

Original Research Article

PROPAGATION, GROWTH AND HAEMATOLOGY OF FISH CULTURED IN WATER FROM A NATURAL STREAM AND UNDERGROUND TUBEWELL SOURCES

ABSTRACT

Propagation, growth and haematology of *Clarias gariepinus* bred and reared in water from a natural stream at 5°28'N, 7°32'E (Nature stream) and two wells at 5.481°N, 7.5370E (well-1) and 5.477°N, 7.539°E (well-2) were studied. The study was carried out in search of suitable water within close proximity for fish propagation. Growth was evaluated once in two weeks. Haematological parameters of the fingerlings were examined at 70 days post hatch. There was significant difference ($P < 0.05$) in egg fertilization, hatchability, larval survival, growth and blood parameters among the treatments. Fish exposed to water from the natural stream entrenched best propagation indices including fastest growth and highest values in all the blood parameters studied. Values of Packed Cell Volume and Molecular Cell Haemoglobin Concentration of fish for well-1 and well-2 were not statistically different ($P > 0.05$). Apart from these two parameters, values of all the other blood parameters including growth were significantly lower ($P < 0.05$) in fish exposed to water from well-2 than those from well-1. The disparities observed signifies differences in water quality which is indicative of presence of contaminant not identified in this study. The report recommended inclusion of heavy metals and organic mineral analysis in future study to unravel the cause of the observed differences.

Key words: Blood indices, Fish propagation, Fish Size, Surface water, Underground water and Water quality

INTRODUCTION

Fishes being poikilothermic are very sensitive to changes in their aqueous environment (Uka, 2021). Fazio *et al.*, (2013) clearly stated that fish live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components. Borkovic *et al.*, (2008) noted that fish are largely used for the assessment of aquatic environment quality and are accepted as bio-indicators of environmental pollution. Zeitoun and Mehona (2014) submitted that studies on fish can play significant role in assessing potential risk associated with contamination of aquatic environment. Fernades and Mazon (2003) had earlier reported that fish blood changes with a change in the environment where it lives. They noted that evaluation of haematology helps to understand the influence of environment on fish wellbeing. Therefore, analysis of haematological parameters is a diagnostic technique to understand health of fish in any environment. De-Pedro *et al.*, (2005) re-echoed that monitoring fish health can be done using haematological and biochemical profile of its blood. Satheeshkumar *et al.*, (2011) observed that haematological and biochemical changes in blood are important indicators used in monitoring physiological and pathological changes in fish. They concluded that qualitative and quantitative variations in haematological parameters including the red blood corpuscles (RBC) and white blood corpuscles (WBC) numbers, cell proportions of leukocyte, the amount of haemoglobin and the size of WBC and

RBC are the most significant findings as regards diagnosis. Bahmani *et al.*, (2001) reported that analysis of haematological and biochemical indices in the blood of farmed fish is good for identifying the health status of farmed fish as they provide reliable information on metabolic disorders and deficiencies. Therefore, haematology has been used as an index of fish health status in a number of fish species to detect physiological changes, as a result of exposure to different stressful condition such as handling, pollutants, metals, hypoxia, anaesthetics and acclimation (Alwan *et al.*, 2009). Yaribeygi *et al.*, (2017) reported that certain environmental stressors can alter blood characteristics with resultant disruptions in metabolic activities leading to reduced growth rate and impairment of reproductive process,

Report of Fazio *et al.*, (2013) showed that data on haematological parameters are good indicators of health status of fish in response to changes related to nutrition, water quality and disease. The health status is reflected on growth and reproduction (London and Volkoff, 2019). Gorjipour (2014) noted that the most common sources of water for aquaculture are wells, springs, rivers and lakes. Of all the sources mentioned, wells and springs are considered to be the best sources of water used for aquaculture. Rivers and other open water bodies have variable chemical and physical characteristics that make them unpredictable in terms of reliability for aquaculture purpose. The present report compared the growth and haematological characteristics of *Clarias gariepinus* bred and reared in water from different sources. It documented differences in haematology of fish attributed to inhabited aqueous environment and therefore defined relationships between blood characteristics and habitat of fish.

MATERIALS AND METHODS

Sources of experimental waters: Sources of the experimented waters were from a natural stream at 5°28'N, 7°32'E (Nature stream) and two neighborhood wells at 5.481°N, 7.537°E (well-1) and 5.477°N, 7.539°E (well-2)

Propagation and rearing: Sexual products were obtained by artificial propagation. Sexually matured female *C. gariepinus* were selected on the basis of ovarian biopsy while males were chosen based on pointed urinogenital papillar. The papillar of selected males were hyperemic at the tip. Hormone administration was as implemented by Uka and Obijiaku (2019). Eggs and sperm were procured as described in Uka and Obijiaku (2019). A total of four females and two males were used. The eggs from the four females were pooled together and were fertilized with pooled sperm from the two males. The fertilized eggs were incubated uniformly in three large concrete hatching tanks each containing water from any of the 3 different sources (Nature stream, Well-1 and Well-2). Hatchlings were nursed in these tanks for 10 days post hatch. Twenty litres of water for each treatment was fetched with measuring cylinder into 25 litres capacity bowls for larval rearing. The larval rearing in each treatment was conducted in quadruplet. Forty healthy fry from each nursing tank were distributed in equal number into their respective treatment water in the bowls. The fish were nursed in these bowls for 60 days. All through the rearing period, the fish were fed to satiation with 42% crude protein commercial diet.

Growth monitoring: Growth was monitored by measuring the weight and length of the fish on fortnight basis. The instruments used for growth monitoring were metre rule, pair of divider and sensitive weighing balance (Mettler Toledo, PL 303). Growth parameters determined were Daily

specific growth rate (DSGR), established from the formula: $DSGR (\%) = \frac{\log W_2 - \log W_1}{T - t} \times 100$,
mean growth rate (MGR): $MGR = \frac{W_2 - W_1}{0.5(W_2 - W_1)t} \times 100$ and percentage weight gain
 $\% WG = \frac{W_t - W_o}{W_o} \times 100$. Where W_2 = final weight, W_1 = initial weight, T = final time and t = initial time.

Analysis of blood parameters: Blood samples were collected after 70 days post hatch from caudal peduncle with insulin needle and syringe. The samples were transferred to heparinized tubes and taken to laboratory for analysis. The haematological parameters analysed were packed cell volume (PCV), Red blood cell (RBC), Haemoglobin (Hg), white blood cell (WBC), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). Packed cell volume (PCV) was estimated after placing sealed micro haematocrit tube in a centrifuge at 10:500 rpm using micro haematocrit and the PCV was read off. Red cell count (RBC) was determined from a small amount of blood diluted 1 in 200ml with a solution of lithium heparin anticoagulant, to preserve the corpuscles. The corpuscles were then counted in a special counting chamber (Neubauer's slide chamber) with the use of microscope. White blood cells (WBC) were counted by Neubauer's improved haemocytometer using Hyem's and Turks solution as a diluting fluid. Haemoglobin was estimated using SAHLI'S method (Acid Hematin Method). Blood was added to N/10 Hydrochloric acid (HCl). Colour change was matched with the standard reference Sahli's apparatus to read concentration of haemoglobin in the blood. Mean corpuscular volume (MCV) was calculated from the volume of red cells and number of red cells in 100ml of blood and expressed in fento litre (FL). MCV was mathematically estimated from the formula: $\frac{\text{Vol. of red cells in 100ml blood}}{\text{Number of red cells in 100ml blood}} = \text{M.C.V.}$ Mean corpuscular haemoglobin (MCH) was calculated by dividing the amount of Hb in 100ml blood with the number of red cells in the same volume of blood and expressed in pictogram. It was mathematically estimated with the formula: $\text{M.C.H} = \frac{\text{Haemoglobin in grams per 100ml blood}}{\text{Number of red cells per 100ml blood}}$. Mean corpuscular haemoglobin concentration (MCHC) was calculated from haemoglobin in grammes and the volume of packed cells in 100ml. It was mathematically estimated with the formula: $\text{M.C.H.C} = \frac{\text{Haemoglobin in grams per 100 ml blood}}{\text{Volume of red cells in ml per 100 ml blood}} \times 100 \text{g per decilitre} = \frac{\text{Haemoglobin content}}{\text{Haematocrit}} \times 100 \text{g/dl}$.

Water quality monitoring

The water quality parameters in the egg incubation and in larval rearing tanks were monitored during the study. The parameters that were monitored included: dissolved oxygen, pH and temperature. Dissolved oxygen was monitored with Dissolved Oxygen meter (EXTECH SDL 150) while temperature and pH were monitored with Hanna Instrument pH metre (HI 991350).

Data analysis

The data obtained were analyzed using SPSS 15.1. One-way ANOVA was employed. Where difference was found, Duncan Multiple Range Test was used to separate the means. Presence or absence of statistical difference was determined at 5% probability level.

RESULTS AND DISCUSSION

Results

Propagation

Percentage egg fertilization in waters from the three sources (nature stream, Well-1 and Well-2) declined from 90.72 % through 79.66 % to 57.50 % respectively (Table 1). The difference in the fertilization rate was significant ($P<0.05$). Egg hatching success (78.0 %, 74.68 % and 49.65 %) and larval survival (86.8 %, 78.7 % and 55.5 %) in the same order were equally significantly different ($P<0.05$) among the treatments (Table 1).

Table 1: Effect of water from different sources on hatchery propagation of *C. gariepinus*

Parameters	Natural Stream	Well-1	Well-2	Statistics
Egg fertilization (%)	90.7	79.7	57.5	*
Egg hatching (%)	78.0	74.7	49.7	*
Larval survival (%)	86.8	78.7	55.5	*

*=Significant at 5% level

Growth

Growth was significantly different among the treatments ($P<0.05$). Final weight attained declined from 26.54 ± 13.27 g through 19.27 ± 7.61 g to 17.71 ± 6.27 g in Nature stream, Well-1 and Well-2 respectively. Mean weight gain of 3.69 ± 2.53 g, 2.01 ± 1.39 g and 1.45 ± 1.13 g were achieved in the same order while Daily specific growth rate was 0.18 ± 0.20 , 0.22 ± 0.16 and 0.16 ± 0.16 respectively (Table 2).

Table 2: Growth performance of *C. gariepinus* in Water from three different Sources

Parameters	Natural Stream	Well-1	Well-2	Statistics
Final Mean wt (g)	26.54 ± 13.27^a	19.27 ± 7.61^b	17.71 ± 6.27^c	*
Mean wt gain (g)	3.69 ± 2.53^a	2.01 ± 1.39^b	1.45 ± 1.13^b	*
DSGR	0.18 ± 0.20^a	0.22 ± 0.16^b	0.16 ± 0.16^b	*

DSGR = Daily Specific Growth rate, *=Significant at 5% probability level ($P<0.05$). Values in the same column with different superscript letter are significantly different.

Haematology

The fish reared in natural stream showed highest values in all the blood parameters studied. Values of PVC and HCMC were not significantly different ($P>0.05$) in water from well-1 and well-2. However, their values in well-1 were higher than in well-2. Apart from these two parameters, values of all the other blood parameters were significantly lower in fish exposed to well-2 than those exposed to well-1 (Table 3).

Table 3: Hematological profile of *clarias gariepinus* cultured in water from 3 different sources

Treatments	Blood parameters						
	RBC	WBC	PCV	HG	MCV	MCH	MCHC
Natural stream	2.5933 ± 0.1484	3.7900 ± 0.1609	28.3333 ± 2.5166	5.9667 ± 0.2081	38.3400 ± 0.9366	14.2100 ± 0.6369	18.3133 ± 1.0424

	4 ^a	3 ^a	1 ^a	7 ^a	4 ^a	5 ^a	2 ^a
Well-1	1.6233 ±0.1817 5 ^b	2.6733 ±0.2138 5 ^b	22.3333 ±1.5275 3 ^b	4.7000 ±0.1000 0 ^b	29.9933 ±0.7563 9 ^b	12.0933 ±0.2968 7 ^b	15.9467 ±0.4061 2 ^b
Well-2	1.3400 ±0.0888 8 ^c	1.9800 ±0.0600 0 ^c	20.0000 ±1.7320 5 ^b	3.5667 ±0.2516 6 ^c	29.8600 ±1.2738 9 ^b	11.3900 ±0.3026 5 ^c	15.3567 ±0.3927 3 ^b
Statistics	*	*	*	*	*	*	*

*=Significant at 5% probability level (P<0.05). Values in the same row with different superscript letter are significantly different.

Water quality

Evaluation of the physicochemical parameters of the experimented waters showed a satisfactory quality for hatching and rearing of *C. gariepinus*. Temperature was not significantly different among the treatments (P>0.05). The mean value of temperature in the incubation and the rearing tanks ranged between 28.00±0.16 and 28.80±0.18°C. The mean pH value ranged from 6.06±0.49 to 6.54±0.87, while the dissolved oxygen ranged from 4.23±0.59 to 6.71±0.08mg/l. There was significant difference (P<0.05) among the treatments in dissolved oxygen concentration. Total dissolved oxygen in Well-2 (4.23±0.59) was significantly (P<0.05) lower than the total dissolved oxygen in Well-1 (6.06±0.49) and nature stream (6.71±0.08mg/l)-Table 4.

Table 4: Water Quality

Treatments	pH	DO(mg/l)	Temperature(°C)
Nature Stream	6.54±0.87	6.71±0.48 ^a	28.80±0.18
Well-1	6.06±0.49	6.34±1.11 ^a	28.00±0.166
Well-2	6.34±0.38	4.23±0.59 ^b	28.50 ^a ±0.20
Statistics	Ns	*	Ns

*=significant at 5% probability, ns=not significant

Discussion

Disparities observed in egg fertilization, hatching and larval survival could emanate from the significant differences observed in level of dissolved oxygen among the treatments. Uka and Kalu (2019) reported increased egg hatching and larval survival with increase in levels of dissolved oxygen. They further reported that fluctuation in hardness enhanced egg hatching and suggested that fluctuation in hardness could be induced to enhance egg hatching during hatchery operations.

The observed differences in growth were evidence of disparities in the chemical, physical and biological state of the waters. Makori *et al.*, (2017) reported increase in growth rate of tilapia as temperature and DO increased. Mannan *et al.*, (2012), revealed that water quality parameters, stocking density and species composition influenced the growth and production of fish in semi-intensively managed aquaculture farm. Imsland *et al.*, (2007) and Bhatnagar and Devi, (2013) recognised the importance of temperature and dissolved oxygen respectively in the growth of

fish. Optimal water quality is therefore essential for fish growth. Fish growth is generally expected better in water of optimal levels of DO and temperature among other parameters.

The disparity in the blood parameters of the fish reared in different waters signifies differences in the quality of the waters. Fernades and Mazon (2003) reported that fish blood changes with a change in the environment where it lives. Fazio *et. al.*, (2012) clearly showed that the environment in which fish live and the conditions governing them influence the metabolite content in their blood. Significantly higher values of all the blood parameters and faster growth rate in fish reared in water from the natural stream implies superiority of the water to the tube-well sources. Fernades and Mazon (2003) further reported that evaluation of haematology helps to understand the influence of environment on fish wellbeing. Therefore, analysis of haematological parameters as a diagnostic technique is appropriate in seeking information on the health of fish cultured in different waters as carried out in this study. The decreased value of RBC recorded in well-2 implies low haemoglobin with resultant low oxygen carriage and poor carbon dioxide excretion. These could be responsible for the poor growth observed in well-2. The poor production of RBC and Hgb which is diagnosed in anaemic malady may be evidence of water quality deterioration as a result of presence of contaminant(s) not identified in this study. Zhang *et. al.*, (2020) reported that presence of benzene derivatives can cause low RBC and low Hgb production in human bone marrow cells. This could also happen to fish in water contaminated with benzene derivatives. Fazio *et. al.*, (2012), reported that the presence of any substance in water produces changes in their quality which are not always favourable to development and survival of aquatic organisms. Mean Cell Haemoglobin Concentration (MCHC) and the values of Packed Cell Volume (PCV) and Mean Cell Volume (MCV) were low either because the cells are small or because the haemoglobin concentration is low. Ishikawa *et. al.*, (2007) stated that when the water quality is affected by toxicant, any physiological change will be reflected in the values of one or more of the biochemical and haematological parameters. Saravanan *et. al.*, (2011) showed that in fish exposed to different agents, the MCHC and MCH values decreased with respect to control group. Low concentration of MCHC in well-1 and well-2 is due to decrease in Hgb that could be attributed to its decreased synthesis or its oxidation into methemoglobin. Kroupovaa *et. al.*, (2008) noted that major outcome of nitrite poisoning is the oxidation of haemoglobin to methemoglobin in erythrocytes. On the other hand, Sahiti *et. al.*, (2018) reported impact of water quality on haematological and biochemical parameters of fish indicative of stress caused by water pollution with heavy metals. It is therefore recommended that a more detailed investigation should be carried out to ascertain the contaminant(s) in the waters that are responsible for the low values of these blood parameters in inhabiting fish. This is crucial in any attempt to purify available water for food fish propagation. Therefore, this report recommends expanded analysis of the waters to include heavy metals and organic mineral parameters in future study to unravel the cause of the observed differences.

Conclusion

Fishes are reported to be sensitive to changes in their aqueous environment. These changes may be reflected in their blood, growth and reproductive parameters. As a result of this, fishes are used as bio indicators of aquatic pollution. This study reported fish growth, haematology, egg hatching and larval development in water from different sources. The study was conducted in search of suitable water within close proximity for fish seed propagation. The findings showed disparities in the impacts of the waters on the parameters studied. It provided a guide in choice of water for fish propagation in the study area. However, it recommended a more detailed investigation to ascertain the contaminant(s) in the waters that are responsible for the disparities observed. Therefore, the report recommended expanded analysis of the waters to include heavy metals and organic mineral parameters in future effort.

REFERENCES

- Alwan, S. F., Hadi, A. A., and Shokr, A. E. (2009). Alterations in hematological parameters of fresh water fish, *Tilapia zillii*, exposed to aluminum. *Journal of Science and its Applications*, 3(1), 12-19.
- Anver, C.E (2004). Blood Chemistry (electrolytes, lipoprotein and enzyme) values of black scorpion fish (*Scorpaena porcus*, 1758) in Dardanelles, Turkey *J. Biol. Sci.* 4:716 -719.
- Bahmani, N; Kazemi, R and Donskaya, P (2001). A Comparative study of some hematological features in young reared sturgeon (*Acipenser persicus* and *Huso huso*). *Fish Physiol. Biochem*, 24: 135-140.
- Bhatnagar, A and Devi, P(2013). Water quality guidelines for the management of pond fish culture. *Introduction Journal of Environmental Sciences* 3(6): 1980 – 1996.
- Borkovic, S. S; Pavlovic, S. Z; Kovacevic, T.B; Stajn, A.S; Petrvic, V.M and Saicic, Z. S (2008). Antioxidant defence enzyme activities in hepatopancreas, gills and muscles of spiny cheek crayfish (*Orconectes limonsus*) from the River Danube. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*. 147 (1): 122-128.
- De Pedro N., Guijarro A.E., Lopez-Patino M.A., Marinez-Alvarez R., and Delgado M. (2005). Daily and seasonal variation in haematological and blood biochemical parameters in tench *Tinca tinca* *Aquaculture Res* 36:85–96.
- Fazio, F; Faggio, C; Marafioti, S; Torre, A; Sanfilippo, M and Piccione, G (2012). Effect of water quality on, haematological and biochemical parameters of *Gobius niger* caught in Faro lake (sicily). *Iranian Journal of Fisheries Sciences*. 12(1): 219 – 231.
- Fazio, F; Faggio, C; Manifiotu, S; Torre A; Sanfilippo, M and Piccione, G (2013). Comparative study of the biochemical and hematological parameters of four wild Tyrrheian fish species. *Veterinarni Medicina*, 58(11):576 – 581.
- Fernades, M. N and Mazon, A. F. (2003). Environmental Pollution and fish gill morphology Fish Adaptations. In Val A.L. and Kapour B.C (eds) Science Publications. Enfield, USA pp. 203-231.
- Gorjipour, E (2014): Effects of water type (River and well) on haematological Parameters in Juvenile Siberian Sturgeon (*Acipenser baeri*). *J. of Fisheries and Aquatic Sciences* 9(4): 213 – 220.
- Ishikawa, N. M., Paiva, Tavares Ranzani-Paiva, M.J., Lombardi, J.V and Ferreira, C. M (2007):

- Haematological parameters in Nile Tilapia, *Oreochromis niloticus* exposed to sub-lethal concentrations of mercury. *Brazilian Archives of Biology and Technology*, 50, 619-626.
- Imslund, A. K., Björnsson, B. T., Gunnarsson, S., Foss, A. and Stefansson, S. O. (2007). Temperature and salinity effects on plasma insulin-like growth factor-I concentrations and growth in juvenile turbot (*Scophthalmus maximus*). *Aquaculture*, 271(1-4), 546-552.
- Kroupovaa, H., Machovaa, J., Piackovaa, V., Blahovab, J., Dobsikovab, R., Novotnyb, L. and Svobodova, Z., (2008). Effects of subchronic nitrite exposure on rainbow trout (*Oncorhynchus mykiss*). *Ecotoxicology and Environmental Safety*, 71, 813- 820.
- London, S., & Volkoff, H. (2019): Effects of fasting on the central expression of appetite-regulating and reproductive hormones in wild-type and Casper zebrafish (*Danio rerio*). *General and comparative endocrinology*, 282, 113207.
- Makori, A. J., Abuom, P. O., Kapiyo, R., Anyona, D. N., & Dida, G. O. (2017). Effects of water physico-chemical parameters on tilapia (*Oreochromis niloticus*) growth in earthen ponds in Teso North Sub-County, Busia County. *Fisheries and Aquatic Sciences*, 20(1), 1-10.
- Mannan, M; Islam, M.S; Rimi, R.H, Suravi and Meghla, N. T (2012). Impact of water quality on fish growth and production in semi-intensively managed aquaculture farm. *Bangladesh J. of Environ. Sci.* 23:108 -113.
- Sahiti, H; Bislimi, K; Dalo, E and Murati, K (2018). Effect of water quality in haematological and biochemical parameters in blood of common Carp (*Cyprind carpio*) in two lakes of Kosovo. *NEScience* 3(3):323 – 332.
- Saravanan, M., Prabhu, K and Ramaesh, M (2011). Haematological and biochemical responses of freshwater teleost fish *Cyprinus carpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. *Pesticide Biochemistry and Physiology*, 100, 206-211.
- Satheeshkumar, P., Senthilkumar, D., Ananthan, G., Soundarapandian, P., & Khan, A. B. (2011). Measurement of hematological and biochemical studies on wild marine carnivorous fishes from Vellar estuary, southeast coast of India. *Comparative Clinical Pathology*, 20(2), 127-134.
- Sayed, A.E.H and Moneeb, R.H (2015). Haematological and biochemical characters of monosex tilapia (*Oreochromis niloticus*; Linnaeus, 1758) cultivated using methyltestosterone. *The journal of Basic & applied Zoology* (72):36 – 42.
- Uka, A and Obijiaku I. C (2019). Potentiation Effect of Vitamin E on Reduced Dosage on Ovaprim Induced Spawning of *Clarias gariepinus*. *Nigerian Journal of Fisheries*. 16(1):1580 – 1583.
- Uka, A and Kalu, S (2019). Differences in physicochemical properties of water from Neighbourhood boreholes and their usefulness in *Clarias gariepinus* egg hatching. *J. Appl. Sci. Environ. Manage.* 23(8): 1567 – 1571
- Uka, A (2021). Chromosomal aberration in fish inhabiting polluted Aquatic System: A Review *Academic Journal of Current Research* 8(4):93 – 101.
- Yaribeygi, H; Panahi, Y; Sahraei, H Johson, T. P AND Sahebkar, A (2017). The impact of stress on body function: A review. *EXCLI J.*16: 1057–1072
- Zeitoun, M.M and Mehona, E.E (2014). Impact of water pollution with heavy metals on Fish health: Overview and update, *Global Veterinaria* 12(2): 219 – 231.
- Zhang, X., Deng, Q., He, Z., Li, J., Ma, X., Zhang, Z and Xiao, Y. (2020). Influence of benzene

exposure, fat content, and their interactions on erythroid-related hematologic parameters in petrochemical workers: a cross-sectional study. *BMC public health*, 20(1), 1-13.

UNDER PEER REVIEW