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Effects of High Stocking Density on Condition Factor and Profile of Free Thyroxine and Cortisol in *Catla catla* (Hamilton, 1822) and *Labeo rohita* (Hamilton, 1822)

Shafaq Fatima*, Shadab Izhar, Zaeema Usman, Farzana Rashid, Zakia Kanwal, Ghazala Jabeen, Asma Abdul Latif

Lahore College for Women University, Department of Zoology, Faculty of Natural Sciences, Lahore, Pakistan
Phone: 00 92 324 8829796
Email: Shafaq.fatima2013@gmail.com

Abstract

High stocking density is a major problem in extensive aquaculture in Pakistan. Present study investigated its effect of growth, levels of thyroxine (T4) and cortisol in two major carps of Pakistan; labeo (*Labeo rohita*) and catla (*Catla catla*). One cohort of labeo (n=30) was kept at high stocking density (HD) (25 g/L). HD cohort was split into two replicates, each containing a total of 15 fish. Second cohort of labeo (n= 10) was reared at low stocking density (LD) of 4.4 g/L. LD cohort was divided into two replicates, each containing a total of five fish. Similar protocol was followed for catla. Trial continued for the period of 30 days. At the end of trial, condition factor significantly decreased in HD cohorts of both species. Levels of cortisol in HD cohorts (210 ± 2.00 nmol/L in *Catla catla*; 425 ± 3.00 nmol/L in *Labeo rohita*) were significantly higher than those in LD cohorts of both species. Values of T4 in HD cohorts were found to be 1.48 ± 0.20 pmol/L in *Catla catla* and 1.74 ± 0.10 pmol/L in *Labeo rohita* at the end of trial which were significantly lower than at the commencement of trial. Suppression of growth, elevation in the levels of cortisol and decrease in profile of T4 in HD cohorts of both species was due to stress induced by high stocking density. Furthermore, present study found that optimum stocking density for extensive farming of both species should be approximately 4.4 g/L.

Key Words: *Labeo rohita*; *Catla catla*; stocking density; condition factor; T4; cortisol

Introduction

Stress in aquaculture is significantly important factor which impairs fish welfare by reduction in growth, abnormal behaviour and immuno-depression (Ellis et al., 2002; Lymbery, 2002; Papoutsoglou et al., 2006; Lupatsch, Santos, Schrana, & Verreth, 2010; Li, Liu & Xie, 2012). One of the major reasons causing chronic stress in farmed fish is stocking high density of fish in a given area to produce maximum commercial output (Iguchi, Ogawa, Nagae, & Ito, 2003; Hussein, 2012). High stocking density has been considered as aquaculture related chronic stressor which causes growth suppression (Barton, 1991; Barton & Iwama, 1991; Wedemeyer, 1997). Reasons of this growth suppression might be deteriorating water quality, abnormal social behavior such as social hierarchy, reduced food consumption and altered metabolic and hematological conditions (Papoutsoglou et al., 2006; Li, Xie, & Zhang, 2008; Lupatsch et al., 2010; Tolussi, Hilsdorf, Canneppele, & Moreira, 2010; Faggio, Fedele, Arfuso, Panzera, & Fazio, 2014; Fazio et al., 2015).

High stocking density has shown to affect some metabolic pathways, such as those related to lipids metabolism (Montero, Izquierdo, Robaina, & Vergara, 1999) and elevating the levels of cortisol Li et al. (2012). Stress induced

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high levels of cortisol might remarkably reduce growth by altering activity of metabolic enzymes such as hepatic aminotransferase in channel catfish (*Ictalurus punctatus*) (Davis, Torrance, Parker, & Suttle, 1985), lipase and trypsin in Japanese flounder (*Paralichthys olivaceus*) (Bolasina, Tagawa, Yamashita, & Tanaka, 2006). Increase in levels of cortisol due to crowding stress has also been described for Atlantic salmon (*Salmo salar*) (Mazur & Iwama, 1993), Ayu (*Plecoglossus altiveli)* (Iguchi et al., 2003) and Jundia (*Rhamdia quelen)* (Barcellos et al., 2004). Elevated levels of cortisol due to density dependent stress have observed to interfere with thyroid function in in certain species such as Nile tilapia (*Oreochromis niloticus*) Walpita, Grommen, Darras, & Geyten, 2007). In Amur sturgeon (*Acipenser schrenckii*), serum concentrations of total triiodothyronine (T3), free T4, and free T3 have been found inversely related to higher stocking densities, whereas serum total T4 level remained stable Li et al. (2012). Crowding stress-induced elevation in levels of cortisol while suppression in profile of T4 might be due to negative feedback mechanism of elevated cortisol level on hypothalamus-pituitary-interrenal axis thus decreasing the release of thyroid stimulating hormone (TSH) and T4 from pituitary and interrenal gland, respectively (Walter et al., 2012). Insufficient levels of circulating T4 levels and their carrier proteins (Fazio, Faggio, Piccione, Bonfiglio, & Marino, 2014) may cause the suppression of fish growth as observed in brook charr (*Salvelinus fontinalis*) (Vijayan, Ballantyne, & Leatherland, 1988). Thus, growth suppression of fish stocked at high rearing density may be related to the reduction in the concentration of T4.

The purpose of present study was to investigate the effects of stocking density on growth and serum concentrations of T4 and cortisol in major commercial carps in South Asia, *Labeo rohita* and *Catla catla*. Considering the negative effects of stress induced by high stocking density, identifying the optimum stocking density for a species is a critical factor not only for designing an efficient culture system. Therefore in present study, an attempt was made to determine the optimum stocking density for these species while maintained at good water quality and feed ration.

Materials and Methods

Trial protocol was approved by Ethical Committee of Lahore College For Women University, Lahore (Approval No. 10031). For trial, mixed sex fish (n=30 of *Labeo rohita*; n=30 of *Catla catla*) were transferred from a local hatchery (Himalaya Fish Hatchery, G.T. Road, Muridke, Lahore) to the Aquaculture and Fisheries lab, Lahore College for Women University, Lahore on May 26, 2014. Fish of each species were stocked in four aquaria, each containing 90 liters of water (3 x 1 x 2 feet). Protocol of trial is given in table 1.

Fish were kept at ambient temperature of (29 ± 0.5 – 33 ± 0.5 ºC) and 12L:12D (L: light; D: Dark) photoperiod over a period of 30 days. Aquaria were supplied with untreated municipal water with 80% water exchange per day. Water quality parameters were maintained at pH 6.8- 7.2, chlorine < 0.02 mg/L, total ammonia < 2 mg/L, nitrite < 1 mg/L, nitrate < 80 mg/L. Fish were fed with standard commercial diet at the rate of 2% of their body weight twice a day (36% protein, 14% fats, 10% ash, 4% fiber, 11% moisture; International fish food, Pakistan).

Final sampling was performed on June 26, 2014. Fish were killed by overdose of anesthesia (10 mg/L of clove oil). Blood was collected from caudal vein and transferred to eppendorf tubes. Samples were centrifuged just after collection at 15000 RPM for 15 minutes to collect serum. Serum samples were stored at -80 °C until assayed to determine the levels of T4 and cortisol. Total body weight and total length were measured to calculate the condition factor according to following formula:

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Condition factor (k) = Total body weight / (Total body length)^3 × 100

Feed conversion ratio was measured following the Luptasch et al. (2010).

Feed conversion ratio (FCR) = Feed intake (g feed on as fed basis) / Body weight gain (g)

Levels of T4 and cortisol were measured by ELISA (Astra Biotech, Germany). Assays were validated for fish serum samples before analyses. Assays of cortisol (detection limit: 10 – 2000 nmol/L, cross-reactivity: < 0.01 %, functional sensitivity: 0.030 µg/dL, intra-assay precision: 9.9%, inter-assay precision: 20%) and T4 (detection limit: 1 – 100 pmol/L, cross-reactivity: < 0.01 %, functional sensitivity: 8.0 nmol/L, intra-assay precision: 3.4 %, inter-assay precision: 4.9 %). Binding capacity of proteins is diminished as treated with salicylates.

Statistical anlaysis was performed following Lupatsch et al. (2010) by using SPSS version 17. Descriptive statistics was performed to calculate the mean and standard error. Paired sample t-test was performed to study differences to compare values of parameters (condition factor, cortisol and T4) between LD and HD cohorts in both species. Null hypotheses were rejected at a probability level of $P < 0.05$.

Results

At the end of trial, condition factor in LD cohorts of both species was significantly higher ($P < 0.05$) than those in HD cohorts (Fig. 1 A). Feed conversion rate was noted as 0.15 and 0.02 in HD cohorts of labeo and catla, respectively. Survival rate in HD cohorts in catla and labeo was 97% and 95%, respectively while 100% survival was recorded in LD cohorts of both species.

Levels of T4, in LD cohorts of both species were found to be below the detection limit of assay (Fig. 1 B). Levels of T4 in HD cohorts were significantly lower ($P < 0.05$) than those in LD cohorts of both species. Profiles of cortisol in LD cohorts of both species were significantly lower ($P < 0.05$) than that in HD cohorts (Fig. 1 C).

Discussion

The present study highlighted the negative effect of high stocking density on growth and feed conversion capacity of labeo and catla as observed in previous studies (Ellis et al., 2002; Kebus et al., 1992; Lupatsch et al. (2010); Li et al. (2012). At this time fish exposed to a higher density showed significantly lower values in total length and weight as indicated by condition factor. Food and water quality were not limiting factors during the experiments because fish were fed 2% of total body weight twice a day and water quality was maintained. Therefore, it can be concluded that suppressed growth in these species was due to reduction in food consumption and feed conversion efficiency caused by crowding stress Li et al. (2012) as previous studies have demonstrated that high stocking density can affect fish growth performance even though water quality in aquaculture systems is well maintained (Karakatsouli, Papoutsoglou, & Manolessos, 2007; Schram, Van der, Kamstra, & Verdegen, 2006; Tolussi et al. (2010). Moreover, competition for food due to crowded environment also plays its role to suppress growth. Crowding stress also resulted in high mortality in these species due to compromised feed consumption as reported in previous studies (Ellis et al., 2002; Bolasina et al., 2006; Lupatsch et al., 2010).

In this study, the stocking density altered circulating levels of T4 and cortisol which are significant metabolic stressors. Similar results have been observed in Amur sturgeon (Li et al. (2012), Nile tilapia (Kpundeh et al., 2013), sea bass (*Dicentrarchus labrax*; Lupatsch et al. (2010), Siberian sturgeon (*Acipenser baerii*) (Hasanalipour, Eagderi, Poorbagher, & Bahmani, 2014) and brook charr (Vijayan et al., 1998). Increased amounts of

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corticosteroid released during stress affect the nonspecific immune system, producing alterations in some processes including non-specific defense (Tort, Sunyer, Gómez, & Molinero, 1996) which is the most important resistance mechanisms in fish (Anderson, 1990). This negative effect on immune system increases the susceptibility to diseases thus utilizing energy to fight against pathogens instead of growth. Elevated levels of cortisol due to stress have observed to interfere with thyroid function in fish Walpita et al. (2007). Chronic elevation in cortisol negatively feeds back the hypothalamus-pituitary-interrenal axis thus decreasing the peripheral circulating levels of T4 and T3 (Walter et al., 2012). Furthermore, prolonged cortisol elevations, caused by chronic stress, decrease the liver's ability to clear excess estrogens from the blood (Santin and Furlanetto, 2011). Excess estrogen increases the levels of thyroid binding globulins (TBG). Thyroid hormones bound to TBG are inactive and must be cleaved from TBG to become "free-fraction" before it can activate cellular receptors (Santin and Furlanetto, 2011). Insufficient levels of circulating T4 levels and their carrier proteins Fazio et al. (2014) may cause the suppression of fish growth as observed in (brook charr) Vijayan et al. (1988). This hypothyroidism due to stress caused by high stocking density might be the main reason of suppression of growth in catla and labeo in present study.

Overall, present study found that high stocking density suppresses growth in catla and labeo. This suppression could be due to crowding stress as indicated by elevated levels of cortisol and decreased profile of T4. This study emphasizes the importance of determining the optimum stocking density in order to minimize the disturbances of fish's physiology and enhance its growth performance in aquaculture system. The most suitable stocking density for catla and labeo might be 4.4 g/L as suggested by present study.

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Table 1. Protocol of trial indicating stocking density groups and number (n) of fish in each replicate. Density in low density cohorts (LD) and high density cohorts (HD) of both species were 4.4 g/L and 25 g/L, respectively in each replicate.

Labeo rohita				*Catla catla*			
Low Density Cohort -LD		High Density Cohort-HD		Low Density Cohort -LD		High Density Cohort-HD	
n	n	n	n	n	n	n	n
05	05	15	15	05	05	15	15

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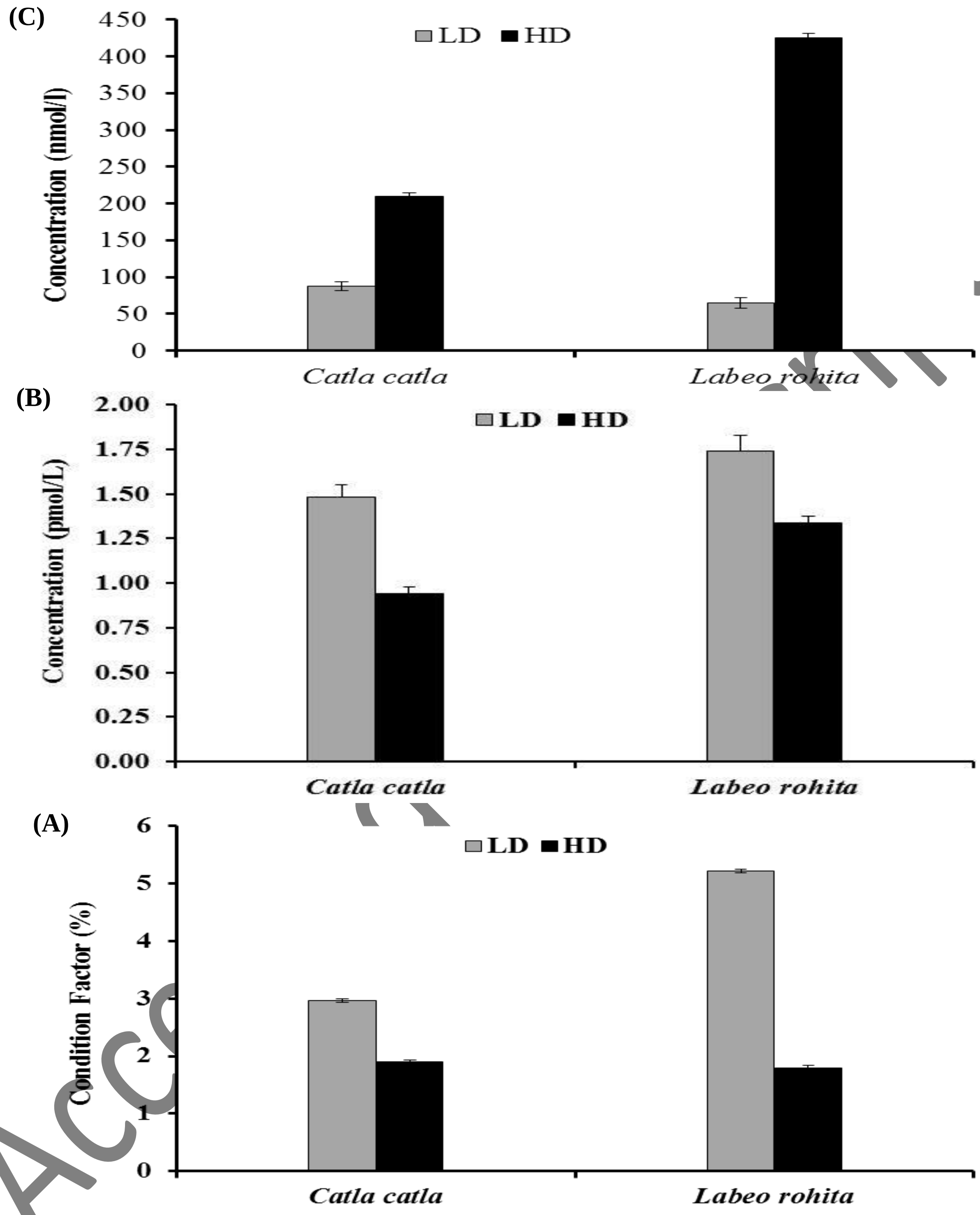

Figure 1. (A) Condition factor (%) (Mean ± SE) in low density (LD) and high density (HD) cohorts of *Catla catla* and *Labeo rohita* at the end of trial period. (B) Profile of (Mean ± SE) T4 in LD and HD cohorts of *Catla catla* and *Labeo rohita* at the end of trial period. (C) Profile of (Mean ± SE) cortisol in LD and HD cohorts of *Catla catla* and *Labeo rohita* at the end of trial period.