on the growth performance of rainbow trout and brown trout grown in recirculation system. Bulg J Agric Sci 14:150–154

Soderberg RW, Meade JW (1987) Effects of rearing density on growth, survival, and fin condition of Atlantic salmon. Progress Fish Cult 49:280–283

Srivastava AK, Sahai I (1987a) Effects of loading density on carbohydrate metabolism and hematology in the Indian freshwater catfish, (*Heteropneustes fossilis*). Aquaculture 66:275–286

Srivastava N, Sahai R (1987b) Effects of distillery waste on the performance of (*Cicer arietinum* L.). Environ Pollut 43:91–102

Tintos A, Míguez J, Mancera J (2006) Development of a microtitre plate indirect ELISA for measuring cortisol in teleosts, and evaluation of stress responses in rainbow trout and gilthead sea bream. J Fish Biol 68:251–263

Tripp RA, Maule AG, Schreck CB et al (1987) Cortisol mediated suppression of salmonid lymphocyte responses in vitro. Dev Comp Immunol 11:565–576

Vijayan M, Leatherland J (1988) Effect of stocking density on the growth and stress-response in brook charr, (*Salvelinus fontinalis*). Aquaculture 75:159–170

Vijayan M, Ballantyne J, Leatherland J (1990) High stocking density alters the energy metabolism of brook charr, (*Salvelinus fontinalis*). Aquaculture 88:371–381

Vinodhini R, Narayanan M (2009) The Impact of toxic heavy metals on the hematological parameters in common Carp (*Cyprinus carpio* L.). Iran J Environ Health Sci Eng 6:23–28

Wedemeyer GA (1996) Physiology of Fish in intensive culture systems. Springer, Berlin

Yada T, Nakanishi T (2002) Interaction between endocrine and immune systems in fish. Int Rev Cytol 220:35–92

Yin Z, Lam T, Sin Y (1995) The effects of crowding stress on the non-specific immune response in fancy carp (*Cyprinus carpio* L.). Fish Shellfish Immunol 5:519–529

Yousefi M, Abtahi B, Kenari AA (2012) Hematological, serum biochemical parameters, and physiological responses to acute stress of Beluga sturgeon (*Huso huso*, Linnaeus 1785) juveniles fed dietary nucleotide. Comp Clin Pathol 21:1043–1048