HAEMATO-PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN *CATLA CATLA* (HAMILTON, 1822) FINGERLINGS SUBJECTED TO VARYING DENSITIES DURING SHORT TERM TRANSPORTATION

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The effect of varying density of *Catla catla* fingerlings packed in oxygen inflated polyethylene bags on haematological, cortisol, glucose, serum biochemistry was investigated. *Catla catla* fingerlings (31.36±1.26 g) were packed in varying densities 25g/L(T1), 50g/L(T2), 75g/L (T3),100g/L (T4) and 125g/L(T5) respectively and at 0 and 6hrs after packing. Results shows that packing density exerts stress which leads to increase in cortisol and glucose level of serum significantly (p<0.05) when compared to control (T0). This decrease in RBC, haemoglobin and NBT (Respiratory Burst) activity recorded when packing density increases but WBC increases as packing density increases. There is no significant difference (p>0.05) between control T0 and T1, T2, T3 but significant difference (p<0.05) in T4 and T5. However, in serum biochemistry, Protein and Globulin do not decrease significantly (p>0.05) when compared to T5 but albumin, albumin/globulin ratio does not significantly increased when compared to the control T0 but significant (p<0.05) when compared to T5. The result reveals that higher density mobilize glycolysis pathway for energy due to activation of cortisol. Overall result suggests that 75g/l is normal density for transportation of semi-adult fingerlings for 6 hrs but if we limit stress there is no problem of immune suppression and good survival.

India is basically a carp producing country and Indian major carps contribute substantially to fresh water fish production. Among the Indian major carps *Catla catla* is very popular due to its high growth rate, compatibility with other major carps, specific surface feeding habit and consumer preference. Availability of quality seed at right time is very important for sustainable aquaculture. Constraint in getting quality seed are; poor pond hygiene, presence of pest, inbreeding, poor management of brood stock and seed, transportation stress, mixed breeding and disease parasite. Transportation is one of the important aspects of aquaculture activity which plays major role in getting the quality fish seed, brood stock. Seed are transported over long and short distance in India making seed packing an integral part of fish husbandry practice. During transportation, seed are subjected to various stressors like netting, handling, crowding and confinement, which may often lead to heavy mortality either during or after transportation. Transportation of fishes creates a stress which has high repercussion during and after transporting the fishes without apparent warning. In order to overcome the transportation stress proper monitoring of stress is to be done so as to get relief for the fish by adopting proper means of transporting fishes. Packing density and duration of transportation are the two parameters that ensure maximum survival at optimum density for a specified duration. The crowding stress increases as we attempt to maximize profit and minimize financial cost. The cartons containing fish seed which are confined in water filled polyethylene bags which was inflated with 2/3rd oxygen. As their number increases, the cost also increases i.e. packing material cost, oxygen gas cost, labour cost, etc.

Hatchery managers and fish seed farmers attempt to pack maximum fish seed per polyethylene bag without knowing the repercussion on fish seed which leads to the economic loss of end user i.e. fish farmer in the form of immediate mortality and delayed mortality of fish seed. Due to increase in number of fish seed per bag without knowing the carrying capacity leads to deterioration of water quality and crowding stress. Water quality is one of the important contributors to fish health and stress level which leads to stimulation of metabolic and osmoregulatory responses and immune suppression. These stresses may leads to reduced growth rate, reduced metabolic activity, decreased disease resistance, decreased reproductive capacity as well as altered behaviour and survivability. They also release catecholamines into blood stream from chromaffin cells. This stress response also stimulates the hypothalamic-pituitary-interrenal (HPI) axis to release corticosteroids (e.g. cortisol) from the interrenal tissue. Although effect of packing...
Table-1. Haematological value of *Catla catla* fingerling transported in oxygen inflated plastic bags in varying loading densities

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Packing Density (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haematological parameters</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$T_0$</td>
</tr>
<tr>
<td>RBC ($10^6$ cell/mm$^3$)</td>
<td>1.033 ± 0.020$^a$</td>
</tr>
<tr>
<td>WBC ($10^3$ cell/mm$^3$)</td>
<td>5.206 ± 0.026$^a$</td>
</tr>
<tr>
<td>Haemoglobin (%)</td>
<td>4.50 ± 0.060$^a$</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>14.80 ± 0.18$^a$</td>
</tr>
<tr>
<td>NBT (O.D.at 620nm)</td>
<td>0.403 ± 0.04$^a$</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>128.29 ± 2.78$^a$</td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>29.63 ± 0.34$^a$</td>
</tr>
</tbody>
</table>

$T_0$ - Control (Before transportation), $T_1$ - 25g/l, $T_2$ - 50g/l, $T_3$ - 75g/l, $T_4$ - 100g/l and $T_5$ - 125g/l.

Values are expressed as mean ± S.E. (n=3) Different superscripts (a,b,c) in same row indicate significant difference (P<0.05) among different treatment (Tukey's HSD test, $\alpha = 0.05$)

Table-2. Effect of packing density on serum biochemistry

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Packing Density (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_0$</td>
</tr>
<tr>
<td>Blood serum</td>
<td></td>
</tr>
<tr>
<td>Protein (g %)</td>
<td>5.41 ± 0.37$^a$</td>
</tr>
<tr>
<td>Albumin (g %)</td>
<td>1.55 ± 0.10$^a$</td>
</tr>
<tr>
<td>Globulin (g %)</td>
<td>3.86 ± 0.276$^a$</td>
</tr>
<tr>
<td>Albumin /Globulin (A/G) ratio</td>
<td>0.401 ± 0.008$^a$</td>
</tr>
</tbody>
</table>

$T_0$ - Control (Before transportation), $T_1$ - 25g/l, $T_2$ - 50g/l, $T_3$ - 75g/l, $T_4$ - 100g/l, and $T_5$ - 125g/l. (After transportation).

Values are expressed as mean ± S.E. (n=3) Different superscripts (a,b,c) in same row indicate significant difference (P<0.05) among different treatment (Tukey’s HSD test, $\alpha = 0.05$)
density and transportation stress on physiological responses, bacterial density, growth and biochemical variables mainly glucose and selected enzymes of *Labeo rohita* have been investigated as Indian major carp is concerned but there is a scarce literature available on *Catla catla*. Some studies on mitigation of transportation stress of *Catla catla* seed by probiotic as feed and also in the water medium in which transportation was done. Effect of transportation stress on humoral immunity of *Catla fish* seed was studied. Quantification of the stress response is useful to device way to ameliorate stress in *Catla catla* seed during transport. In this context present study was conducted to evaluate the effect of transportation stress on selected haematological, serum biochemical, cortisol and glucose aspects of *Catla catla* fingerlings.

**MATERIALS AND METHODS**

Before proceeding to the experiment, *Catla catla* fingerlings are procured from Kaharland Research Station, Panvel of Dr. B.S.Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India and were acclimatized for 30 days in 2000 lit. fibreglass tank at the wet lab laboratory of Aquaculture Division, Central Institute of Fisheries Education, Versova, Mumbai with proper aeration and 25 percent water replenishment on daily basis. During this acclimatization process they were fed with 2% body weight twice daily with formulated diet containing groundnut oilcake, fishmeal, soyabean flour, rice powder, carboxymethyl cellulose, Cod liver oil, Sunflower oil, Vitamin and mineral premix. Water parameters were fortnightly taken and were in optimum range. Feeding was stopped to *Catla* fingerling for 24hrs prior to commencement of the transportation experiment.

*Catla* fingerlings body measurements were taken prior to packaging i.e. average weight and length. Healthy and disease free fingerlings weighing average weight (31.36±1.26 g) were selected for further transportation experiment. The fingerlings were packed in five densities i.e. 25g/L(*T_1*), 50g/L(*T_2*), 75g/L(*T_3*), 100g/L(*T_4*) and 125g/L(*T_5*) in polyethylene bag of dimension(L-77.8cm x B-40cm) which was filled with 5 liters i.e. 1/3rd water and inflated with 2/3rd oxygen. These five group of packing density were packed in triplicate. Three fish from each replicate were drawn from these five groups after six hours of transportation. Sample fishes were anesthetized by using clove oil @50µl/liter. The blood was drawn from caudal vasculature of anesthetized fingerling into 1ml tuberculin syringe flushed with 2.7% EDTA solution. The blood was collected and then transferred into the flat bottomed plastic tubes which were coated with EDTA so as to avoid coagulation of blood. These tubes are rolled between the palm for thorough mixing of EDTA. This blood is been used for determination of RBC, WBC, Haemoglobin, Haematocrit, NBT and Glucose.

For collection of serum, the blood was drawn from caudal vasculature of anesthetized fingerlings using 1 ml uncoated syringe. Blood collected was immediately transferred to dried eppendorf tube. These tubes are allowed to stand in tilted position in room temperature for clotting. After some time due to clotting of blood, the yellow straw colour serum was carefully separated out and collected and transferred to another tube which was kept at -20°C with proper labelling for further analysis i.e. Protein, Albumin, Globulin, Albumin Globulin (A:G ratio). Cortisol was quantified using Caymans Cortisol Enzyme Immunoassay kit. It is a competitive assay that has been used for estimating or quantifying of cortisol in serum.

Haemoglobin content in the blood was estimated using Cyanomethaemoglobin method using Drabkins Fluid (Qualigen). Here 20 µl of blood was taken in a test tube and mixed thoroughly with Drabkins working solution. Then the absorbance was measured using UV-VIS Spectrophotometer at wavelength 540nm, The final concentration was calculated by comparing with standard Cyanomethaemoglobin (Qualigen Diagnostic). Haematocrit was estimated by spinning blood sample contained in EDTA coated capillary tube in microhematocrit centrifuge. RBC and WBC were estimated by using Neubauers counting chamber of haemocytometer. Serum Protein was estimated by biuret method using the kit. Albumin was estimated by Bromocresol green binding method.

Globulin was calculated by subtracting albumin value from total protein value, Albumin Globulin ratio was calculated by dividing albumin value by globulin value. Nitroblue Tetrazolium (NBT) assay was done to estimate Respiratory Burst Activity by the modified methods. All data obtained was subjected to One way ANOVA procedure of IBM SPSS(Ver.16.0) and significant means were separated by Tukey’s HSD option of same software.
RESULTS AND DISCUSSION

There was an apparent effect on the packing density on Cortisol, glucose, haematological and serum biochemistry with respect to the unpacked fingerlings of *Catla catla* as in Table-1. Stress in fish results in a number of physiological changes pertaining to neuroendocrine system (blood cortisol), inducing change in metabolism (i.e. blood glucose), osmosality regulatory (blood ions and osmolarity) and haematological (RBC, WBC, Haemoglobin, Haematocrit etc.) as reported 14 Monitoring these physiological changes leads to track the health status and also the stocking density of fingerlings undergoing transportation stress.

The cortisol level was significantly (p<0.05) different before transportation (T0) and after transportation (Packed fingerlings in varying densities i.e. T1, T2, T3, T4 and T5 respectively). Post hoc Tukey HSD test reveals that the mean score for T5 was significantly different (p<0.05) than T1, T2, T3, T4 and T5 respectively. However when T1 was compared with T2 and T3, there was no statistical difference(P>0.05) between them. When T3 was compared with T4 and T5 the results were statistical significant (P<0.05) as in Table-1. The glucose level of fingerlings in five different packing densities increased significantly after 6hrs (p<0.05).Post hoc comparison using Turkey HSD p> 0.05 reveals that there is no statistical difference between unpacked fingerlings and packed fingerlings of densities T1, T2, T3 while there is a statistical difference between unpacked and packed fingerlings of densities T3 and T5. While T5 is not significant (p>0.05) with T0, T1, T3 but T5 is significant (p<0.05) compare to T4 and T6. It is concluded that after T3 density there is sudden higher increase in glucose as in Table-1. Fish transported in high densities are usually under stress 15 but there is a lack of information available on the stress responses of fish transported at high densities. On other hand in all other densities tested there was significant increase in plasma glucose and cortisol after transportation while comparing it with the control i.e. before transportation. This was supported by researchers who reported that while transporting four species of salmonoid in open system for two hours the plasma cortisol level remain elevated even 48hrs after transportation and did not return to its pre-transportation values16. Plasma glucose increase is the secondary response induced mainly by the catecholamines, as an important energy supply, which permits fish to withstand the stressful situation17.

There is significant (p<0.05) decrease in RBC count from T1 to T5 as compared to control (T0). The RBC count of packing density T1 is not significant (p>0.05) as compared to T2 and T3 while in T4, the RBC count is significantly different (p<0.05) as compared to T0, T2 and T5 respectively. While RBC count in T5 is not significantly different (p>0.05) from T1 and T3 but significant (p<0.05) decrease in RBC count in T0, T4 and T5 packing densities. It is concluded that after T3 density there significant drop in RBC count as in Table-1. There is significant (p<0.05) decrease in haemoglobin from T1 to T4 as compared to control (T0). The packing density T1 is not significant (p>0.05) as compared to T2 and T3 while in T1 the haemoglobin percentage is significantly different (p<0.05) as compare to T0, T2 and T5 respectively. While haemoglobin percentage in T2 is not significantly different (p>0.05) from T1 and T3 but significant (p<0.05) decrease in haemoglobin in T0, T4 and T5 packing densities. It is concluded that after T3 density, there is significant drop in haemoglobin as in Table-1. There is significant (p<0.05) decrease in Haematocrit value from T1 to T5 as compared to Control (T0). The packing density T1 is not significant (p>0.05) as compared to T2 and T3 while in T1, the haematocrit value is significantly different (p<0.05) as compare to T0, T2 and T5 respectively. While haematocrit value percentage in T2 is not significantly less (p>0.05) from T1 and T3 but significant (p<0.05) decrease in Haematocrit in T0, T4 and T5 packing densities. It is concluded that after T3 density, there drop in haematocrit value as in Table-1.

There was a significant reduction in RBC, Haemogloblin, Hematocrit values of fingerlings packed with different densities as compared to unpacked fingerlings of *Catla catla*.RBC and Hemoglobin reduction is observed due to stress which affects the metabolism and normal functioning of fish physiology. Red blood cells is composed of haemoglobin surrounded by a flexible protein membrane and outer lipid bilayer. The energy required for the maintenance of red cell shape, flexibility and osmotic pressure is provided by the adenosine triphosphate (ATP) generated by anaerobic glycolysis18. The depletion of ATP as a result of imposed stress due to acclimation, results in inability of the red blood cells to transport excess sodium outer cell membrane and subsequent haemolysis of the red cells19. Thus red blood cell’s life become short that cells are destroyed much faster than they can be formed20.
The decrease in haemoglobin contents in the fingerling of control to the test fingerlings is due to the inhibition of erythropoiesis, haemoglobin synthesis, osmoregulatory dysfunction or due to an increase in the rate of erythrocytic destruction in haematopoietic organs, which may cause anaemia in the exposed fish as observed in *Cyprinus carpio*\(^2\). There is increase in trend in W.B.C. when packing density increases. There is no significant (p>0.05) increase in WBC count from T\(_0\) to T\(_1\) but significant (p<0.05) increase in T\(_2\) and T\(_3\) as compare to Control (T\(_0\)). The WBC count of packing density T\(_1\) is not significant (p>0.05) as compared to T\(_0\), T\(_2\) and T\(_3\) respectively. While in T\(_1\), the WBC count is significantly different (p<0.05) as compare to T\(_2\) and T\(_3\). It is concluded that after T\(_2\) density there significant increase in WBC count as in Table-1. The change in composition of circulating white blood cells is more reliable indicator of chronic crowding stress \(^2\). This results are confirmed with the findings of other researchers\(^2\) in Black chin Tilapia *Sarotherodon melanotheron* when subjected to transportation stress and is also confirmed in *Tilapia zillii* when subjected to transportation and handling stress \(^2\). This is due to the stress of handling and transportation. White blood cells of fish or any animal has been reported to be a function of the immunity and the animal resistance to some vulnerable diseases\(^2\). There is significant (p<0.05) decrease in NBT value from T\(_1\) to T\(_2\) as compared to Control (T\(_0\)). The NBT value of packing density T\(_1\) is not significant (p>0.05) as compare to T\(_2\) and T\(_3\) while in T\(_1\) the NBT value is significantly different (p<0.05) as compared to T\(_0\), T\(_2\) and T\(_3\) respectively. While NBT value in T\(_2\) is not significantly different (p>0.05) from T\(_1\) and T\(_3\) but significant (p<0.05) decrease in NBT value in T\(_0\), T\(_1\) and T\(_3\) packing densities. It is concluded that after T\(_1\) density there significant drop in NBT value as in Table-1. NBT or respiratory burst activity is a production of superoxide anions by fish phagocytes and its reactive derivatives i.e. hydrogen peroxide and hydroxyl radicals during a period of intense oxygen consumption\(^2\). These reactive oxygen species are considered to be toxic for fish bacterial pathogens are generated by phagocytes after stimulation\(^2\). Increase in respiratory burst activity can be correlated with increased bacterial pathogen killing activity of phagocytes\(^2\). Respiratory burst activity of phagocytes was measured by reduction of nitroblue tetrazolium (NBT) by intracellular superoxide radicals produced by leucocytes. In this study decreasing trend of NBT value was observed with increase in packing density of fingerlings in polyethylene bags. Serum biochemical parameters are good indicators of the physiological state of organism as there is a close association between circulatory system of the fish and environment \(^2\). Serum contains important proteins of nutritive function (albumin) Component of immune system (globulin), blood clotting factors, hormones and enzymes \(^3\). There is no significant decrease (p>0.05) in protein level in serum when compared to control T\(_0\) and T\(_1\), T\(_2\) and T\(_3\) while there is significant difference between control T\(_0\) and T\(_4\) and T\(_5\). Tukey’s HSD test reveals that T\(_1\) is not significant (p>0.05) with the treatment T\(_0\), T\(_2\), T\(_3\), T\(_4\) but is significant (p<0.05) with treatment T\(_5\) as in Table -2. The albumin content increases as the packing density increases. Tukey’s HSD test reveals that T\(_1\) is not significantly higher (p<0.05) as compared to T\(_1\), T\(_2\), T\(_3\) but significant higher in T\(_4\) and T\(_5\). T\(_1\) is not significantly different when compare to T\(_0\), T\(_2\), T\(_3\) and T\(_4\) but significant difference (p<0.05) in T\(_5\) as in Table -2. There is decreasing trend in globulin level when packing density increases. Turkey’s HSD test reveals that there is no significance (p>0.05) decrease between T\(_0\) and T\(_1\), T\(_2\), T\(_3\) but there is significance difference(p<0.05) between T\(_4\) and T\(_5\). However packing density T\(_1\) is not significantly different from T\(_0\), T\(_1\), T\(_2\) and T\(_3\) but significantly different when compared to T\(_4\) and T\(_5\) as in Table -2. There is increasing trend in A:G ratio as packing density increases. There is no significant difference (p>0.05) between Control (T\(_0\)) and packing densities T\(_1\), T\(_2\), T\(_3\) and T\(_4\) but significant difference (p<0.05) compared to T\(_5\). T\(_1\) is not significantly different (p>0.05) from T\(_0\), T\(_2\), T\(_3\) but significant (p<0.05) to T\(_5\) as in Table-2. Plasma biochemistry of fingerlings exposed to transportation and handling stress results in decrease in protein and globulin value but increase in the value of albumin and albumin globulin ratio which is in conformity to the findings of others \(^2\). Stress due to capture, handling and sampling affects plasma protein in fish and is linked with increased secretion of catecholamine, increased concentration of adrenaline and nor adrenaline in the blood of rainbow trout (*Salmo gairdneri*) in response to physical disturbance\(^3\). Reduction in plasma value has implication on physiological activity and may be vital in immunosuppression of the fingerlings which may have strong negative impact on subsequent performance of the fish. In the present study, Catla catla fingerling transported at the density of 75g/L (T\(_1\)) could
withstand measurable change in plasma cortisol, glucose concentration, haematocrit value, haemoglobin, RBC, WBC, NBT and plasma biochemical parameters (i.e. Protein, Albumin, Globulin and Albumin and Globulin ratio).

Overall results reveal that Catla catla fingerlings demonstrate increase in immunosuppressant with increase in packing density. This base line laboratory study is used for standardsizing the packing density of fingerlings of Catla catla at optimum density of 75g/L for 6hrs is suitable for haematological parameters but when serum biochemistry is also studied then 100g/L density is also seems suitable. Contrast to that when cortisol and glucose parameters are been taken into consideration then 75g/L is more suitable as after this haematological parameters, cortisol and glucose levels drastically change. This study will be helpful to the farmers who are transporting advanced fingerlings of Catla catla for 6hrs duration in polyethylene bags with no severe stress.

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