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HAEMATOLOGICAL RESPONSES TO STRESS OF TRANSPORTATION AND ACCLIMATION ON THE AFRICAN CATFISH, H*eterobranchus bidorsalis* (Geoffrey, Saint Hilare)

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Abstract

Stress due to transportation and one week laboratory acclimation on the African catfish, *Heterobranchus bidorsalis* procured from New Calabar River, Aluu in Rivers State of Nigeria caused significant reduction of Red Blood Cell (RBC), Blood Platelet (BP) counts, Packed Cell Volume (PCV) as well as haemoglobin contents (HC) $p<0.05$**.** Conversely, stress of transportation and acclimation led to significant elevation of total leucocyte (TL) counts, mean corpuscular volume (MCV) indices, blood osmolality and glucose levels ($p<0.05$).The reduction in erythrocyte and thrombocyte counts is as a result of reduced activity of haematopoietic organs due to stress of transportation and acclimation. The elevated glucose and white cell counts is attributed to alarm reaction, preparing the fish to defend itself against invading pathogens. Consequently, the body builds up a reserve of energy which ought to be expended. The stress of transportation and acclimation (handling, sorting, injury, overcrowding,) and poor water quality normally damage the mucous or slime layer that surrounds the fish. Under such a circumstance, *H. bidorsalis,* being a freshwater species, absorbs much of the acclimation water and this contributes substantially to the reduced haematocrit and elevated osmolality observed.

Key words: stress, transportation, acclimation, haematological, *H.bidorsalis.*

INTRODUCTION

The stress of transportation and acclimation (handling, sorting, overcrowding, physical injuries, poor water quality) are the primary contributing factors to fish diseases and mortality in aquaculture. (Wedemeyer and Yasutake, 1977) Stress is defined as any condition that causes physical or mental discomfort which either results in the release of stress related hormones (eg adrenaline) or in bringing about specific physiological responses (Okafor, 2011_a, Uzebu and Mba, 2015) Long term stress or severe short term stress ,can contribute to many of the illnesses and deaths in aquarium fish. Chronic stress can lower the immune system of fish (Wedemeyer and Yasutake, 1977) There are many potential stressors of fish, but some of the common ones include: harassment by other fish, inadequate tank size which leads to overstocking of tank, inadequate nutrition, poor sanitation, lack of hiding places, injury from bigger fishes or during handling (capture, sorting, transportation) poor water quality (low dissolved oxygen, undesirable temperature, salinity and pH, increased levels of CO_2 NH_3, NO_3, $N0_2$, H_2S and organic matter) etc. (Uzebu and Mba, 2015) Therefore stress is that force that brings about physiological changes and adjustments. The nature of keeping aquatic species in confined environments generates many stresses that are unique to aquarium fish (Okafor, 2011_b)

H. bidorsalis belongs to the Clariidae family of bony fish (Osteichthyes) which are economically important food fish in Nigeria and in many other parts of the world. (Owodeinde et al. 2012) The Clariid catfish have gained prominence as important culture fish in tropical and subtropical parts of the world such as south East Asia as well as in West Africa. (Dada and Olarewaju, 1996; Owodeinde and Ndimele, 2011) The genus, *Heterobranchus* consists of four known species which are: *H. bidorsalis, H. longifilis, H.isopterous* and *H. boulengeri* (Babatunde and Aminu, 1998). The Clariids exhibit many qualities which make them suitable for culture in West Africa. Some of these qualities include: fast growth, wide distribution, easy to culture, and high potential yield (Ugwumba and Ugwumba, 2003) In addition, the Clariid catfish have the ability to st grow on a wide range of natural and low cost artificial feed and can withstand low oxygen and pH levels (Fagbenro and Sydenham, 1998). *H. bidorsalis,* however, is one of the most common species of catfish cultured in West Africa (Okafor et al. 2011). Culture of *H. bidorsalis* and some species of *Clarias* dominate aquaculture production in West African countries. (Yisa and Olufeagba, 2005; Owodeinde et al. 2012) It is therefore not surprising that this predominantly indigenous species because of its delicacy has a high demand amongst the local populace.

One of the production and management techniques commonly used in fisheries is acclimation, which is pre-requisite for stocking of ponds, both for culture and experimental purposes. (Akinrotimi, 2006) Acclimation (adaptation to a new situation or environment) is the modification of biological structures to maintain or minimize deviation from homeostasis, despite changes in some environmental factors such as temperature, salinity, pH, light, hardness or toxicant concentration (Akinrotimi et al. 2007) Therefore, distinction should be made between *acclimatization* which is a shift taking place under natural conditions and *acclimation* which is a shift taking place in artificial conditions. Thus most fish physiologists recommend the practice of subjecting fish species to be used for laboratory experiments to acclimation period of about seven days (Gabriel et al. 2004) Generally, it is believed that during this period, the fish may manifest symptoms of diseases that may assist in the separation of apparently healthy fish from sick individuals thereby ensuring that only healthy fish are used for the experiment. This helps to testify that the result thus obtained are not influenced by the state of health of the fish. (Wedemeyer and Yasutake, 1977) The effect of transportation stress on blood parameters of tilapia fish has been studied (Orji, 1998, 2005). Also the effects of acclimation stress on some blood parameters of certain Clariids have been studied (Gabriel et al. 2004, Akinrotimi et al. 2007) However, there is little or no report on the effects of stress of transportation and acclimation (a common practice in aquaculture) on some physiological responses of *Heterobranchus* species. Consequently, this study aims to determine more precisely some haematological responses of *H. bidorsalis* to stress of transportation and laboratory acclimation.

MATERIALS AND METHODS

One hundred disposable 2cm^3 syringes, one hundred 2cm^3 EDTA (ethylene diamine tetra acetic acid)bottles, metre rule, weighing balance, ten rolls of sterile cotton wool and five plastic buckets (0.25 x 0.25 m) but only one was filled to the brim with ice chips, were all conveyed to New Calabar River side at Aluu, in Rivers State, Nigeria. New Calabar, River lies between Longitude 6^0 30 and 7^0 .00 East and Latitude 4^0 30 and 5^0.00 North

Collection of blood samples from *H. bidorsalis* specimens at New Calabar River side

As soon as a live *H. bidorsalis* specimen was brought out of the River using hook and line as well as basket traps, its standard length and weight were quickly determined using metre rule and weighing balance respectively. Then a 2cm^3 disposable plastic syringe was used to collect 0.5ml of blood from the caudal blood vessels beside the anal fin and immediately transferred into a 2cm^3 EDTA bottle. The hundred blood samples in test tubes collected in this manner (sixty from juveniles and forty from adult *H. bidorsalis)* were surrounded by ice chips and kept inside two of the five plastic buckets (0.25 x 0.25 m) All the one hundred and fifty live specimens of *H. bidorsalis* were put inside the remaining three plastic buckets (0.25 x 0.25 m). Finally, the five plastic buckets (0.25 x 0.25 m) were transported to Physiology laboratory of Animal and Environmental Biology Department of Abia State University, Uturu-Nigeria within less than 4 hours.

Stressful acclimation in the laboratory.

In the laboratory the blood samples in test tubes were immediately kept in a Refrigerator while the one hundred and fifty live specimens of *H. bidorsalis* were randomly introduced in three plastic tanks (0.54 x 0.38 x 0.30 m) at a stocking density of 50 fish per tank, however, ensuring that each tank contained both the small (30 juveniles) and large (20 adults) specimens. The

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acclimation temperature was 33.4± 1.1°C. The tanks were neither aerated nor covered and the water in them was not renewed throughout the seven day period of acclimation. However, they were provided daily with pelleted fish feed obtained from the Regional Aquaculture Centre, Aluu, Port Harcourt in Rivers State, Nigeria.

Haematological analysis of blood samples

The red blood cell count (RBCC) total leucocyte count (TLC)and blood platelet count (BPC) were determined in an improved Neubauer Haemocytometer following the methods of Blaxhall and Daisely (1973) and Cheesbrough (2007). The haemoglobin content (HC) was determined by cyanmethaemoglobin method (AACC, 1984., Tietz, 1995). Packed cell volume (PCV) was determined by the method of Blaxhall and Daisley (1973) The osmolality and glucose level of the supernatant were estimated by the use of Karl Kolb osmometer (Model 650) and Folin and Wu standard methods respectively (Brewer et al, 1974.,AACC, 1984., Tietz, 1995). Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular haemoglobin concentration (MCHC) were calculated by the formula of AACC (1984) and Cheesbrough (2007) given below:-

$$MCV(\mu m) = \frac{PCV(\%) \times 10}{RBCC(mm^3)}$$

$$MCH(pg) = \frac{Haemoglobin\ (g\%) \times 10}{RBCC(mm^3)}$$

$$MCHC(\%) = \frac{Haemoglobin\ (g\%) \times 100}{PCV(\%)}$$

After 7 days of stressful laboratory acclimation another 0.5ml of blood was collected from the tail blood vessels beside the anal fin of each surviving but stressed *H. bidorsalis* for another set of blood analyses which followed exactly the pattern that had just been described. A hundred blood samples were collected.

Statistical Analysis

Blood parameters of each of the two groups, (non-acclimated and acclimated) were compared using the students' t-test to verify whether any observed difference in each parameter was statistically significant at 5% probability level.

RESULTS

The haematological parameters before transportation and after the one week of stressful laboratory acclimation are shown in Tables 1 and 2. The one week period of acclimation in both the juvenile and adult specimens of *H.bidorsalis* caused significant reduction of RBCC, HC, PCV, BPC, MCH, and MCHC indices ($p<0.05$) but led to significant elevation of TLC, osmolality, glucose and MCV indices ($p < 0.05$)

DISCUSSION

The significant reduction of RBC count ($p < 0.05$) due to stress of acclimation is in accordance with the work of Gabriel et al (2004) and Akinrotimi (2006) on *Clarias gariepinus* and *Sarotherondon melantherone* respectively. The reduction in RBCC and BPC could be attributed to reduced activity of haematopoietic organs such as bone marrow and spleen (due to stress of acclimation) to manufacture more red cells and blood platelets. Similarly, the reduction in HC (the oxygen carrier) observed in the study is attributed to concomitant reduction in the number of red cells where haemoglobin is contained. The elevated blood glucose level also observed in the study conforms to the result obtained by Hattingh (1976) on the fresh water fish, *Labeo capensis*, and also on the nile tilapia, *Oreochromis niloticus* (Orji, 1998). It is interpreted to be an 'alarm' reaction (fight or flight response) that was triggered by the stress. The elevated glucose level produced a burst of energy which prepared the animal for an emergency situation. This was coupled with an inflammatory response, that prepared the fish to defend itself against invading pathogens and this explains the rise in TLC observed in this study.

The reduction of packed cell volume (PCV) conforms to results obtained by Soivio and Oikari (1976) on *Esox lucius* and also by Madden (1976) on the rainbow trout, *Salmo gairdneri.* Nomura and Kawatsu (1977) as well as Sikoki et al. (1989) got such similar results in the rainbow trout, *Salmo gairdneri* and in the African catfish, *Clarias gariepinus*

Table 1.

Mean values of Haematological parameters of sixty juvenile *Heterobranchus bidorsalis* specimens before transportation and after stressful laboratorys acclimation. (mean length 27.6±3.4cm, mean weight, 118.5±9.3g)

BEFORE ACCLIMATION **AFTER ACCLIMATION**

		Range				Range		**
Parameters	Mean value	Minimum	Maximum	Parameters	Mean value	Maximum	Minimum	
RBCC	1.62±0.50	1.52	1.72	RBCC	1.28±0.05	1.18	1.38	*
TLC	13.68±0.56	12.56	14.79	TLC	16.02±0.56	14.89	17.13	**
HC	5.78±0.56	4.66	6.91	HC	4.09±0.56	2.96	5.21	**
PCV	23.49±0.48	22.54	24.44	PCV	20.94±0.48	19.98	21.89	
BPC	66.92±4.44	58.07	75.76	BPC	54.25±4.44	45.40	63.09	**
Glucose	85.00±20.50	60.40	90.20	Glucose	125.00±10.00	85.40	136.70	*
MCV	145.00±3.85	148.29	142.09	MCV	163.59±3.66	169.32	158.62	
MCH	35.68±1.11	30.66	40.17	MCH	31.95±1.02	25.08	37.75	*
MCHC	24.61±0.97	20.67	28.27	MCHC	19.53±0.88	14.81	23.80	*
Osmolality	135.80±44.20	93.80	181.90	Osmolality	179.40±35.60	155.10	196.70	

Table 2.

Mean values of Haematological parameters of forty adult *Heterobranchus bidorsalis* specimens before transportation and after stressful laboratory acclimation. (mean length,42.3±6.1cm, mean weight, 236.9±22.5g)

BEFORE ACCLIMATION		Range		AFTER ACCLIMATION		Range		
Parameters	Mean value	Minimum	Maximum	Parameters	Mean value	Maximum	Minimum	
RBCC	2.12±0.05	2.02	2.22	RBCC	1.94±0.50	1.84	2.04	**
TLC	34.79±0.56	33.68	35.91	TLC	36.82±0.56	35.70	37.94	*
HC	10.14±0.56	9.02	11.26	HC	8.58±0.56	7.46	9.70	**
PCV	28.60±0.48	27.65	29.55	PCV	26.54±0.48	25.58	27.49	**
BPC	170.56±4.44	161.71	179.40	BPC	145.95±4.44	137.11	154.79	**
Glucose	92.00±18.50	65.00	98.80	Glucose	138.50±8.50	100.40	152.00	*
MCV	134.91±6.52	136.88	133.11	MCV	136.80±4.78	139.02	134.75	*
MCH	47.83±1.49	44.65	50.72	MCH	44.23±1.17	40.54	47.55	
MCHC	35.45±1.06	32.62	38.10	MCHC	32.33±1.21	29.16	35.29	
Osmolality	176.30±19.50	92.80	188.50	Osmolality	204.10±18.80	130.60	229.70	*

Key: **RBCC:** Red Blood Cell Count in million/mm^3
TLC: Total leucocyte count in thousand/mm^3
HC: Haemoglobin Content (g%)
PCV: Packed cell volume (%)
BPC: Blood Platelet count in thousand/mm^3
Glucose: mg/dl
MCV: mean Corpuscular Volume (µm)
MCH: Mean Corpuscular Haemoglobin (pg)
MCHC: Mean Corpuscular Haemoglobin Concentration (%)
Osmolality: mosmol/litre

** significant reduction ($p < 0.05$)
* significant increment ($p < 0.05$)

respectively. Orji (2005) also reported significant reduction of haematocrit level by the nile tilapia, *Oreochromis niloticus* (Linnaeus) due to stress of transportation.

The stress of acclimation (handling sorting, injury, overcrowding transportation and poor water quality e.t.c) removes or damages the mucous or slime layer that surrounds the fish, thereby reducing its effectiveness as a barrier against infection at a time when it is most needed. (Sharp et al. 1998) Under such a circumstance, *H bidorsalis,* being a fresh water fish absorbs excessive amount of water from the environment (over hydrate) and this logically accounts for the reduced PCV observed. The stress of acclimation also elevated the blood osmolality level, an indication of a likely elevation of osmotically active particles (solutes). Further work is needed to establish a link between acclimation and the levels of some plasma solutes such as urea, uric acid, creatinine. e.t.c.

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