

The Effect of Transportation and Handling Stress on Haematology and Plasma Biochemistry in Fingerlings of *Clarias gariepinus* and *Tilapia zillii*

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Abstract: Blood samples collected from fingerlings of *Clarias gariepinus* and *Tilapia zillii* obtained from State Government Hatchery, Ilesha, Osun State were used to evaluate the effects of stress due to handling and transportation on haematology and plasma biochemistry in the fingerlings of the 2 species of fish. Blood samples were collected from 15 samples of 50 fingerlings of each species into 2 separate bottles- one containing EDTA, an anticoagulant and the other without EDTA, before and after transportation. The parameters evaluated from the blood samples with EDTA are the haematocrit, hemoglobin (Hb), erythrocyte count and leukocyte count, while the blood samples collected in bottles without EDTA were used to determine the plasma total protein (TP), albumin and globulin. The results indicate reduced values for all the parameters examined except for the leukocyte, Hb, albumin and albumin-globulin ratio for *T. zillii*, while the blood constants, albumin, albumin-globulin ratio values increased for *C. gariepinus*. The changes in the Hb, leukocyte, MCHC and the TP were more significant ($p < 0.05$) for fingerlings of *T. zillii* compared to those of *C. gariepinus*. It can be deduced from this study that fingerlings generally are susceptible to stress but those of *T. zillii* are more susceptible to physical stresses than those of *C. gariepinus*.

Key words: *Clarias gariepinus* • *Tilapia zillii* • haematology • biochemistry and stress

INTRODUCTION

Fish is very important to man and is one of the most readily available and valuable sources of high graded, relatively cheap protein available to man [1, 2]. Furthermore, of all the sources of animal protein, fish is the easiest to digest with most species showing protein digestibility of between 90 and 98% [3].

The prominence of fish as food source has been growing, with the rapid expansion in food industry as a result of increasing population awareness and demand for quality food. Currently, about 4.3 billion fingerlings of desirable fish species are required annually for stocking about 25% of the available 1,175,510 hectares of fresh and brackish water bodies at 10,000 fingerlings per hectare in Nigeria [4].

In aquaculture, transporting fish from one location to another is inevitable. The transportation of live fish is a widespread practice, particularly in rural areas of developing countries, often representing the only means of supplying fry or juveniles to small-scale aquaculturists [5]. Fry and fingerlings are moved from hatchery to fish

farms, adult fish are moved from fish farms to market and processors, etc. Very often, large numbers of fry, fingerlings and adult fish are being transported.

The major problems encountered in these transportation operations according to Taylor and Ross [5], are usually unstable water temperatures control of metabolic wastes and supply of dissolved oxygen, which constitute physical and chemical stresses and produce unwanted variations in qualitative and quantitative haematological parameters.

Blood chemistry and haematological measurements can provide valuable physiological indices that may offer critical feedback on transportation and handling stress in aquaculture as haematological measures have been reported (Bridges *et al.* [6]; Warner and Williams [7] and Folmar [8]) to be useful indicators of sub lethal environmental stress in fish.

The application of haematological techniques has proved valuable in monitoring stress responses [9]. However, there is paucity of information on the use of plasma indices in the assessment of physiological stress arising from handling and transportation of fish in Nigeria.

This study was therefore designed to assess the effect of transportation and handling stress on haematological and plasma parameters in fish, with a view to proffer suitable and appropriate technique(s) for handling and transporting live fish to enhance hatchery-to-farm survival.

MATERIALS AND METHODS

Source of fingerlings: Fifty live fingerlings each of the African catfish (*Clarias gariepinus*) with mean weight of 1.89 ± 0.17 g and *Tilapia zillii* with mean weight of 6.06 ± 0.29 g were obtained from Government Hatchery, Ilesha, Osun State, Nigeria. The fingerlings were stabilized and starved for 24 h. Thirty five of each species were transported to Adekunle Ajasin University, Akungba Akoko (a distance of about 150 km) in an open container with 4 L of the hatchery water, lasting 3 h.

All the fish fingerlings were considered as healthy on the basis of their external appearance, the absence of obvious sign of disease and their calculated condition factor $K = WL^{-3} \times 10^2$; where W = weight and L = length.

Blood collection and analysis: Blood samples were collected from the caudal vein of 15 fingerlings of each species immediately after capture at the fish farm with the aid of a heparinised 5 mL disposable plastic syringe and a 21 gauge disposable hypodermic needle according to the method of Haruna and Adikwu [10] into 2 vacutainers, one with EDTA and the other without EDTA.

The same process of blood collection was performed after the exposure of the fingerlings to stress due to handling and transportation to Adekunle Ajasin University, Akungba-Akoko.

The blood samples collected in the EDTA bottles were used for the determination of haematocrit (PCV), haemoglobin (Hb) concentration, erythrocyte count and leukocyte count. Plasma was obtained from blood samples without EDTA by centrifugation and then drawn into a plastic syringe transferred into non-heparinised bottle and stored in refrigerator and later used for the determination of total protein, albumin and globulin. The blood samples collected at the hatchery were labelled initial and those collected after transportation were labelled the final samples.

The microhaematocrit method of Snieszko [11] was used to determine the haematocrit. The red blood cells were enumerated in an improved Neubauer haemocytometer, using Hendricks' [12] diluting fluid. The total white blood cell counts were similarly

enumerated in an improved Neubauer haemocytometer using Shaw's diluting fluid. The haematological indices- mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the equations given by Anderson and Klontz [13].

The total plasma protein was determined by the Biuret method of Reinhold [14] using a commercial kit, while albumin value was estimated by bromocresol green [15]. The globulin and albumin-globulin ratio was calculated according to Coles [16]. All determinations were carried out in duplicates for each sample.

Mortality rate determination: Mortality rate, which is the number of dead fish divided by number of fish transported multiplied by 100, was calculated for each species on arrival at Adekunle Ajasin University, Akungba-Akoko.

Statistical analysis: All data obtained were subjected to one-way ANOVA procedure of SAS[®] [17] and the significant means separated by Duncan option of the same software.

RESULTS

The initial and final haematological parameters of fingerlings of *T. zillii* and *C. gariepinus* exposed to transportation and handling stress are shown in Table 1. The results show that haematocrit, erythrocyte count, MCV and MCHC of the fingerlings of *T. zillii* decreased following their exposure to handling and transportation stress, the leukocyte count and Hb values increased significantly ($p < 0.05$), while the other haematological parameters increased but not significantly after stress. The haematocrit, erythrocyte count, leukocyte count and Hb value of the fingerlings of *C. gariepinus* decreased following exposure to handling and transportation stress, while MCHC, MCH and MCV increased after stress.

The initial and final serum biochemistry of fingerlings of *T. zillii* and *C. gariepinus* exposed to transportation and handling stress are summarized in Table 2. The results show that the total protein of *T. zillii* fingerlings declined significantly ($p < 0.05$) and their globulin also reduced but not significantly, while albumin and albumin-globulin ratio increased. However, the total protein and globulin of the fingerlings of *C. gariepinus* decreased while their albumin and albumin/globulin ratio increased after stress following their exposure to handling and transportation stress.

Table 1: Changes in haematology of *T. zillii* and *C. gariepinus* fingerlings exposed to transportation and handling stress (Mean±SEm)

Parameter	<i>T. zillii</i>		<i>C. gariepinus</i>	
	Initial	Final	Initial	Final
Haematocrit (%)	20.07±0.07	19.00±0.02	20.78±0.02	19.30±0.07
Erythrocytes (×10 ¹² L ⁻¹)	1.10±0.04	1.05±0.01	0.73±0.02	0.70±0.03
Haemoglobin (%)	6.60±0.14 ^b	14.80±0.71 ^a	3.30±0.04	3.25±0.03
Leukocytes (×10 ⁹ mm ⁻³)	1.29±0.12	4.20±0.17	1.80±0.35	1.72±0.14
MCV* (μ ³)	181.78±0.06	181.06±0.95	200.93±0.31	213.72±0.42
MCH** (pg)	46.48±2.49	35.29±0.28	51.39±0.04	54.80±0.01
MCHC*** (%)	33.14±1.88	25.36±1.32	15.87±0.03	17.01±0.04

^{a,b}: Mean values for each species with different superscripts differ significantly (p<0.05)

*MCV- Mean Corpuscular Volume; **MCH- Mean Corpuscular Haemoglobin; ***MCHC- Mean Corpuscular Haemoglobin Concentration

Table 2: Changes in plasma biochemistry of *T. zillii* and *C. gariepinus* fingerlings exposed to transportation and handling stress (Mean±SEm)

Parameter	<i>T. zillii</i>		<i>C. gariepinus</i>	
	Initial	Final	Initial	Final
Total protein (g/100 mL)	2.37±0.01 ^a	2.21±0.07 ^b	5.29±0.04	5.06±0.03
Albumin (g/100 mL)	0.90±0.07	0.98±0.08	2.35±0.03	2.45±0.04
Globulin (g/100 mL)	1.47±0.04	1.23±0.01	2.93±0.07	2.59±0.02
Albumin-Globulin ratio	0.61±0.02	0.80±0.15	0.78±0.02	0.96±0.04

^{a,b}: Mean values for each species with different superscripts differ significantly (p<0.05)Table 3: Percentage change in haematology of fingerlings of *T. zillii* and *C. gariepinus* exposed to transportation and handling stress (Mean±SEm)

Parameter	<i>T. zillii</i>	<i>C. gariepinus</i>
Haematocrit	-5.01±0.25 ^{NS}	-4.86±2.34 ^{NS}
Haemoglobin	124.24±2.95*	-1.40±3.60*
Erythrocytes	-3.91±3.19 ^{NS}	-3.45±0.17 ^{NS}
Leukocytes	225.71±1.14*	-4.70±0.38*
Mean corpuscular volume	-0.99±3.77 ^{NS}	1.47±1.70 ^{NS}
Mean corpuscular haemoglobin	-23.96±6.18 ^{NS}	2.39±3.26 ^{NS}
Mean corpuscular haemoglobin concentration	-23.33±3.32*	3.60±1.22*

* Significant means (p<0.05); ^{NS} Non-significant means (p>0.05)Table 4: Percentage change in plasma biochemistry of fingerlings of *T. zillii* and *C. gariepinus* exposed to transportation and handling stress (Mean±SEm)

Parameter	<i>T. zillii</i>	<i>C. gariepinus</i>
Total Protein	-6.75±0.45*	-4.52±0.19 ^{NS}
Globulin	-13.27± 2.00 ^{NS}	-12.37±0.51 ^{NS}
Albumin	-3.87±0.34 ^{NS}	-5.32±0.24 ^{NS}
Albumin/Globulin	19.75±0.42 ^{NS}	20.19±0.96 ^{NS}

* Significant means (p<0.05); ^{NS} Non-significant means (p>0.05)Table 5: Percentage change in water quality parameters and mortality rate of *T. zillii* and *C. gariepinus* exposed to transportation and handling stress

Parameter	<i>T. zillii</i>	<i>C. gariepinus</i>
Initial temperature (°C)	28.00	28.00
Final temperature (°C)	29.00	30.00
(%) change in temperature	3.40	6.60
Initial pH	7.37	7.39
Final pH	7.04	6.87
(%) change in pH	4.48	7.04
No transported	35.00	35.00
No dead	20.00	2.00
(%) mortality	57.10	5.70

Table 3 shows the percentage change in haematology parameters of fingerlings of *T. zillii* and *C. gariepinus* exposed to transportation and handling stress. The results show that PCV, Hb, RBC, MCH and MCHC increased in *T. zillii* but decreased in *C. gariepinus*, while WBC and MCV decreased in *T. zillii* but increased in *C. gariepinus* due to handling and transportation stress.

The percentage change in plasma biochemistry of fingerlings of *T. zillii* and *C. gariepinus* exposed to transportation and handling stress are shown in Table 4. The results show that total protein and globulin increased in *T. zillii* but decreased in *C. gariepinus* following the exposure of the fingerlings to handling and transportation stress.

Table 5 shows the percentage change in water temperature and mortality rate of *T. zillii* and *C. gariepinus* exposed to transportation and handling stress. Percent temperature change of water for transporting *T. zillii* is lower than that of *C. gariepinus*

(3.40 vs. 6.60), but mortality rate of 57.10% recorded for *T. zillii* fingerlings is higher than the 5.70% recorded for the fingerlings of *C. gariepinus*.

DISCUSSION

The results of the present study revealed changes in haematology and plasma biochemistry of fingerlings of *T. zillii* and *C. gariepinus* exposed to transportation and handling stress. Stress of handling has been shown to produce haemoconcentration and that the haematocrit of fish blood decreases after the stress of capture and transportation [18]. The final haematocrit and erythrocyte values reduced (20 vs. 19%) and (1.10 vs. 1.05), respectively, but the Hb value increased following the exposure of the fingerlings of *T. zillii* to handling and transportation stress.

These findings may be attributed to the exposure of the fingerlings of *T. zillii* to handling and transportation stress. Erythrocyte value is a function of oxygen absorption and transportation within a living cell and depletion in the count may weaken and lead to death in fish [19]. Since oxygen in blood depends on the iron compound, haemoglobin, this result shows that respiration in fingerlings of *T. zillii* might have been impaired. The final Hb concentration value of 14.80 ± 0.71 recorded for fingerlings of *T. zillii* is above the reference intervals of 7.0-9.8 reported by Hrubec *et al.* [20] for cultured tilapia. This may be responsible for the relatively high mortality (Table 5) recorded for the fingerlings of *T. zillii*.

Similarly, the final erythrocyte value recorded in this study was below the erythrocyte range of 1.91-2.83 also reported by Hrubec *et al.* [20]. Since the reduction in the erythrocyte value may be due to destruction of the red cells which might have resulted in the liberation of the Hb content of the cells, the reduction in final values for blood constants (MCHC, MCH and MCV) of the fingerlings of *T. zillii* can be attributed to transportation and handling stress since the Hb and erythrocytes are the components utilized in the evaluation of the MCH. Although the final MCH value was within the reference value reported [20].

The final haematocrit, erythrocyte and Hb values of the *C. gariepinus* fingerlings exposed to transportation and handling stress also decreased as observed in the fingerlings of *T. zillii*. However, the mean final erythrocyte value of $0.70 \times 10^{12} \text{ L}^{-1}$ obtained for the fingerlings of *C. gariepinus* in this study was within the physiological range for catfish species raised in freshwater ponds in Nigeria [21]. The implication of this is that, the fingerlings of *C. gariepinus* were still within the

physiologically effective respiratory range, below which the normal functioning of their respiratory mechanisms would be significantly impaired as observed in the fingerlings of *T. zillii*.

The increased leukocyte value for *T. zillii* in this study might be due to the stress of handling and transportation. White blood cell of fish or any animal has been reported to be a function of the immunity and the animal resistance to some vulnerable diseases [19]. The final leukocyte value for *T. zillii* increased while that of *C. gariepinus* fingerlings did not in this study. The final value of the total leukocyte observed in the fingerlings of *T. zillii* was higher than the reference interval of $0.22-1.55 \times 10^8 \text{ mm}^{-3}$ ($=21,559-154,690/\mu\text{L}$) given by Hrubec *et al.* [20], while the final value of the total leukocytes for *C. gariepinus* was within the physiological range of $1.00-4.30 \times 10^8 \text{ mm}^{-3}$ [21]. This result indicates leukocytosis in the fingerlings of *T. zillii* which has been reported by Coles [2] as a consequence of tissue destruction, irrespective of its cause. This result may lend credibility to the hypothesis of red cell destruction in the fingerlings of *T. zillii* earlier discussed in this study.

In plasma biochemistry of fingerlings of *T. zillii* and *C. gariepinus* exposed to transportation and handling stress, the final values of total protein and globulin decreased but there were increases in the values of albumin and albumin-globulin ratios. Wedemeyer [22] observed that stress due to capture, handling and sampling, affect plasma proteins in fish and was 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. The reduction in the final plasma total protein value may have implication on the physiological activity and may be vital in immunosuppression of the fingerlings which may have a strong negative impact on subsequent performance of the fish.

The percentage changes in the Hb values of the fingerlings of *T. zillii* and *C. gariepinus* which were significant may have fatal consequence on the respiratory physiology of the fingerlings. However, a higher percentage change in the Hb of *T. zillii* compared to that of *C. gariepinus* (124.24 ± 2.95 vs. -1.40 ± 3.60) might have resulted in the higher mortality rate observed for *T. zillii* (Table 5) exposed to similar handling and transportation stress.

Similarly, the percent change in the leukocyte values may indicate the level at which the immunity of the fingerlings of the two species of fish might have been compromised when exposed to stress of handling and

transportation. The significant change in the leukocyte values of *T. zillii* fingerlings as compared to that of *C. gariepinus* fingerlings (Table 3) revealed that the fingerlings of *T. zillii* are more sensitive and respond to physical stresses than those *C. gariepinus*.

The percent change in the total plasma protein of the fingerlings, which was significant for *T. zillii* also lend credibility to higher susceptibility of this species to physical stresses than of *C. gariepinus* which has been hypothesized in this report.

CONCLUSIONS

This study has shown that in exposing fingerlings of *T. zillii* and *C. gariepinus* to stress of handling and transportation, the physiological state of the fingerlings were compromised, with the fingerlings of *T. zillii* more susceptible than those of *C. gariepinus*. This may affect the subsequent production performances of the fishes if not minimized.

REFERENCES

- Murray, J. and J.R. Burt, 1991. The composition of fish. Her Majesty Stationary Office (HMSO) Press, Edinburgh.
- Choo, P.S. and M.J. Williams, 2003. Fisheries production in Asia: Its role in food security and nutrition. Paper presented at the IX Asian Congress of Nutrition, February 23-27. New Delhi, India. In: NAGA, WorldFish Centre Quarterly, 26: 11-16.
- Acton, J.C. and C.L. Rudd, 1987. Protein quality methods for sea foods. In: Seafood Quality Determination. D.E. Kramer and J. Liston (Eds.). Elsevier. Amsterdam, pp: 453.
- FMARD., 2003. Aquaculture development. In: Presidential Forum on the Fisheries Development Subsector, Federal Department of Fisheries, Federal Ministry of Agriculture and Rural Development, October, 2003, pp: 68.
- Taylor, N.I. and L.G. Ross, 1988. The use of hydrogen peroxide as a source of oxygen for the transportation of live fish. Aquaculture, 70: 183-192.
- Bridges, D.W., J.J. Cech and D.N. Pedro, 1976. Seasonal hematological changes in winter flounder, *Pseudopleuronectes americanus*. Trans. Am. Fish. Soc., 105: 596-600.
- Warner, M.C. and R.W. Williams, 1977. Comparisons between serum values of pond and intensive raceway cultured channel catfish *Ictalurus punctatus* (Rafinesque). J. Fish Biol., 11: 385-391.
- Folmar, L.C., 1993. Effects of chemical contaminants on blood chemistry of teleost fish: a bibliography and synopsis of selected effects. Environ. Toxicol. Chem., 12: 337-375.
- Soivio, A. and A. Oikari, 1976. Hematological effects of stress on a teleost *Esax luscious* L. J. Fish Biol., 8: 397-411.
- Haruna, A.B. and I.A. Adikwu, 2001. Haematological responses to non-familiar diets: A study of the African mud catfish, *Clarias gariepinus*. J. Arid Zone Fish., 1: 12-22.
- Snieszko, S.F., 1960. Microhaematocrit as a tool in fishery research and management. Spec. Scient. Rep. US Fish and Wild Serv. No. 341.
- Hendricks, L.J., 1952. Erythrocyte counts and haemoglobin determinations for the species of suckers, *Gesur catotomus* from Colorado. Copea, 4: 265-266.
- Anderson, D. and G.W. Klontz, 1965. Basic haematology for the fish culturist. Ann. Northw. Fish Cult. Conf., 16: 38-41.
- Reinhold, J.G., 1953. Standard Methods of Clinical Chemistry. Academic Press New York.
- Doumas, B.T., W. Watson and H. Briggs, 1971. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta., 31: 87-89.
- Coles, E.H., 1986. Veterinary Clinical Pathology. 4th Edn. W.B Saunders Co. Philadelphia, pp: 56-58.
- SAS Institute Inc., 1999. SAS/STAT. User's Guide. Version 8 for windows. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA.
- Hattingh, J. and A.J.J. Van Pletzen, 1974. The influence of capture and transportation on some blood parameters of fresh water fish. Comp. Biochem. Physiol., 49: 607-609.
- Akinwande, A.A., F.O. Moody, O.A. Sogbesan, A.A.A. Ugwumba and S.O. Ovie, 2004. Haematological response of *Heterobranchus longifilis* fed varying dietary protein levels. A paper presented at FISON Conference, Ilorin.
- Hrubec, T.C., J.L. Cardinale and S.A. Smith, 2000. Hematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis Hybrid*). Veterinary Clinical Pathol., 29: 7-12.
- Erondu, E.S., C. Nnubia and F.O. Nwadukwe, 1993. Haematological studies on four catfish species raised in freshwater ponds in Nigeria. J. Appl. Ichthyol., 9: 250-256.
- Wedemeyer, G.A. and W.T. Yasutake, 1981. Clinical methods for the assessment of the effect of environmental stress on fish health. US Tech Pap. US Fish Wildl. Serv., Wash., DC., 89: 1-17.