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CHANGES IN SELECTED HEMALOLOGICAL PARAMETERS OF CATLA CATLA (HAMILTON, 1822) FINGERLING EXPOSED TO TRANSPORTATION STRESS

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Abstract

Catla fingerlings' weighing average weight (31.36±1.26 g) were selected for further transportation experiment. Catla catla fingerlings were packed at two densities, 25 g/l (optimum) and 50 g/l (double of the optimum) of ten replicates each for 6 h ,12 h,18 h and 24 h respectively in two sets in polyethylene bags of dimensions (L-77.8 cm x B-40 cm), which were filled with 5 liters water, i.e., 1/3rd water and 2/3rd oxygen. The samples were regularly collected at CIFE Aquaculture Wet Laboratory at every 6 h (i.e., 6, 12, 18 and 24 h) intervals for taking stress parameter i.e. hemalological parameters of catla fingerlings. The vehicle was continuously running for 24 hrs in and around Mumbai from 8.00 A.M to 8.00 A.M. covering a distance of about 640 Km. One set of packing was used for determining stress parameters hemalological parameters like RBC,WBC and Haematocrit which was determined from blood of Catla catla fingerlings. Another set was used for determining survival percentage after 7 days post-transportation. After transportation fishes were reared in separate tanks with aeration and water exchange. Fingerlings mortality was monitored for 7 days. The seven days post-transportation survival was determined by rearing the fishes in separate tanks for 25 g/l and 50 g/l packing densities with regular water exchange. It was observed that haematological parameters like RBC,WBC and Haematocrit are a good indicators of stress during transportation of Catla catla fingerlings packed at high density and increasing transportation duration. Hence, 25 g/l was the optimum packing density of Catla catla fingerlings for 24hrs duration. It was also concluded that increase in packing density resulted in increase of stress. There was a statistical significant interaction between the packing density (i.e., 25g/l and 50g/l) and transportation period (i.e., 0h, 6h, 12h, 18h and 24h) on glucose level. In case of double the optimum density results, it was found that up to 12hrs, this density was optimum and after that the stress parameters i.e. hemalological parameters like RBC,WBC and Haematocrit got drastically changed.

Key words: *Catla catla, fingerling, transportation, stress, haematological parameters .*

Introduction

In context to Indian fish farming carps are main stay and contributing 85%of total aquaculture production. The three Indian major carps, viz. catla, rohu and mrigal contribute major share of the carp production while and second important carps group i.e. exotic carps such as silver carp, grass carp and common carp. Indian major carps enjoy prime position in the Indian aquaculture scenario are due to their fast growth,compatibility and complimentary food habit and taste. (Laxmappa, 2014).Intensification of Indian major carp farming and also coverage of new area under culture has one limiting factor i.e. availability of quality seed of these species. Availability of seed statisfactory in one area of the country while other areas farmers faces difficulties to procure quality seed in desirable quantity. To overcome this problem farmers used to transport fish seed for long distance to the deficit area which adds to the input cost . (NFDB, 2009).

The three Indian major carps, viz. catla, rohu and mrigal are the mainstay of Indian Carp fish farming contributing over 85% of the total aquaculture production. represent the bulk of the production in the country, and other important carps group i.e. exotic carps such as silver carp, grass carp and common carp.

Fingerlings of 80-100 mm is desirable for reservoir fisheries and in culture system since survival percentage will be more if large size fingerling are stocked. As per NFDB,2009 large (above 100mm) fingerling fetches almost double price than the smaller one. Kumar, 1992 revealed that fingerlings of grass carp, silver carp and catla are sold at about double the price of fingerlings of the same size of species like rohu, mrigal and common carp.

These fingerlings are been transported by two system open and closed system. Most preferred technique is closed system where all the basic requirements for fish survival are self-contained. Recommended technique for fish transport is continuous bubbling of oxygen in sealed container during transportation or oxygen injected into a plastic bag containing water and fish which is then sealed air-tight for transport.

Singh etal.,2004 revealed that fish seed are exposed to various stressors during the entire process of transportation, the seed is exposed to various stressors like netting, handling, crowding and confinement and this often results in high mortality either during or after transportation. Detectable physiological changes which are very useful indicator of stress

experienced due to handling and transportation in fishes during hatchery operations. (Wedemeyer et al. 1990; Iwama et al. 1995, 1997; Wendelaar Bonga 1997)

In hatchery operations fish undergoes variety of handling and transport related stress such disturbance leads to detectable physiological changes which are very useful indicator of degree of stress experienced by fishes in overall aquaculture operations. (Wedemeyer et al. 1990; Iwama et al. 1995, 1997; Wendelaar Bonga 1997). Physiological response experienced by fishes are grouped as primary response(Hormonal changes), secondary response(change in metabolites,blood ions and hematology) and last tertiary response(whole animal performance) (Wedemeyer et al. 1990; Iwama et al. 1995, 1997; Wendelaar Bonga 1997). Packing density and duration of transport are two parameters that can vary for successful transportation aiming to ensure maximum survival at an optimum packing density for a specified duration (Carmichael,1984). Literature on the transportation of Indian and exotic major carps has specified various packing densities for different durations (Alikunhi, 1957; Ramachandran, 1969); however, on-site farmers and hatchery managers often decide the packing density based upon the size, duration and mode of transport. The stress exists in fish acts through the hypothalamus pituitary chromaffin axis and the hypothalamus pituitary inter renal axis which respectively stimulate the production of catecholamines and cortisol. Catecholamine activates glycogenolysis and cortisol gluconeogenesis resulting in increased production of glucose which is needed to combat stress (Pickering 1993; Schreck 1996). Transportation stress elicits the same responses as other forms of stress (Maule et al.,1988).
Therefore, the present study was undertaken to evaluate the secondary response i.e. hemalological parameters in Catla catla fingerlings exposed to transportation stress

Methodology

The fingerlings of Catla catla were packed at Aquaculture Division Wet Lab of Central Institute of Fisheries Education, Versova, Mumbai and placed in motorized vehicle for transportation in and around Mumbai for 24 hrs. The sampling of the catla fingerlings was done in Aquaculture Biology Lab.

Experimental fish and their maintenance

Before proceeding to the experiment, *Catla catla* fingerlings were procured from Khar Land Research Station, Panvel of Dr. B. S. K. K. V., Dapoli, Maharashtra, India and were acclimatized for 30 days in 2000 L fibreglass tank at the wet 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% of their body weight twice daily with formulated diet containing groundnut oilcake, fishmeal, soybean flour, rice powder, carboxymethyl cellulose, cod liver oil, sunflower oil, vitamin and mineral premix. Water parameters were fortnightly observed and found in optimum range. Feeding was stopped to fingerlings 24 hrs prior to commencement of the transportation experiment.

Experimental design and sampling

Catla fingerlings' body measurements, *i.e.,* average weight and length were taken prior to packaging. Healthy fingerlings weighing average weight (31.36±1.26 g) were selected for further transportation experiment. The fingerlings were packed at five densities, *i.e.,* 25 g/L (T_1), 50 g/L (T_2), 75 g/L (T_3), 100 g/L (T_4) and 125 g/L (T_5) in polyethylene bags of dimensions (L-77.8 cm x B-40 cm), which were filled with 5 liters water, *i.e.,* 1/3rd water and 2/3rd oxygen. These five groups of packing densities were packed in triplicate.

Transportation Protocol

Fishes were packed at different stocking densities, *i.e.,* 25 g, 50 g, 75 g, 100 g and 125 g per litre in triplicate at Aquaculture Wet Laboratory, Central Institute of Fisheries Education, Versova, Mumbai and transported for a period of 24 hrs in a motorized vehicle. The samples were regularly collected at CIFE Aquaculture Wet Laboratory at every 6 h (*i.e.,* 6, 12, 18 and 24 h) intervals for taking various stress parameters of catla fingerlings. The vehicle was continuously running for 24 hrs in and around Mumbai from 8.00 A.M to 8.00 A.M. covering a distance of about 640 Km .

Three fish from each replicate were drawn from these five groups after 6, 12, 18 and 24 h of transportation. Various stress indicators like Cortisol, Glucose, NBT, RBC, WBC, Haematocrit, Protein, Albumin, Globulin and Albumin Globulin ratio (A:G ratio) were estimated from blood and serum of fingerlings. It was observed from these estimations that optimum packing density for 6 h, 12 h, 18 h and 24 h transportation of catla fingerlings was 75 g/l, 75 g/l, 50 g/l and 25 g/l respectively. From the above trials, it was concluded that for 24 h transportation, 25 g/l was optimum packing density for *Catla catla* fingerlings.

Catla catla fingerlings were packed at two densities, 25 g/l (optimum) and 50 g/l (double of the optimum) of ten replicates each for 6 h ,12 h,18 h and 24 h respectively in

two sets. Above said transportation protocol was followed. One set of packing was used for determining stress parameters i.e. hemalological parameters , which was determined from blood of *Catla catla* fingerlings. Another set was used for determining survival percentage after 7 days post-transportation. After transportation fishes were reared in separate tanks with aeration and water exchange. Fingerlings mortality was monitored for 7 days. The seven days post-transportation survival was determined by rearing the fishes in separate tanks for 25 g/l and 50 g/l packing densities with regular water exchange. Fingerlings were kept according to the duration of transport in tanks, *i.e*., 0 h, 6 h, 12 h,18 h and 24 h for 25 g/l and 50 g/l packing densities.

Collection of blood and haematological parameters

Blood was collected by puncturing the *Venacaudal,* using a tuberculin medical syringe, which was previously rinsed with 2.7% EDTA solution. Fingerlings were anesthetized with clove oil (MERCK, GERMANY) @ 50 µl per litre of water before taking their blood. Collected blood was then transferred immediately to test tube coated with thin layer of EDTA powder (as an anticoagulant) and shaked well in order to prevent haemolysis of blood.

TLC/WBC (Total Leukocyte count), TEC /RBC(Total Erythrocyte Count) was determined using Hemocytometer or Neubauer chamber (Blaxhall and Daisley,1973) and Packed Cell Volume / Haematocrite Value as per the method given by Snieszko,1960.

Physico-chemical parameters of Water

Water quality parameters, *viz,*.temperature, pH (pH meter having the temperature probe), dissolved oxygen by azide modification method (APHA-AWWA-WEF.,1998), free carbon dioxide titrimetric method (APHA-AWWA-WEF,1998), ammonia by spectrophotometerically at 640nm wavelength by phenate method (APHA-AWWA-WEF,1998), nitrite was estimated spectrophotometerically at 543nm wavelength (APHA-AWWA-WEF,1998) and nitrate was estimated spectrophotometerically at 543nm wavelength (APHA-AWWA-WEF.,1998) were recorded in this experiment.

Statistical Analysis

All data obtained were subjected to Two way ANOVA procedure of IBM SPSS(Ver.16.0) and significant means were separated by Tukey's HSD option of same software and further data obtained were subjected to Independent sample t-Test procedure

of IBM SPSS(Ver.16.0) and significant means were separated by Tukey's HSD option of same software.

Results

There was a decreasing trend of RBC count in both packaging densities (*i.e.*,25 g/l and 50 g/l) as the duration of time increased. The packing density of 25 g/l had high RBC count as compared to 50 g/l. Two way ANOVA revealed that there was a statistical significant interaction ($p<0.05$) between packing densities (25 g/l and 50 g/l) and transportation time (*i.e.*, T_1 (0h) T_2,(6h), T_3 (12h),T_4 (18h) and T_5 (24h) on RBC count. There was a significant difference ($p<0.05$) between packing density and also in transportation time.

RBC count varied significantly ($p<0.05$) for different transportation durations T_1 and T_2,T_3 ,T_4, T_5 ; but there was no significant difference between T_2,T_3 , T_4, T_5 undertaken in the experiment when the fishes were packed at a density of 25 g/l. However, RBC count was significantly different ($p<0.05$) for the transportation duration T_1 and T_2,T_3,T_4,T_5; but no significant difference was noticed between T_4 andT_5 when they were packed at a density of 50 g/l.

There was an increasing trend of WBC count in both packaging densities (*i.e.*, 25 g/l and 50 g/l) as the duration of time increased. The packing density of 25 g/l had low WBC count as compared to 50 g/l .Two way ANOVA revealed that there was a statistical significant interaction ($p<0.05$) between packing densities (25 and 50 g/l) and transportation time (*i.e.*, T_1(0h), T_2,(6h),T_3 (12h),T_4 (18h) and T_5 (24h) on WBC count. WBC did not significantly vary ($p>0.05$) for different transportation durations T_1 ,T_2,T_3 ,T_4 and alsoT_1 ,T_2,T_3,T_4,T_5 but there was significant difference ($p>0.05$) between T_1 and T_5 when the fishes were packed at a density of 25 g/l. WBC count was not significantly different ($p>0.05$) for the transportation durations T_1, T_2, T_3 and not significantly different ($p>0.05$) for T_4,T_5; but there was significant difference ($p<0.05$) between T_3 and T_4, T_5 when they were packed at a density of 50 g. Hence in packing density of 50 g/l, the WBC count did not differ significantly up to 12 h of transportation when packed at a density of 50 g/l.

There was an decreasing trend of haematocrit value in both packaging densities (*i.e.*, 25 g/l and 50 g/l) as the duration of time increased. The packing density of 25 g/l had high haematocrit value as compared to 50 g/l. Two way ANOVA revealed that there was a statistical significant interaction ($p<0.05$) between packing densities (25 and 50 g/l)

and transportation time (*i.e.*, T_1 (0h) T_2, (6h),T_3 (12h), T_4 (18h) and T_5 (24h) on haematocrit value. There was a significant difference between transportation time and packing densities.

Haematocrit value significantly varied ($p<0.05$) for different transportation durations T_1 and T_2, T_3, T_4, T_5 but there was no significant difference ($p>0.05$) between T_2,T_3, T_4,T_5 when the fishes were packed at a density of 25g/l. Haematocrit value was significantly different ($p<0.05$) for the transportation duration T_1 and T_2,T_3 ,T_4,T_5 and no significant difference ($p>0.05$) was noticed between T_2 and T_3 when they were packed at a density of 50 g. Hence in packing density of 50 g/l, the haematocrit value did not differ significantly up to 12 h of transportation when packed at a density of 50g/l.

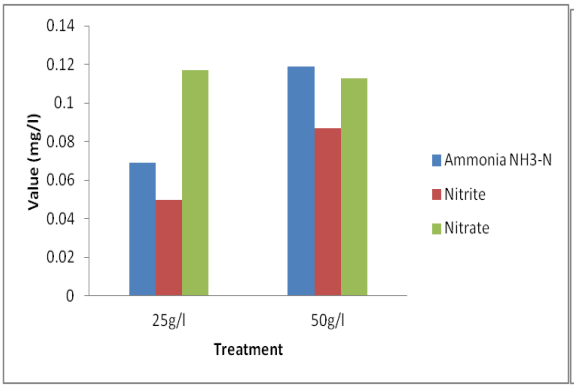
Table1. Haematological values of *Catla catla* fingerlings transported in oxygen inflated plastic bags for varying time period.

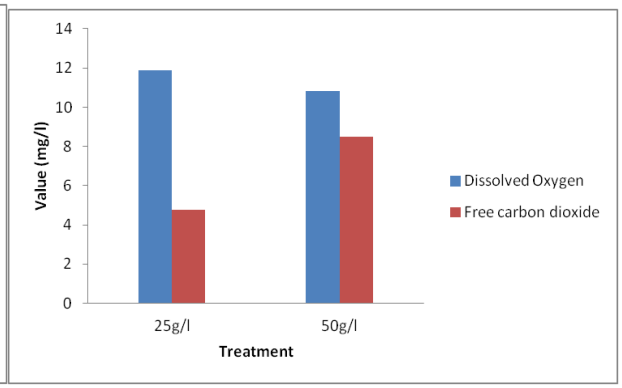
Treatment		**RBC**	**WBC**	**Haematocrit**
Packaging density				
25g/l		0.804^{a}	528.20^{a}	10.93^{a}
50g/l		0.766^{b}	540.25^{b}	10.38^{a}
SEM		0.006	1.15	0.075
P-value		S(0.00)	S(0.00)	S(0.00)
T_1(0h)		1.050^{c}	521.37^{a}	14.77^{c}
T_2 (6h)		0.763^{b}	528.65^{ab}	10.09^{b}
T_3 (12h)		0.743^{b}	530.71^{b}	10.03^{b}
T4(18h)		0.692^{a}	544.29^{c}	9.33^{a}
T_5 (24h)		0.677^{a}	546.12^{c}	9.07^{a}
SEM		0.010	1.83	0.11
P-value		S(0.00)	S(0.00)	S(0.00)
Packaging Density*Duration				
25g/l	**T_1(0h)**	1.033^{c}	520.60^{a}	14.80^{d}
	T_2 (6h)	0.770^{b}	527.00^{ab}	10.13^{c}
	T_3 (12h)	0.757^{b}	528.89^{ab}	10.00^{bc}
	T4(18h)	0.738^{b}	530.53^{ab}	9.8^{c}
	T_5 (24h)	0.723^{b}	533.94^{b}	9.23^{b}
50g/l	**T_1(0h)**	1.067^{c}	522.14^{ab}	14.74^{d}
	T_2 (6h)	0.757^{b}	530.30^{ab}	10.26^{c}
	T_3 (12h)	0.730^{b}	532.53^{ab}	10.13^{c}
	T4(18h)	0.647^{a}	557.98^{c}	9.8^{bc}
	T_5 (24h)	0.631^{a}	558.31^{c}	8.23^{a}
	SEM	2.59	0.163	0.003
	P-value	S(0.00)	S(0.012)	S(0.00)

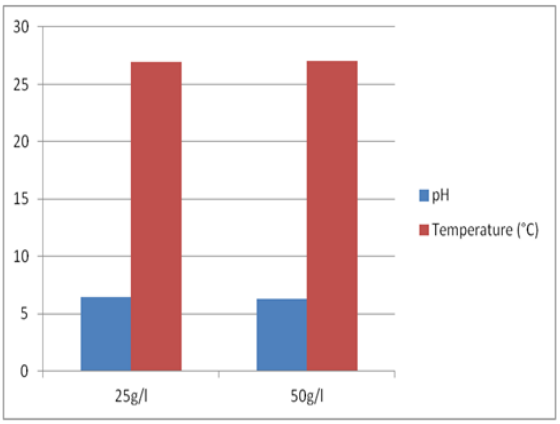
*Treatment means represent the average values of three plastic tubs per treatment. Tukey HSD range test was conducted for treatment means only if there was a significant interaction

(ANOVA, $p < 0.05$). Means value in same column with different superscript differ significantly ($p<0.05$). S-Significant, NS-Nonsignificant.

Fig.1: Water quality parameters after different transportation durations at two packing densities







Survival after 7 days period

Survival percentage of fingerlings after transportation are given in Table 15. Two way ANOVA reveals that there is a significant ($p<0.05$) interaction between packing density and transportation duration on survival of fingerlings. It also reveals that there was a significant ($p<0.05$) effect on the packing and transportation duration on survival of fingerlings. Survival after seven days was non significantly different ($p>0.05$) for transportation durations T_1 ,T_2,T_3 ,T_4,T_5 when the fishes were packed at a density of 25 g/l. However, survival after seven days was non-significantly different ($p>0.05$) for the transportation durations T_1,T_2, T_3; but there was significant difference ($p<0.05$) between T_3 and T_4, T_5 when they were packed at a density of 50g/l.

Table 2. Post survival after transportation for first seven days in two packing densities

Treatment		**Survival (%)**
Packing density		
25g/l		100
50g/l		90.83
SEM		0.589
P-value		S(0.00)
Duration		
T_1 (0h)		100^a
T_2 (6h)		100^a
T_3 (12h)		100^a
T4(18h)		89.58^b
T_5 (24h)		87.50^b
SEM		2.08
P-value		S(0.00)
Packing Density*Duration		
25g/l	T_1 (0h)	100^a
	T_2 (6h)	100^a
	T_3 (12h)	100^a
	T4(18h)	100^a
	T_5 (24h)	100a
50g/l	T_1 (0h)	100^a
	T_2 (6h)	100^a
	T_3 (12h)	100^a
	T4(18h)	79.16^b
	T_5 (24h)	75^b
SEM		1.31
P-value		S (0.00)

***Treatment means represent the average values of three plastic tubs per treatment. Tukey HSD range test was conducted for treatment means only if there was a significant interaction (ANOVA, $p < 0.05$). Means value in same column with different superscript differ significantly ($p<0.05$). S-significant.**

The effect of higher packing density and increased duration of transport on the survival and stress indicators of *Catla catla* fingerlings was investigated. *Catla catla* fingerlings were packed at two densities, 25 and 50g/l and sampled at 0, 6, 12, 18 and 24h after packing. There was also statistical significant ($p<0.05$) interaction between packing density and transportation time on haematological parameters (*i.e.*, RBC, WBC and haematocrit), From these results, it was observed that haematological parameters are good indicators of stress during transportation of *Catla catla* fingerlings packed at high density and increasing transportation duration.

Discussion

There was a decreasing trend of RBC count in both packaging densities (*i.e.*,25 g/l and 50 g/l) as the duration of time increased. Adeyemo *et al.*, 2009 revealed that haematocrit and red blood cell counts (RBC) was generally higher in the control relative to the test populations however, the differences in values was not statistically significant ($p<0.05$).

There was an increasing trend of WBC count in both packaging densities (*i.e.*, 25 g/l and 50 g/l) as the duration of time increased. The packing density of 25 g/l had low WBC count as compared to 50 g/l. Crowding stress caused leukocytes release from head-kidney and their cumulation in blood circulation (Ortuno *et al.*, 2001) There is an increased number of leukocytes, particularly phagocytes and damaged cells, in peripheral blood of stressed dab, *Limanda limanda*, along with a decrease in lymphocyte, thrombocyte and erythrocyte counts.(Pulsford et al.,1994) .

There was an decreasing trend of haematocrit value in both packaging densities (*i.e.*, 25 g/l and 50 g/l) as the duration of time increased. The packing density of 25 g/l had high haematocrit value as compared to 50 g/l. These results are conformity with the observations of De Abreu et al.(2008) in juvenile matrinxa, *Brycon amazonicus* subjected to 4 h transportation stress as he did not observe any variation in Ht; rather it decreased to 32% after 24 h of transportation. Stress of handling has been shown to produce haemoconcentration and that the haematocrit of fish blood decreases after the stress of capture and transportation (Hattingh and van Pletzen, 1974). Lawani and Alawode, 1987 reported a significant($p<0.05$) decrease in the haematocrit value of *Oreochromis niloticus* confined for thirty minutes and one hour respectively relative to control.

Conclusion

It was observed that haematological parameters like RBC,WBC and Haematocrit value , are good indicators of stress during transportation of *Catla catla* fingerlings packed at high density and increasing transportation duration. Hence, 25 g/l was the optimum packing density of *Catla catla* fingerlings for 24hrs duration. It was also concluded that increase in packing density resulted in increase of stress. There was a statistical significant interaction between the packing density (*i.e.*, 25g/l and 50g/l) and transportation period (*i.e.*, 0h, 6h, 12h, 18h and 24h) on glucose level. In case of double the optimum density results, it was found that up to 12hrs, this density was optimum and after that the stress parameters i.e. haematological parameters got drastically changed.

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