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Studies of the Effect on the Haematology and Immuno-System of *Clarias gariepinus* Fed Graded Levels of *Ocimum gratissmum* as Feed Additive

Ekelemu Jerimoth Kesena & Maroh Eseoghene

Department of Fisheries, Delta State University, Asaba Campus, Asaba-Nigeria
jerimothekelemu@yahoo.com

Abstract: *Varying levels of Ocimum gratissimum were added to the diet of juveniles of Clarias gariepinus to ascertain its effect on the haematological profile of experimental fish. The study which lasted for 4 months was conducted using 15 concrete tanks of dimension 1m x 1m x 1m. One hundred and fifty juveniles of C.gariepinus used were acclimated for three days and distributed in 10s per tank which were grouped in triplicates, to form five treatments (1 - 5). Treatment 1, served as the control. The diets contained 0g, 10g , 20g, 30g, and 40g of O. gratissimum per kg of 2mm Coppens feed and labeled Diets 1 – 5 respectively. Fishes were fed rations of 3% body weight twice daily, with diets having corresponding numbers with the tanks. Blood samples were taken from one fish in each treatment at the end of the culture period to determine the blood quality of the fish in each treatment. Data collected were subjected to correlation analysis, one way analysis of variance and means were separated using Duncans multiple range test. C. gariepinus fed 10g inclusion of O. gratissimum performed better haematologically with an increase in Red Blood cell, White Blood Cell, Plasma protein, and Mean Corpuscular Haemoglobin Concentration. It is therefore concluded that the optimum level of inclusion of O. gratissimum to achieve improved blood quality and boost immunity is 10g/kg of feed.*

Keywords: *Ocimum gratissimum,Clarias gariepinus, immunity.*

1. INTRODUCTION

Catfish (Clariidae) is very popular in Nigeria due to its culturable attributes which has endeared it to many fish farmers (FAO, 2004). Expansion and growth of the aquaculture industry in Nigeria, has been slow due to challenges faced by fish farmers. These problems include lack of feed and quality fish seed for stocking. Due to the growing concern over the discovery of the side effects in using synthetic drugs in medicare, there is a renewed interest in the use of organic materials from plants in fish culture and care.

In recognition of the value of traditional medical systems, and the identification of medicinal plant from indigenous pharmacopoeias, which have significant healing powers, medicinal plants has contributed immensely to healthcare in Nigeria (John *et al*,2000). As a result the search for other protein sources in aqua feeds has gained much recognition (Ali *et al*., 2003; Erdal *et al*., 2004; Adewolu, 2008). Accordingly, protein sources of leaf meal origin such as duckweed have been given much attention as replacement for fish meal (Hassan and Edwards, 1992; Leng, *et al*., 1995; Erdal *et al*., 2004). Other examples of plant protein sources are alfalfa meal (Ali *et al*., 2003) and also potato leaf meal (Adewolu, 2008).

O. gratissimum (*lamiaceae*), commonly known as “alfavaca” is naturally used in the treatment of different diseases for example upper respiratory tract infections (Ilori *et al*, 1996).

C. gariepinus is known to be omnivorous and due to its high quality, market potentials and acceptability among others is an economically important fish food in various countries (Babalola and Apata, 2006; Ibrahim et al., 2007; Wikipeadia, 2008). *O. gratissimum* has been researched on and is used as a natural antibiotic because of its antibacterial and antifungal properties. Thus this study seeks to determine the use of *O. gratissimum* in improving the haematological quality of *C. gariepinus.*

2. Materials and Methods

2.1. Preparation of Experimental Leaf Meal

Fresh leaves of *O.gratissimum* were collected, rinsed with clean water to remove any foreign matter, chopped and air-dried. The dried leaf of *O. gratissimum* was subjected to proximate analysis to ascertain the phyto-chemicals present in it. The dried leaves of *O. gratissimum* were milled in a nearby hammer mill. Grounded *O. gratissimum* was thoroughly mixed with 2mm coppens fish feed at a rate of 0g, 10g, 20g, 30g, and 40g per 1kg of feed and labeled as diets D1, D2, D3, D4 and D5 respectively. Diet D1 without *O.gratissimum* inclusion, was the control.

One hundred and fifty juveniles of *C. gariepinus* used for the study were acclimated in a 1m x 1m x 1m concrete tank for one week before commencement of the experiment. During acclimation the fishes were fed to satiation with coppens feed twice daily.

2.2. Experimental Design

The design was a complete randomized block design (CRBD). Fish were randomly distributed in tens into 15 concrete outdoor tanks of 1m x 1m x 1m. Fish and tanks were grouped in threes, to form five treatments in triplicates labeled T1 – T5. Treatment 1 without *O.gratissimum* inclusion served as control. Fish in the Tanks were cultured for four (4) months and fed diets having the same numbers as the tanks. Rations fed were 3% body weight twice daily.

2.3. Collection of Blood Samples

At the end of the culture period, a fish sample was caught from each Tank and blood samples was collected for analysis in the laboratory. After the preliminary investigation of the length and weight of the fish, the fishes were placed on a tray and a towel was used to clean the body to avoid the mixture of blood and water. Blood samples were obtained from the caudal fin with the aid of a clinical disposable plastic syringe. The needle was inserted at right angle to the vertebral column of the fish and was gently aspirated during penetration. The needle was pushed gently down until blood started to enter as the needle. The blood was drawn gently until about 3 cm^3 of blood was obtained.

Thereafter, the needle was withdrawn and the blood immediately transferred into a heparinized plastic tube. The sample was gently but thoroughly mixed to avoid coagulation. Blood samples were used for the measurement of haematocrit (Pack Cell Volume) haemoglobin concentration, red blood cell count, white blood cell count and plasma protein.

2.4. Blood Analysis

Data collected from the blood were used to determine

- Hemoglobin concentration
- Haematocrit (Packed Cell Volume)
- Plasma Protein
- The Red Blood Cell.
- White Blood Cell Count (WBC)

Data collected on blood parameters were subjected to Pearson's correlation matrix, Analysis of Variance and means were separated using New Duncan Multiple Range Test (NDMRT).

3. Results

Table1. *Proximate Analysis of Ocimum Gratissimum*

S/N	Parameters	Conc(g/100g)	S/N	Phytochemical	Status
1	Carbohydrates	3.92	1	Alkaloid	+
2	Protein	6.24	2	Saponin	++
3	Ash.	1.98	3	Tannin	+
4	Crude fibre	2.41	4	Phlobatannins	+
5	Crude lipids	4.81	5	Anthraquinone	++
6	Energy	83.16	6	Steroids	+
7	Sodium	5.11	7	Terpenoids	+
8	Potassium	0.98	8	Flavonoids	+++
9	Calcium	4.82	9	Reducing sugar	+
			10	Carotinoid	-

Note: Results are average of triplicate analysis, sample used in dried mass.

+ low concentration, ++ moderate concentration, +++ high concentration, - absent

The result of the phytochemical screening in Table 1 revealed the presence of tannins, phlobatannins, anthraquinone, flavonoids, reducing sugar, terpenoids, steroids, saponins and alkaloids in *O. gratissimum*. In particular the flavonoids, saponins and anthraquinone were detected in high and moderate concentrations.

Table2. *Mean Variation Of Blood Parameter Of Clarias Gariepinus Fed Graded Levels Ofocimum Gratissimum As Feed Additive.*

	Treatment				
Parameters	**0g(T1)**	**10g(T2)**	**20g(T3)**	**30g(T4)**	**40g(T5)**
RBC	1.60 ± 0.17^{d}	2.37 ± 0.12^{a}	1.83 ± 0.33^{b}	1.70 ± 0.15^{c}	1.63 ± 0.24^{c}
WBC	1.33 ± 0.15^{e}	4.63 ± 1.85^{a}	3.33 ± 1.14^{b}	2.77 ± 2.51^{c}	1.97 ± 0.38^{d}
P.P.	7.20 ± 0.37^{a}	7.27 ± 0.61^{a}	7.13 ± 0.41^{ab}	7.00 ± 0.58^{b}	6.70 ± 0.15^{c}
H.B	11.10 ± 0.61^{a}	8.83 ± 0.49^{b}	8.83 ± 0.49^{b}	7.87 ± 0.47^{c}	6.60 ± 0.61^{d}
PCV	35.00 ± 2.89^{a}	32.35 ± 1.73^{a}	32.00 ± 1.53^{ab}	26.00 ± 1.53^{b}	22.33 ± 1.45^{bc}
MCHC	30.24 ± 0.02^{b}	30.78 ± 0.33^{a}	30.52 ± 0.36^{a}	30.20 ± 0.30^{b}	30.08 ± 0.21^{bc}
MCH	6.78 ± 1.11^{a}	5.87 ± 0.58^{b}	5.44 ± 0.66^{bc}	4.79 ± 0.75^{c}	2.84 ± 0.30^{d}
MCV	2.29 ± 0.37^{a}	1.93 ± 0.1^{b}	1.80 ± 0.21^{bc}	1.71 ± 0.29^{c}	1.52 ± 0.25^{d}

Means with the same superscript on the same row are not significantly different (P>0.05). Where; ± =standard error of the mean, PCV = Packed Cell Volume, Hb= Haemoglobin, PP= Plasma Protein, RBC = Red Blood Cell , WBC= White Blood Cell, MCHC = Mean Corpuscular Haemoglobin Concentration, MCH = Mean Corpuscular Haemoglobin, MCV = Mean Corpuscular Volume.

Table 2 showed a decreasing trend in RBC count in all experimental groups, this decreasing trend is statistically significant (P < 0.05). The highest mean value of $2.37+0.1210^{4}$/ul was observed in T2 while the lowest value was recorded for T1. Increase in inclusion level beyond 10 %, resulted in a decrease in RBC concentration. A similar observation was made for the WBC, PP and MCHC concentrations. The RBCs count shows significant decrease in T4 and T5 (P < 0.01) when compared to T2. The T2 group has shown significant increase when compared to T3 , T4 and T5 in mean value of WBCs count. Plasma protein count showed significant increase (P < 0.05) in T2 compared to any other treatment except the control T1.The PCV in all experimental groups reported lower values than the control. The PCV value of the T2 and T3 did not vary much from the control (T1).The MCHC registered a higher value than that of control group for the experiment T2 (30.78± 0.33%) and for all other groups a decline is observed.

From Table 2 with the use of one-way ANOVA and Duncan Multiple Range Test to separate the means it shows that most of the parameters are statistically significant (p<0.05) in response to changes in different level of *O. gratissimum* inclusion in the feed.

In Treatment 1, Table 3, WBC is positively correlated with MCH but not significantly related to other blood parameters, HB is also positively correlated with MCV, MCH is positively correlated to WBC , MCV is also positively correlated to HB. All correlations are related significantly at a level of (r=0.55, p<0.05)

Table3. *Pearson's Correlation matrix for the haematological profile of C. gariepinus fed 0g O.gratissimum inclusion in Diet 1*

Correlations									
Treatment		RBC	WBC	PP	HB	PCV	MCHC	MCH	MCV
RBC	Pearson	1	.932	.812	.985	.264	.123	.928	.973
	Correlation		.237	.397	.111	.830	.921	.243	.149
	Sig. (2-tailed)		3	3	3	3	3	3	3
	N		1	.968	.981	.596	.475	1.000*	.991
WBC	Pearson			.161	.126	.593	.685	.006	.088
	Correlation			3	3	3	3	3	3
	Sig. (2-tailed)			1	.901	.778	.680	.971	.925
	N				.286	.433	.524	.155	.248
PP	Pearson				3	3	3	3	3
	Correlation				1	-427	.294	.979	.998
	Sig. (2-tailed)					.719	.810	.132	.038
	N					3	3	3	3
HB	Pearson					1	.990	.064	.480
	Correlation						.091	.587	.681
	Sig. (2-tailed)						3	3	3
	N						1	.484	.350
PCV	Pearson							.679	.772

	Correlation							3	3
	Sig. (2-tailed)							1	.989
	N								.094
MCHC	Pearson								3
	Correlation								1
	Sig. (2-tailed)								
	N								
MCH									
MCV									

**. Correlation is significant at the 0.05 level (2-tailed).*

***. Correlation is significant at the 0.01 level (2-tailed).*

PCV = Packed Cell Volume, Hb= Haemoglobin, PP= Plasma Protein, RBC = Red Blood Cell, WBC= White Blood Cell, MCHC=Mean Corpuscular Haemoglobin Concentration, MCH = Mean Corpuscular Haemoglobin, MCV = Mean Corpuscular Volume.

RBC had a positive correlation with WBC and PP (r=0.55, p<0.05) for T2 but was not significantly related to the other blood parameters (Table 3). WBC is positively correlated with RBC and PP (r=0.55, p<0.05) but was not significantly related to the other blood parameters. PP is positively correlated with RBC and WBC* (r=0.55, p<0.05) for T2 but was not significantly related to the other blood parameters. HB had no correlation with the other blood parameters. PCV was not significantly related to the other blood parameters. MCHC was not significantly related to the other blood parameters. MCH had a positive correlation with MCV(r=0.55, p<0.05) but was not significantly related to the other blood parameters. MCV is positively related to MCH(r=0.55, p<0.05) but was not significantly related to the other blood parameters.

Table4.*Pearson's Correlation matrix for the haematological profile ofClariasgariepinus fed 10g(T2) Ocimum gratissimum*

Correlations									
Treatment		RBC	WBC	PP	HB	PCV	MCHC	MCH	MCV
RBC	Pearson	1	.999*	1.000*	.529	.500	.022	.948	.942
	Correlation		.023	.017	.645	.667	.989	.205	.219
	Sig. (2-tailed)		3	3	3	3	3	3	3
	N		1	.998*	.559	.468	-.014	.959	.953
WBC	Pearson			.040	.622	.690	.991	.182	.196
	Correlation			3	3	3	3	3	3
	Sig. (2-tailed)			1	.506	.524	.050	.939	.932
	N				.663	.649	.968	.223	.236
PP	Pearson				3	3	3	3	3
	Correlation				1	-470	-.837	.771	.784
	Sig. (2-tailed)					.688	.369	.440	.427
	N					3	3	3	3
HB	Pearson					1	.877	.200	.179
	Correlation						.319	.872	.885
	Sig. (2-tailed)						3	3	3
	N						1	-.296	-.316
PCV	Pearson							.809	.796
	Correlation							3	3
	Sig. (2-tailed)							1	1.000*
	N								.013
MCHC	Pearson								3
	Correlation								1
	Sig. (2-tailed)								
	N								
MCH									
MCV									

**. Correlation is significant at the 0.05 level (2-tailed).*

***. Correlation is significant at the 0.01 level (2-tailed).*

PCV = Packed Cell Volume, Hb= Haemoglobin, PP= Plasma Protein, RBC = Red Blood Cell, WBC= White Blood Cell, MCHC = Mean Corpuscular Haemoglobin Concentration, MCH = Mean Corpuscular Haemoglobin, MCV = Mean Corpuscular Volume.

For T3, there was no correlation between RBC and the other blood parameters. There was also no correlation amongst WBC ,PP ,HB ,PCV and any other blood parameter. MCHC was positively related to MCV (r=0.55, p<0.05) and MCV is also positively related to MCHC(r=0.55, p<0.05).

Table5. *Pearson's Correlation matrix for the haematological profile of Clarias gariepinus fed 20g (T3) Ocimum gratissimum*

Correlations									
Treatment		RBC	WBC	PP	HB	PCV	MCHC	MCH	MCV
RBC	Pearson	1	.985*	.346	.222	.756	.348	.429	.451
	Correlation		.110	.775	.857	.454	.774	.718	.702
	Sig. (2-tailed)		3	3	3	3	3	3	3
WBC	N		1	.502	.386	.633	.813	.577	.597
	Pearson			.665	.748	.564	.883	.608	.593
	Correlation			3	3	3	3	3	3
PP	Sig. (2-tailed)			1	.992	.352	.759	.996	.994
	N				.082	.771	.451	.057	.072
	Pearson				3	3	3	3	3
HB	Correlation				1	-470	-.837	.976	.971
	Sig. (2-tailed)					.688	.369	.140	.155
PCV	N					3	3	3	3
	Pearson					1	.877	.267	.244
	Correlation						.319	.828	.843
MCHC	Sig. (2-tailed)						3	3	3
	N						1	-.698	.680
	Pearson							.509	.524
	Correlation							3	3
MCH	Sig. (2-tailed)							1	1.000*
	N								.015
	Pearson								3
MCV	Correlation								1
	Sig. (2-tailed)								
	N								

**. Correlation is significant at the 0.05 level (2-tailed).*

**. Correlation is significant at the 0.01 level (2-tailed).*

PCV = Packed Cell Volume, Hb= Haemoglobin, PP= Plasma Protein, RBC = Red Blood Cell, WBC= White Blood Cell, MCHC = Mean Corpuscular Haemoglobin Concentration, MCH = Mean Corpuscular Haemoglobin, MCV = Mean Corpuscular Volume.

For T4, all blood parameters were not positively related to each other except for PP and MCV that were positively related to each other(r=0.55, p<0.05) but was not significantly related to the other blood parameters (Table 6)

Table6. *Pearson's Correlation matrix for the haematological profile of Clarias gariepinus fed 30g(T4) Ocimum gratissimum*

Correlations									
TREATMENT		RBC	WBC	PP	HB	PCV	MCHC	MCH	MCV
RBC	Pearson	1	.910	.961	.661	.795	-.238	.950	.969
	Correlation		.272	.179	.540	.415	.847	.203	.156
	Sig. (2-tailed)		3	3	3	3	3	3	3
	N		1	.989	.290	.472	.186	.734	.984
WBC	Pearson			.093	.812	.687	.881	.475	.114
	Correlation			3	3	3	3	3	3
	Sig. (2-tailed)			1	.472	.596	.040	.826	.999*
	N				.719	.593	.974	.382	.021
PP	Pearson				3	3	3	3	3
	Correlation				1	.981	-.886	.863	.457
	Sig. (2-tailed)					.126	.307	.337	.698
	N					3	3	3	3
HB	Pearson					1	.778	.945	.622
	Correlation						.432	.212	.572
	Sig. (2-tailed)						3	3	3
	N						1	-.531	.007
PCV	Pearson							.644	.995
	Correlation							3	3
	Sig. (2-tailed)							1	.844
	N								.361
MCHC	Pearson								3
	Correlation								1
	Sig. (2-tailed)								
	N								
MCH									
MCV									

**. Correlation is significant at the 0.05 level (2-tailed).*

***. Correlation is significant at the 0.01 level (2-tailed).*

PCV = Packed Cell Volume, Hb= Haemoglobin, PP= Plasma Protein, RBC = Red Blood Cell, WBC= White Blood Cell, MCHC=Mean Corpuscular Haemoglobin Concentration, MCH = Mean Corpuscular Haemoglobin, MCV = Mean Corpuscular Volume.

For T5, Table 7, RBC had a positive relationship with PCV. WBC had a positive correlation with MCH. HB is positively correlated with PCV. PCV is positively correlated with RBC and HB. MCH is positively correlated with WBC. All correlations are significantly related at($r=0.55, p<0.05$) .

Table7. *Pearson's Correlation matrix for the haematological profile of Clarias gariepinus fed 40g(T5) Ocimum gratissimum*

Correlations									
TREATMENT		RBC	WBC	PP	HB	PCV	MCHC	MCH	MCV
RBC	Pearson	1	.948	.908	.993	.999	-.355	.958	.693
	Correlation		.207	.275	.076	.033	.769	.185	.512
	Sig. (2-tailed)		3	3	3	3	3	3	3
	N		1	.726	.903	.929	.038	.999*	.887
WBC	Pearson			.483	.283	.241	.976	.023	.305
	Correlation			3	3	3	3	3	3
	Sig. (2-tailed)			1	.951	.929	.715	.750	.327
	N				.199	.242	.493	.460	.788
PP	Pearson				3	3	3	3	3
	Correlation				1	-998	-.464	.917	.603
	Sig. (2-tailed)					.043	.693	.261	.588
	N					3	3	3	3
HB	Pearson					1	-.404	.942	.655
	Correlation						.735	.218	.546
	Sig. (2-tailed)						3	3	3
	N						1	-.073	.427

PCV	Pearson							.953	.719
	Correlation							3	3
	Sig. (2-tailed)							1	.870
	N								.328
MCHC	Pearson								3
	Correlation								1
	Sig. (2-tailed)								
	N								
MCH									
MCV									

**. Correlation is significant at the 0.05 level (2-tailed).*

**. Correlation is significant at the 0.01 level (2-tailed).*

PCV = Packed Cell Volume, Hb= Haemoglobin, PP= Plasma Protein, RBC = Red Blood Cell, WBC= White Blood Cell, MCHC = Mean Corpuscular Haemoglobin Concentration, MCH = Mean Corpuscular Haemoglobin, MCV = Mean Corpuscular Volume.

4. DISCUSSION

There is a high value for RBC in treatments T2 to T5 when compared with T1(0g). This result is supported by Sahu *et al.* (2007) who reported that Red Blood Cells count were higher in *Labeo rohita* fingerlings fed *Magnifera indica* kernel and they postulated that this increase is an indication of enhanced cellular immunity. This increase in Red Blood Cells values in the treated groups, may be because of the possible mechanism by which the *Ocimum gratissimum* extract may have triggered erythropoiesis and also decrease the rate of oxidant induced haemolysis, due to the presence of the antioxidants present in the plant extract as reported by Sheeja *et al.* (2006). Contrary to the result of this study, the administration of *O.gratissimum*to rats did not have any significant effect on RBC, PCV, Hb, MCV, MCH and MCHC as reported by Okon *et al*. (2011).

In this study, with the inclusion of *Ocimum gratissimum* to the diet of *C. gariepinus*, there was a significant increase in total White Blood Cell count for T2-T5. Similar results were obtained by Sahu *et al*. (2007) when *L. rohita* was fed with *Magnifera indica* kermel and by Dada and Ikuerowo (2009) who studied the effect of ethanolic extract of *G. cambogia* in catfish, *C. gariepinus* brood stock. Contrary to the reports of Ephraim *et al*. (2000) and Jimoh *et al*. (2008), the White Blood Cell count level significantly increased in the test groups when compared to the control group. On the basis of the present study, the increase neutrophil (White Blood Cell) level reemphasizes its anti-bacterial and anti-fungal properties and justifies the use of the plant by traditional medicine practitioners; considering that neutrophils constitute the first line of defense.

The blood indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC), are particularly important for the diagnosis of anemia in most animals. In this study, the Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) values decreased in all the other treatments (T2-T5) compared with the control. This could be the reason for the high mortality rate observed in T4 (with the highest amount of *Ocimum gratissimum* inclusion of 40g / Kg of feed) and lowest values of MCV and MCH. Mean Corpuscular Haemoglobin Concentration (MCHC) maintained a steady value in all experimental groups. The maintenance of constant level or increase of Mean Corpuscular Haemoglobin Concentration (MCHC) in *Clarias gariepinus* fed *Ocimum gratissimum* as feed additive, may bring an improvement in fish health as suggested by Suresh and Amoikumar (2009).

Obaji *et al.* (2005) had reported a generalized anti-haematinic effect of *O. gratissimum* and cautioned on the adverse consequences of its prolonged use. It is possible that *O. gratissimum* could induce haemolysis and can even suppress haemopoiesis because of its phytochemical constituent, such as saponin. However the detaiedl specific mechanism of action is not clear. Nevertheless, it appears that the anti-haematinic effect of saponins to some extent could be cell linage selective. There is also a possibility that other constituents of *O. gratissimum* could stimulate the activity of some haemopoietic growth factors while inhibiting others. It is therefore possible that some of the active ingredients in *O. gratissimum* could promote the action of growth factors related to neutrophil production, while at the same time inhibiting growth factors associated with platelets and lymphocytes production.

5. Conclusion

In conclusion, the optimum level of inclusion of *Ocimum gratissimum* to *C. gariepinus* is 10g/kg of diet. The haematological profile of C. *gariepinus* fed 10g/kg inclusion of *Ocimum gratissimum* performed better with an increase in RBC, WBC, PP and MCHC. Therefore, it can be concluded that an inclusion of 10g/kg of *Ocimum gratissimum* in the diet of *Clarias gariepinus* will yield optimum results for better immunity, growth and survival under culture conditions.

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Authors' Biography

EKELEMU Jerimoth Kesena, holds a B.Sc , M.Sc and Ph.D degree in Fish Biology, Hydrobiology and Fisheries/Limnology respectively. He is currently an Associate Professor of Fisheries/Limnology in the Department of Fisheries/Aquaculture Technology in Delta State University, Abraka Nigeria.

MARROH Eseoghene, holds a B. Fisheries degree from Delta State University, Abraka Nigeria. She is currently pursuing a Master of Science degree in fisheries from the same University.