stocking densities in cages were: 10, 30, 60, 90, 120, 150 and 180 fish m^{-3}. All treatments were executed in triplicate. The ponds were stocked with juveniles at a stocking density of 2 fish m^2. The fish were hand fed sinking diet in the morning (0800 h) and in the evening (1700 h). Feed were supplied at 2.5% of the caged biomass.

24 hours after the transfer, a total of 20 fish were sampled from each pond and a similar number from the cages using seine and dip nets respectively. Water samples were taken from four different locations (near inlet and outlet, in the middle of the pond and close to the shore along the pond length) in each pond and within the cages, approximately two hours after morning and evening feeding using 1.12-m long column sampler. Samples from the ponds and those from the cages were pooled separately to provide an integrated sample then a sub sample was drawn from the integrated sample for the analysis of the various water quality parameters. Dissolved oxygen (DO), pH and salinity were measured in situ in the cages and in ponds, using a calibrated JENWAY 3405 electrochemical analyser (Barloword Scientific Ltd, Essex, UK). Water sampled were filtered through Whatman glass filter paper GF/F and analysed for nitrate nitrogen (NO$_3$-N) using cadmium reduction method, phosphate phosphorus (PO$_4$-P) by the standard ascorbic acid method and total ammonia nitrogen (TAN) by the indophenol blue method following detailed procedures in APHA (1998) [10].

A total of 20 fish from each cage and pond, were randomly captured with dip nets and quickly anaesthetized with benzocaine (5 mg/L) for 2-3 min. Blood was withdrawn from the caudal vein of each sampled fish into 1 ml heparinized insulin syringe. Blood glucose was measured according to King and Garner (1947) [11]. Heparinized blood was centrifuged at 3000 r.p.m. for 10 min and plasma frozen at -196°C in liquid nitrogen for further plasma cortisol (Radioimunoassay with a Coat-to-Count Kit,

Diagnostic Products Corporation, Los Angeles, CA, USA), chloride (SIGMA kit 461, Sigma Diagnostics, USA), blood ammonia (Nessler's method modified by Gentzkow and Masen, 1942) and plasma sodium (flame photometry) analyses. Capillary tubes with blood samples were centrifuged to separate cells from plasma. Plasma osmolality was then determined using Wescor 5520 vapour pressure osmometer.

Normality of the data was determined using Shapiro-Wilk test while homogeneity of variance was ascertained using Levene's test. Difference in stress response was determined using ANOVA. Multiple comparisons for significantly different means was done using Tukey test. All the statistical analyses were done using the SPSS 17.0. Differences were considered significant at $p < 0.05$.

3. Results

Changes in water quality parameters during transfer of fish from the hatchery to the cages and ponds at different stocking densities are provided in Fig. 1. DO concentration decreased significantly from 10.6 to 2.83 mg/l in cages and 8.89 to 0.78 mg/l in ponds. Cages had significantly ($P < 0.05$) higher DO than ponds. Similar patterns of reduction were also observed for pH: in cages, pH reduced from 7.7 to 5.6 while in ponds it reduced from 7.4 to 5.1. Salinity, NO$_3$-N, PO$_4$-P and T-NH$_4$-N all increased significantly with increasing stocking density. Salinity values between 4 to 8 ppm were recorded in cages and ponds when fish was transferred to cages and ponds of stocking density 10, 30, 60 and 90. However above 90 fish/m^3, salinity values increased significantly with increase in ponds being significantly ($P < 0.05$) higher at all stocking densities. Concentration of NO$_3$-N, PO4-P and T-NH$_4$-N increased with each stocking density however, there were no significant differences in the increase between cages and ponds ($P > 0.05$).
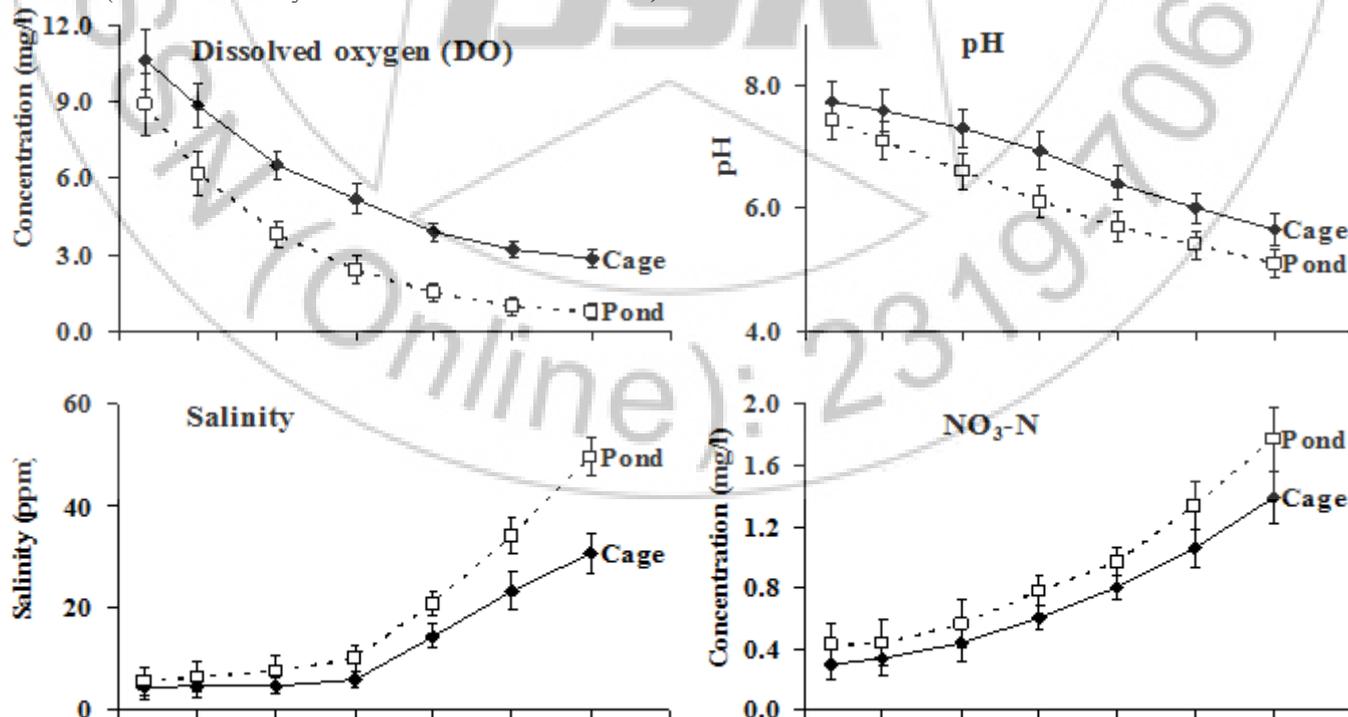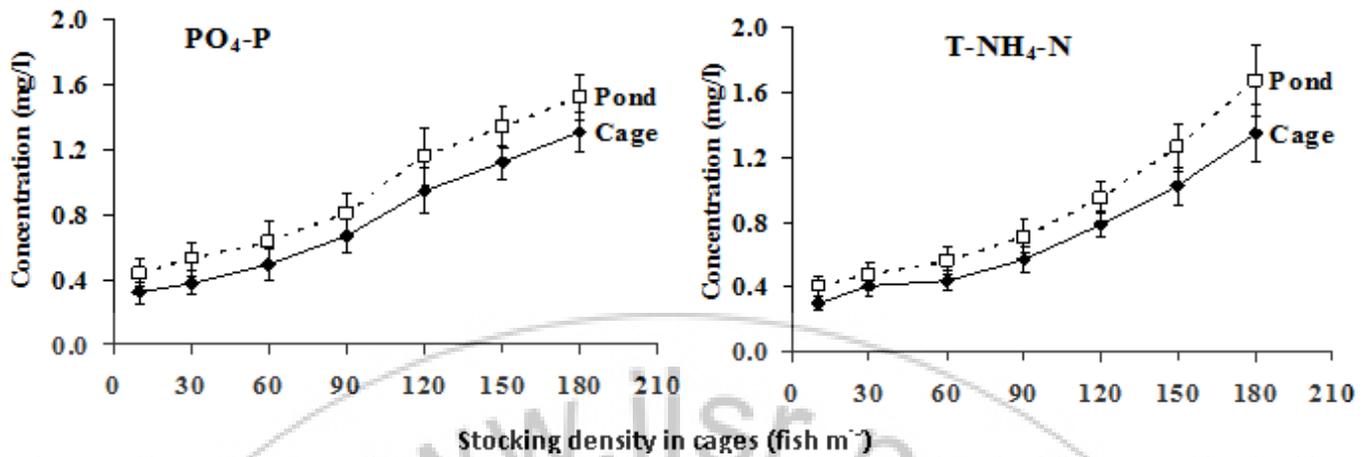
Figure 1: Changes in water quality parameters during transfer of fish to the cages and ponds of different stocking densities

Stocking fish in cages and ponds directly from the hatcheries resulted in significant (P < 0.05) increase in all physiological parameters (Fig 2). Plasma cortisol increased above the normal values in fish (80-90 ng/mg) at stocking densities $\geq$ 90 fish/m^3 in cages and at stocking density $\geq$ 120 fish/m^3 in ponds. Above density of 60 fish/m^3 cortisol levels was significantly higher in cages than ponds. Plasma glucose displayed similar changes being above that for fish under normal condition (70-90 mg/dl) at stocking density $\geq$ 120 fish/m^3 in cages and $\geq$ 150 fish/m^3. Secondary stress parameters i.e. plasma sodium, plasma chloride and blood ammonia, all displayed similar changes when fish was transferred from the hatchery at different caged density; they all increased above that of normal physiological levels in cages at density $\geq$ 90 fish/m^3 and in ponds $\geq$ 120 fish/m^3 respectively. In cages and in the ponds, osmolality of the fish increased above fish at normal physiological condition at caged density of $\geq$ 120 fish/m^3 and $\geq$ 90 fish/m^3 respectively.
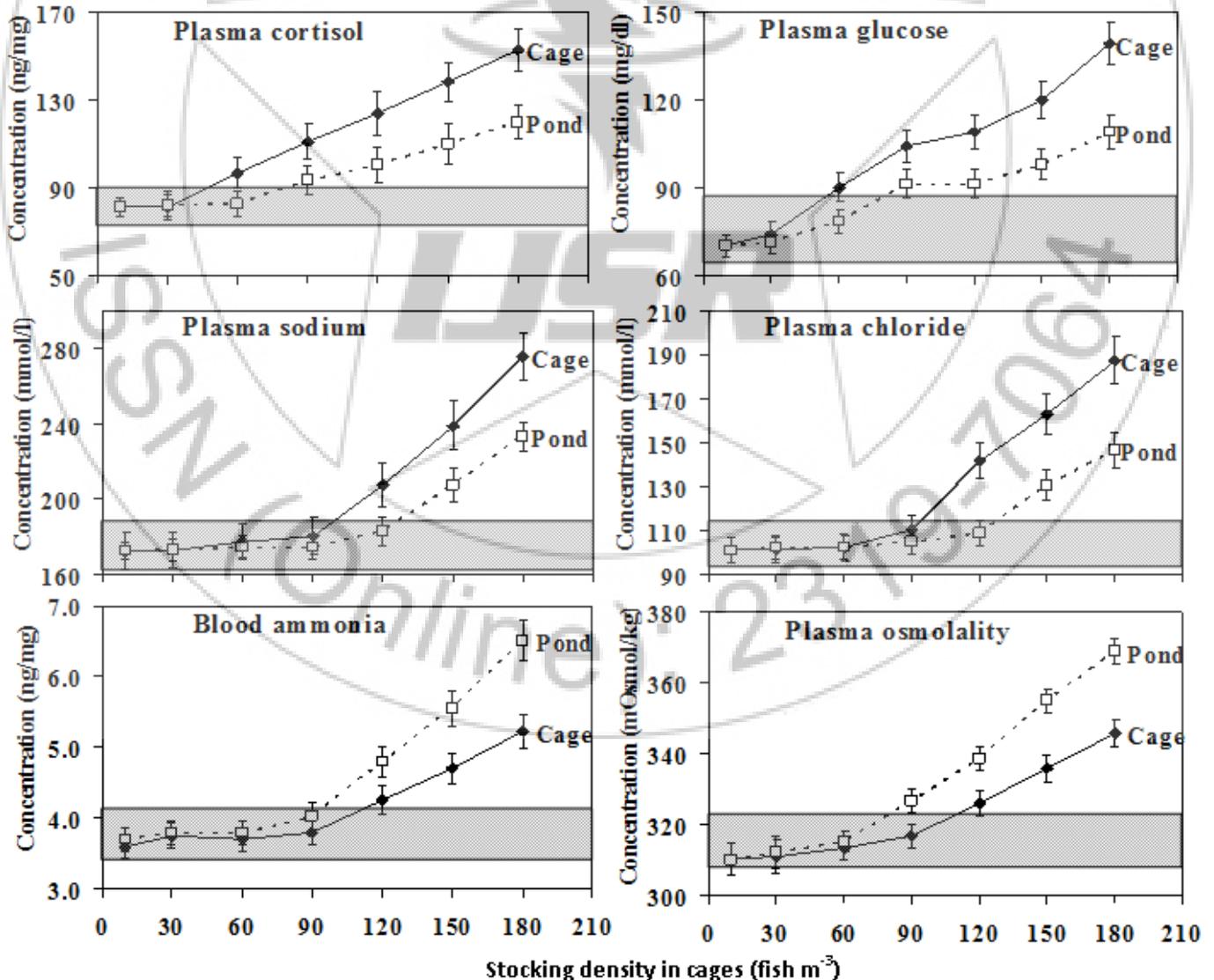
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Figure 2: Changes in the mean (± SEM) plasma cortisol, blood glucose, plasma sodium, plasma chloride and blood ammonia levels in *Labeo victorianus* under various stocking density in cages. Shaded areas represent the normal ranges of fish under recommended stocking densities.

4.Discussion

Management practices used daily in aquaculture necessarily generate stressors, to which the fish being grown may respond dissimilarly. Moreover, most freshwater fish have their body fluids hyper-osmotic (osmolality of 260–330 mOsm/kgH$_2$O) with respect to their external medium and may experience osmoregulatory disturbances in freshwater if stressors are introduced. In the current study, directly transferring *L. victorianus* from the hatchery to cages and ponds resulted in a characteristic increase in primary response parameters (cortisol and glucose levels) at $\geq$ 90 fish/m^3 in cages and at stocking density $\geq$ 120 fish/m^3 in ponds. Similar, stress responses have been observed in other species subjected to different forms of abrupt shock or stressful conditions [12-14]. Plasma cortisol and glucose concentrations observed in our study were generally within the range reported for stress elevated values of most teleost fishes (30–300 ng/mL; [5]). Prestress plasma cortisol concentrations in *L. victorianus* (80-90 ng/mg) are somewhat higher relative to that of several other fish species examined. The pre- and poststress concentrations of plasma cortisol of 12 species indicate ranged from 1.0 to 11 ng/mL [5] suggesting that in the environment where the current fish was reared may have been stressful. The increase in cortisol and glucose as primary response factor may have been caused by exhaustion of the endocrine system as a result of prolonged hyperactivity [15].

The secondary response parameters (plasma sodium, plasma chloride and blood glucose) were induced at stocking desnity $\geq$ 90 fish/m^3 in cages and $\geq$ 120 fish/m^3 in ponds. Significant increase in plasma sodium and plasma chlorides of fish after transfer to ponds and cages at high stocking densities could be due to gains of Na$^+$ and Cl$^-$. The increase in the plasma sodium and plasma chloride is a possible indicator of impairment in ionic (both Na+ and Cl−) regulation of fish due to the stressful condition. The high TAN after transfer suggests an increased physiological activity of the fish during osmoregulation. The accumulation of ammonia nitrogen due to increased metabolites may cause serious problems to the fish, such as increased oxygen consumption and heart rate, decreased plasma sodium and alteration of the acid–base balance [16].

This is the first evaluation of stress response during transfer of fish from hatchery to the ponds and cages. Differences in the magnitude of stress responses among fish species is common. Barton's review describes post-stress concentrations that ranged from as low as 3.0 ng/mL in pallid sturgeon to as high as 229 ng/mL for walleye *Sander vitreus* [5]. Several factors can influence the stress response, including prior stress exposure and the intensity and duration of the stressor. In our study the changes in primary and secondary stress responses was closely correlated with water quality changes and is our first suspicion as the main cause

of stress response during transfer of fish from grow-out to the hatcheries.

5.Conclusion

Abrupt transfer of fish from hatcheries to the cages and ponds resulted in increased primary and secondary stress response in tandem with water quality changes. The observed levels of stress response parameters are within the range of values observed for other species and provide a baseline for future evaluations of stress physiology and the development of aquaculture conditions and techniques suitable to this species.

6.Future Prospects

Protocol for culture of fish involves larval production and subsequent transfer of fish from the hatchery to the ponds and in the cages for stocking purposes. The present study reveal that such abrupt transfer result in stress response when done at different densities above which some are found to induce both primary and secondary response. Such information is vital for future management of stock recruitment.

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