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VARIATION IN CORTISOL LEVEL IN CATLA CATLA (HAMILTON, 1822) FINGERLING EXPOSED TO TRANSPORTATION STRESS

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Abstract

Catla fingerlings' weighing average weight (31.36±1.26 g) was 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 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. cortisol level 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. It was observed that cortisol level, is 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 cortisol 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 parameter i.e. cortisol level got drastically changed.

Key words: *Catla catla, fingerling, transportation, stress, cortisol level.*

Introduction

One of the world's fastest growing food production sectors is aquaculture with an annual growth rate of 8%. Indian fresh water is dominated by carps with a lion share of 87% of the total freshwater fish production. Catla Catla is one of the most preferred fish of Indian major carp species due to its high commercial value, growth rate ,most preferred by the consumers, compatible with other carps, complimentary food habits (Laxmappa, 2014).

In aquaculture practises stress is an unavoid-able component, which is associated with handling, netting, transportation, water and soil quality of bottom,vaccination and disease treatment. Due to this fishes undergoes various physiological changes including immunosuppression. eventually succumb to disease (Barton, 2002; Oliva-Teles, 2012). Acquaint with fish stress responses during aquaculture practices is very essential to avoid the stress related problems which helps in improving the fish quality and to get optimum production with ease. Biochemical and haematological parameters are known as diagnostic tool for monitoring fish health. (Sebastiao et al., 2011). In aquaculture stress mitigation isone of the most challenging tasks and one of the promising area of research. In aquaculture transportation is inevitable multiphase operation. .In transportation of fishes optimizing stocking density for specified duration is required to reduce stress related mortality during transportation or after transportation. Diagnosis of metabolic disturbance and structural and functional status of the fish body can successfully do by haematology and biochemistry. (Wagner and Congleton, 2004; Mohammadizadeh et al., 2013) .).

Scanty literature is available regarding the stressors like packaging and transportation etc on the biochemical and haematological parameters of carps especially Catla ,Catla catla . This present study is therefore carried out to assess the stress response.i.e. Cortisol level in serum of Catla fingerlings when subjected 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 transporation 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 Glucose, which was determined from serum 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.

Serum Collection

For collection of serum, the blood was drawn from caudal vasculature of anesthetized fingerlings using 1 ml uncoated syringe. Collected blood was immediately transferred to dried eppendorff tube. These tubes were allowed to stand in tilted position at 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 ^{o}C with proper labelling for further analysis, *i.e.*, Glucose.

Estimation of Cortisol

Cortisol level in serum was determined with a was quantified using Caymans Cortisol Enzyme Immunoassay kit (Cortisol EIA Kit Item No. 500360) .It is a competitive assay that has been used for estimating or quantifying of cortisol in serum.

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

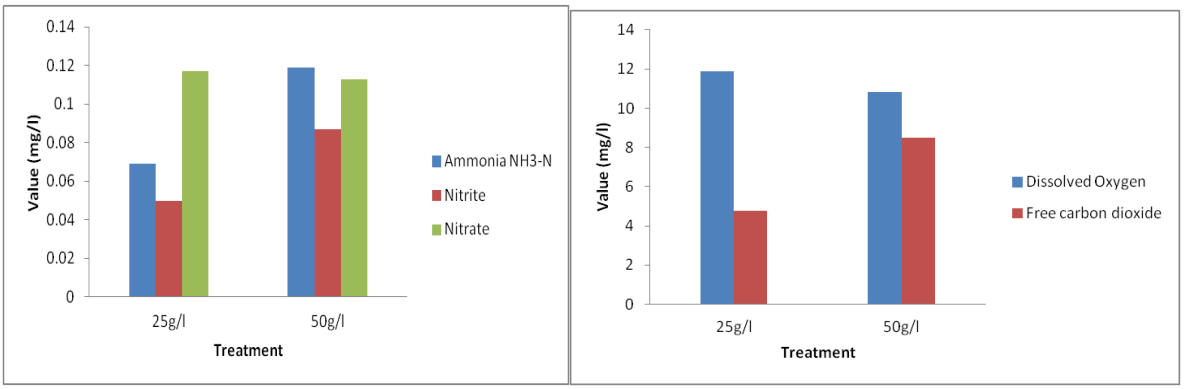
There was an increasing trend of cortisol level in both packaging densities (*i.e.,* 25 g/l and 50 g/l) as the duration of time increased. The packing density of 25 g/l had low cortisol level as compared to 50 g/l. Two way ANOVA revealed that there was a statistical significant interaction ($p<0.05$) between packing densities (25 g and 50 g/l) and transportation time (*i.e.*, T1 (0h) T2, (6h),T3 (12h),T4 (18h) and T5 (24h) on plasma cortisol level (Table1). There was a significant difference ($p<0.05$) between packing density and also in transportation time. Plasma cortisol level varied significantly ($p<0.05$) for different transportation durations T1 and T2,T3,T4,T5; but there was no significant difference ($p>0.05$) for the transportation durations T2, T3, T4, T5 when the fishes were packed at a density 25 g/l. However, there was a significant difference in plasma cortisol level for different transportation durations T1 and T2,T3,T4, T5; but there was no significant difference ($p>0.05$) between T2, T3 when they were packed at a density of 50 g/l. At this density plasma cortisol level was found to be significantly higher when transported for 18 h and more. Plasma cortisol level did not differ significantly up to 12 h of transportation when packed at a density of 50g/l

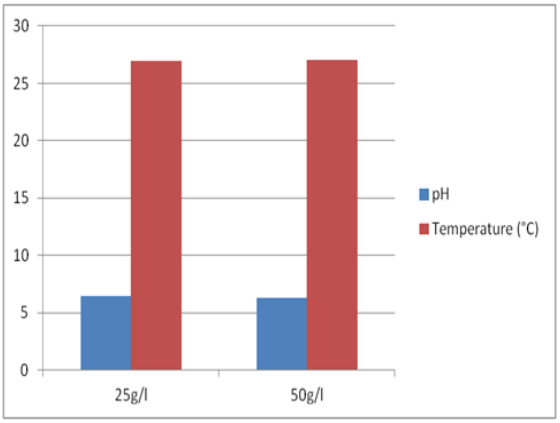
Table 1. Cortisol level of *Catla catla* fingerlings transported in oxygen inflated plastic bags for varying time period.

Treatment		**Cortisol (ng/ml)**
Packaging density		
25g/l		47.24[a]
50g/l		63.51[b]
SEM		0.84
P-value		S(0.00)
T_1(0h)		30.07[a]
T_2 (6h)		47.34[b]
T_3 (12h)		51.62[b]
T4(18h)		64.14[c]
T_5 (24h)		83.73[d]
SEM		1.33
P-value		S(0.00)
Packaging Density*Duration		
25g/l	**T_1(0h)**	29.63[a]
	T_2 (6h)	46.62[b]
	T_3 (12h)	50.51[b]
	T4(18h)	53.52[b]
	T_5 (24h)	55.93[b]
50g/l	**T_1(0h)**	30.51[a]
	T_2 (6h)	48.06[b]
	T_3 (12h)	52.73[b]
	T4(18h)	74.75[c]
	T_5 (24h)	111.53[d]
	SEM	1.88
	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, NS-Nonsignificant.

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





Discussion

During transportation of fishes many stress like crowding, handling, water quality, oxygen deficit stress used to act of fish body. Fish body releases stress hormones, viz., cortisol and catecholamine (epinephrine) in to the blood stream by the endocrine system as a primary response under stress condition. (Randalland Perry, 1992).Measuring cortisol level is an good option for measuring acute stress in fishes(Martínez-Porchas et al. ,2009) In this study there was an increasing trend of cortisol level in both packaging densities (*i.e.*, 25 g/l and 50 g/l) as

the duration of time increased. The cortisol levels in the Catla fingerlings prior to transportation increased significantly as primary response with the transportation procedures. Immediately after transportation, cortisol level increases in fingerlings packed at different densities compared to control. These results conformity to the earlier finding on common carp (Dobsikova et al.,2009), juvenile matrinxa (De Abreu et al., 2008) and juvenile tam-baqui (Gomes et al., 2003) subjected to transportation and packing density stress. The degree of stress experienced by fishes are indicated by cortisol (Barton and Iwama 1991;Wendelaar-Bonga, 1997).are conformity to our results. Carmichael (1984)revealed that advanced fingerlings (15–24 cm) of largemouth bass were transported at higher densities, the levels of corticoids and glucose in the plasma increased.

Primary stress response is the activation of two main components of neuroendocrine system; the first to be activated in chromaffin system of the head kidney which is under the control of sympathetic nervous system. This results in the secretion of catecholamine, adrenaline noradrenaline and to lesser extent dopamine. The other neuroendocrine system is the hypothalamus pituitary interregnal axis . This system results in the secretion of cortisol, the principal corticosteroid in fish (Henderson and Garland,1980; Pickering,1993; Schreck,1996). Elevation of cortisol levels, a global response to stress, has been observed during transport.(Hur *et al.*,2007; Bendhack and Urbinati,2009; Iversen *et al.*, 1998 and Dhanasiri *et al.*, 2013).

Elevated plasma cortisol level has been seen to increase after acute and chronic stress as reported by Farbridge and Leatherland,1992; Vijayan and Moon,1992. Gomes *et al.*(2003), reported that there was a significant increase in plasma glucose and cortisol immediately after transportation in juvenile Tambaqui, *Colossoma macropomum.*

The present study results are in conformity with the results obtained by Chatterjee *et al.*, 2010, where cortisol and glucose level were found to increase both in response to higher packing density and increased in length of confinement, indicating an increased energy demand. Similar results are also reported by other workers (Specker and Schreck,1980; Iverson *et al.*,1998; Perez-Casanova *et al.*, 2008) in response to various stressors like transportation, confinement and handling. Therefore, it can be concluded that cortisol parameter is a good marker of stress during transporation of catla fingerlings. Further, it is

also concluded that 25 g/l packing density of catla fingerlings can be transported upto 24 hrs as far as cortisol level is concerned.

Conclusion

It was observed that cortisol level, is 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 cortisol 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 parameter i.e. cortisol level got drastically changed.

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