GENETIC CHARACTERIZATION AND EARLY LIFE CULTURE PERFORMANCE EVALUATION OF FOUR GHANAIAN POPULATIONS OF *Tilapia guineensis* (PISCES: CICHLIDAE)

This thesis is submitted to the University of Ghana, Legon, in partial fulfillment of the requirements for the award of Master of Philosophy degree in Fisheries Science

By

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DEDICATION

To my mother **Mrs. Florence Agyakwah** for appreciating my zeal for higher education.

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DECLARATION

I, Seth Koranteng Agyakwah, do hereby declare that this thesis consists entirely of my own work done under supervision and that no part of it has been previously published or submitted for a degree or diploma elsewhere.

.....26/01/2005.....

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ABSTRACT

Genetic characteristics of four populations of the cichlid *Tilapia guineensis* were studied using allozyme (starch gel) electrophoresis and early life culture performance characterization to generate information on genetic structure and characteristics of major culture performance traits during early life of the species. Samples of the fish populations were collected from three freshwater reservoirs: (i) the Volta reservoir at Kpong, (ii) the Okye-Aminsa reservoir at Akotogua and (iii) the Ayensu reservoir at Okyereko; and a coastal open lagoon of the Volta system at Aglorkpovie. Observed heterozygosity (Ho) and polymorphism (P) of allozymes as indices of genetic diversity were relatively low in Akotogua (Ho = 0.077; P = 0.077) and Aglorkpovie (Ho = 0.077; P = 0.077) populations compared to those of Kpong (Ho = 0.308; P = 0.089) and Okyereko (Ho = 0.462; P = 0.087) populations. Early life culture performance characteristics of populations evaluated over 84 days showed significantly higher final mean length, weight gain and condition factor in Akotogua populations compared to growth characteristics of Aglorkpovie and Kpong populations. Results suggest that heterozygosity and polymorphism might be important in determining culture performance traits. Additionally, high genetic diversity did not confer high culture performance characteristics on the early life of the fish. The biochemical genetic structure and growth characteristics observed in Akotogua population identify it as a population with a potentially better early life culture performance that could be used by culturists for improvement of *T. guineensis* in aquaculture.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

Ghana is endowed with rich natural marine and freshwater fishery resources (FAO, 1990; Danquah *et al,* 1999). These resources have contributed significantly to national fish consumption which constituted between 60 % and 70 % of total animal protein intake, thus making fish the most important source of animal proteins to Ghanaians (Owusu *et al,* 2001). There is however a growing concern that fisheries resources of Ghana are being exploited beyond sustainable limits with particular reference to local food species (Blay and Asabere-Ameyaw, 1993; Koranteng *et al,* 1998; Danquah *et al,* 1999). A consequence of the unsustainable exploitation level has been reduction in fish availability, which has resulted in decline of per capita fish consumption from about 29.3 kg in the 1970s to about 22.0 kg in the 1990s (Owusu *et al,* 2001). The low fish supply together with increasing population size (currently growing at a rate of 3 % per annum) is expected to further reduce fish availability in the years ahead, if local production is not augmented.

Currently, it is a worldwide consideration, as it is in Ghana, that fish culture and culture based fisheries practices have more certainty to increase fish production compared to management of wild stocks (FAO, 1995). Among resources of Ghana that have potential to make fish culture and culture based fisheries a viable industry and in the process enhance livelihoods and income earnings of farmers is the Tilapia group of fishes (family Cichlidae). The tilapias of Ghana include species of three genera namely *Oreochromis, Sarotherodon* and *Tilapia.*

All the tilapias of Ghana, as elsewhere in Africa, serve as important food fishes, some being of great commercial importance (Lazard and Legendre, 1996; Ruddle, 1996; Diallo, 1997; Van der Bank, 1997). For fish culture, the tilapias are also the fishes of primary importance in Africa. Coward and Bromage (2000) attributed the success of tilapias in aquaculture to several inherent characters of the fish including adaptation to wide ranges of salinity and oxygen tension, relatively short reproductive cycles, ability to reproduce under culture conditions, resistant to diseases and amenability to handling.

Of the three genera, *Oreochromis* species, especially *O. niloticus* has been used extensively in freshwater aquaculture (Pullin, 1991; Coward and Bromage, 2000). The potential of other tilapias for saline water (e.g. lagoon and estuary) aquaculture development has been proposed by many authors including Payne (1983), Campbel (1987), Gilles (1995), Wokoma and Marioghae (1996) and Diallo (1997). In Ghana, two tilapias that have received some attention for saline water fish culture related development have been *T. guineensis* and *S. melanotheron* (Ofori-Danson *et al* 1993).

Justification for development of *S. melanotheron* and *T. guineensis* in Ghana for aquaculture include the situation that the two species are indigenous to all national river basins in the country while the single indigenous *Oreochromis* species of the country, *O. niloticus* is restricted naturally to the Volta basin (Ofori, 1988). Broadly therefore, the development of the two national widely distributed species *(X guineensis* and *S. melanotheron)* would be expected to limit the uses of *O. niloticus* in several basins in especially rural aquaculture, so as to reduce genetic implications of their (*O. niloticus)* translocation, thus the necessity to obtain genetic information on the species and their populations.

THE ISSUE

Fish culture and culture-based fisheries are accepted potential approaches to increase fish production in Ghana. Major food fishes, which have shown potential for use in culture fisheries, including tilapias, especially the Nile tilapia *{Oreochromis niloticus*), mango tilapia (*Sarotherodon galilaeus),* black-chinned tilapia *(S. melanotherori)* and the red-chinned tilapia (*Tilapia guineensis)*. Among the fishes indicated *T. guineensis* (Plate A) has least been studied to facilitate its use in culture fisheries. However, the fish is widely distributed in West Africa including Ghana (Philipart and Ruwet, 1982; Oberst *et al,* 1996). The present work is aimed at generating information on the genetic structure and characteristics of major culture performance traits such as spawn frequency, growth rate, condition factor and survival during early life of some populations of *T. guineensis,* which could be used by culturist while providing the beginning of culture evaluation of identifiable populations of the fish in Ghana.

1.1 ALLOZYME ELECTROPHORESIS

Electrophoresis, refers to migration of proteins in a buffered medium under the influence of an electric field. Allozymes are different forms of enzymes (proteins), which are the products of alternate alleles segregating at a locus of a gene within a species. The systematic value of allozyme electrophoresis is therefore achieved from protein mobilities, which are used as indicators of similarity (or dissimilarity) when comparing orthologous proteins (equivalent proteins in different taxa) from different individuals (Ferguson, 1980).

Application of allozyme electrophoresis using starch gel as medium, became a common biochemical approach used in characterization and identification of several groups of organisms including fish and more specifically, tilapiine species natural populations and aquaculture strains

in the 1980s (Abban, 1988; Me Andrew and Majumdar, 1983; Seyoum, 1990; Macaranas *et al,* 1995; Falk *et al,* 1996; Oberst *et al,* 1996; Quarcoopome, 2000). Its use in species characterization reflects two basic advantages. First, the technique involves the use of protein characters (first product of the gene) that can be used for delimitation of species and populations. Secondly, degrees of phenetic relationship can be determined and phylogenetic relationship inferred.

According to Richardson *et al* (1986), the technique is most useful in providing information on levels and distribution of genetic variability in relation to mating patterns, life history, population structure, migration and environment. For instance, in a pedigree determination and analysis of captive strains of brood stocks of Domsea coho salmon *(Oncorhynchus kisutch),* Myers *et al* (2001) demonstrated the potential of allozyme starch gel electrophoresis in revealing loss of or changes in genetic variability due to inbreeding depression in the brood stocks, though there was insignificant phenotypic expression of such. Desvigne *et al* (2001) and Dufresne *et al* (2002) postulated that, an important characteristic of allozymes in population genetics is their selectivity role in habitat differentiation. This postulation makes allozyme electrophoresis an important biotechnological approach in assessing levels of allelic variations in relation to selection. Particularly on tilapias, Naish and Skibinski (1997) noted that the use of allozyme electrophoresis yields acceptable results on phylogenetic studies, population genetic studies and studies on hybridized species.

The technique has gained widespread usage as an accepted standard biotechnology method in population genetic studies, due to the facts that it is reproducible, fairly rapid, requires few specialized equipments, allows for quick simultaneous screening of many samples involving many structural loci, provides acceptable precise estimates of genetic variables, is inexpensive and

reduces labor cost (Park and Moran, 1994; Ward and Grewe, 1994). Therefore the use of starch gel electrophoresis in the present study is scientifically credible for the genetic characterization study of *T. guineensis* populations.

1.2 CHARACTERIZATION OF *T. guineensis* (SLEEKER, 1862)

Early classification placed most tilapiine fishes into a single genus, *Tilapia* with several species based on morphological characteristics (Trewavas, 1966). However, subsequent studies subdivided species groups belonging to the original *Tilapia* genus mainly based upon differences in their mode of reproduction into three new genera i.e. *Oreochromis, Sarotherodon* and *Tilapia* (Trewavas, 1982). Those species that had evolved as substrate spawners or spawn guarders were retained in the *Tilapia* genus, while species rearing their young orally by females or by both sexes were put into the genera *Oreochromis* and *Sarotherodon* respectively (Trewavas, 1983).

According to Danquah *et al* (1999), six *Tilapia* species are known to occur within Ghanaian inland waters. These include, *T. guineensis* (Bleeker, 1862), *T. busumana* (Gunther, 1903), *T. discolor* (Gunther, 1903), *T. dageti* (Thys van den Audemaerde, 1971), *T. zillii* (Gervais, 1848) and *T. mariae* (Boulenger, 1899). Species of *Tilapia* however, are known to show striking similarities and considerable overlap of morphometric, morphological and meristic characteristics (Trewavas, 1983; Tuegels and Thys van den Audenaerde, 1992). For example, *T. guineensis* and *T. zillii* often naturally found to be sympatric are closely related meristically (i.e. both having 14 - 15 dorsal fin spines; 10-14 dorsal soft rays for *T. zillii* whiles *T. guineensis* has 12-13) (Danquah *et al,* 1999).

Although *T. guineensis* could be distinguished from *T. zillii* by having base of scales on flanks with dark line, and lower half of caudal fin having yellowish blue blotches, discrimination of the two species undoubtedly poses some problems and therefore renders species discrimination using morphometry, morphology or meristic approach ambiguous. Therefore starch gel electrophoresis with long standing scientific credibility in providing unambiguous results for species discrimination was adopted in the present study.

I guineensis (Plate A) is thus classified after Tuegels and Thys van den Audenaerde (1992) as follows:

PHYLUM: Vertebrata

SUBPHYLUM: Craniata

SUPERCLASS: Gnathostoma

SERIES: Pisces

CLASS: Teleostomi

SUBORDER: Percoidae

FAMILY: Cichlidae

GENUS: *Tilapia*

SPECIES: *Tilapia guineensis*

Plate A: Specimen of a seventy-five gram *Tilapiaguineensis*

Scale: 1:1

3 HYPOTHESIS AND GENERAL OBJECTIVES

The hypothesis leading to the current work on genetic characterization is that since by locality, different stocks of the same species could vary in their genetic structure (e.g. variations in allelic frequency or heterozygosity) and growth characteristics (e.g. condition factor, growth rate), stocks of *T. guineensis* in Ghana are likely to show differences in genetic structures and culture performance characteristics.

The general objective of the genetic structure study was to describe and document the biochemical genetic structure (e.g. level of heterozygosity and polymorphism) of four locally distinguishable *T. guineensis* stocks in Ghana. The Data generated will provide some information on genetic resources available in the species, which could contribute to scientific knowledge of the species

and be useful for conservation management programs. Results of culture evaluation of the specimens studied will also be a characterization output, which could be taken advantage of to enhance culture based fisheries programs involving the fish. For instance, such results could be useful in identifying brood stock populations for culture projects.

CHAPTER TWO

2.0 STUDY AREA, MATERIALS AND METHODS

2.1 Study Area

The present study was conducted at Akosombo (latitude 6° 13' N and longitude 0° 4' E) in Ghana, at the premises of the Aquaculture Research and Development Center (ARDEC) of Water Research Institute (WRI) of the Council for Scientific and Industrial Research (CSIR). Biochemical discriminatory study (electrophoresis analysis) and culture experiments were conducted in the Center's laboratories and out - door concrete tanks respectively, from October 2002 to July 2003.

2.1.1 Fish Sampling Stations

Population representatives of *Tilapia guineensis* studied were obtained from locations within three inland water systems of Ghana namely, Ayensu, Okye and Volta (Fig. 1). For the electrophoresis study, fresh dead specimen were labeled and bagged on wet ice for transport to laboratory for later study. Live samples (for the generation of progeny for early life culture performance evaluation) were transported in water - filled transparent polythene bags infused with oxygen. Fish from the Volta system were sampled from two environments; one from Kpong reservoir (freshwater) and the other from Aglor lagoon (a coastal open lagoon of the Volta system) at Aglorkpovie. The Kpong reservoir of the Volta system lies between two dams, one at Akosombo and the other at Akuse. Before the construction of the two dams, the Volta River was contiguous with Aglor lagoon over a stretch of about 100 kilometers. The Ayensu river system was sampled in a reservoir behind a dam at Okyereko, while samples of fish were collected from a dammed reservoir of Okye River at Akotogua.

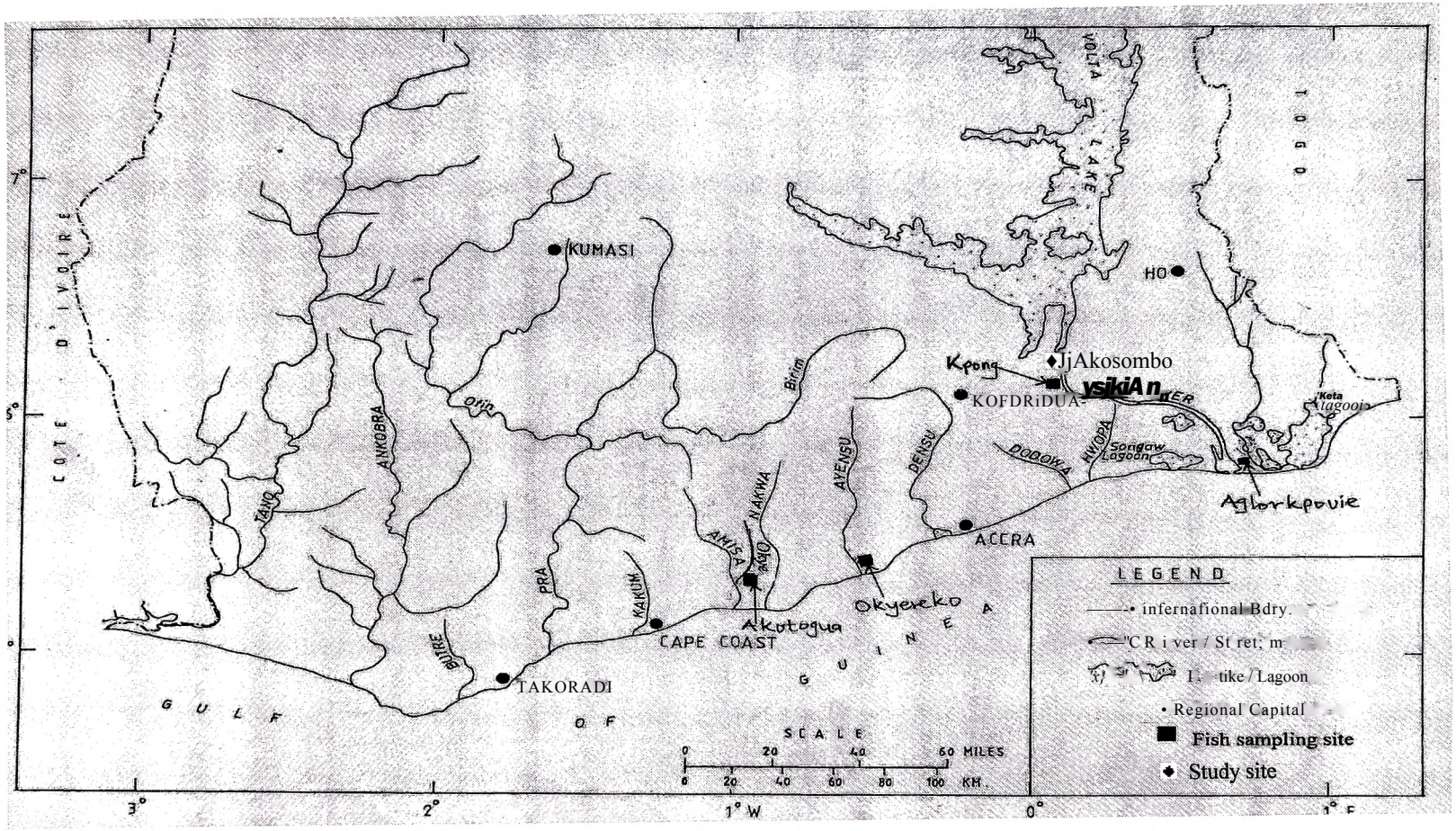


Figure 1: Drainage map of southern Ghana and locations of fish sampling sites

1.1.1.1 Environment of Volta Reservoir at Kpong

The Kpong reservoir (0 ^{0} 4' E, 6^{0} 7' N) lies between two dams (Akosombo dam at Akosombo and Kpong dam at Akuse), on the Volta River. Ranges of mean monthly physico-chemical characteristics of the reservoir (GWC, 1996 to 2000 report) are summarized in Table 1.

2.1.1.2 Environment of Aglor lagoon

Aglorkpovie lagoon is located in the marshy flood plains of the Volta estuary. The lagoon has a mean depth of 1.7 m and covers a total surface area of 10 km^{2} (Ghana Survey Dept.). It lies on longitude 0 ^{0} 44' E and latitude 5^{0} 53' N, westward of Keta lagoon. A small stream, Kpenu, feeds the lagoon with freshwater, while seawater influences it at high tides through the Keta lagoon complex. A survey of the physico-chemical parameters by Water Research Institute, between April and November 2002 are presented in Table 1.

2.1.1.3 Environment of Ayensu River at Okyereko

The Okyereko reservoir on Ayensu River lies on longitude 0^{0} 33' W and latitude 5 ^{0} 25' N. The reservoir is used for fishing and irrigating farmed crops within its catchment area. Ranges of mean monthly physico - chemical characteristics of Ayensu Basin, studied between 1995 and 1996 (Nana-Amankwaah and Paintsil, 1996) are summarized in Table 1.

2.1.1.4 Environment of Okye Reservoir at Akotogua

The Akotogua reservoir (1° W, 5° 11' N) was established on a tributary of Okye River in 1970. The reservoir has a mean total water volume of 5.6 million m^{3} and used purposely for irrigating farmed crops within a 250 ha. irrigation project scheme (MAEDA, 2003).

Table 1. Ranges of mean monthly Physico - chemical characteristics of four water bodies used as sources of *T. guineensis* where samples were collected

Parameter	Water Body			
	Volta Reservoir (GWC, 1996 to 2000 report)	Aglor Lagoon (WRI, 2002)	Ayensu River (Nana-Amankwaah and Paintsil, 1996)	Okye Reservoir (NA*)
PH	6.9-7.13	7.9-8.5	7.7-7.8	NA
Temperature (°C)	NA	27.0-29.0	25.0-29.0	NA
Turbidity (NTU)	NA	NA	3.0-75.0	NA
D 0 (mg/1)	5.3-7.3	6.5-8.5	2.1-8.7	NA
BOD_s (mg/1)	NA	NA	0.8-2.6	NA
Total Alkalinity (mg/1)	35.5-46.9	NA	56.0-86.0	NA
Total Hardness (mg/1)	NA	NA	49.8 - 83.4	NA
TDS (mg/1)	NA	NA	93.4-228.0	NA
Phosphates (mg/I)	NA	0.01-0.025	<0.01-0.22	NA
Sulphates (mg/1)	NA	NA	1.0-17.0	NA
Nitrates (mg/1)	0.1-0.66	<0.01	<0.03	NA
Nitrite (mg/1)	NA	<0.001	NA	NA
Ammonia (mg/1)	0.00-0.4	0.24-0.57	NA	NA
Salinity (ppt.)	0.00	3.0-5.0	NA	NA
Conductivity (|iS / cm)	72-78	NA	NA	NA

NA* = Data or source of data not available

2.2 Materials and Methods

For biochemical genetic characterization study the approach used was starch gel electrophoresis. For evaluation of early life culture performance characteristics, spawn frequencies (number of spawning over a period), spawn sizes (number of fry per spawn), means of daily weight gain (in grams) and specific rates of populations studied were compared.

.2.1 Procedure for Biochemical Genetic Characterization

To assess the population genetic structure of four populations of *T. guineensis,* nine (9) soluble proteins (enzymes) (Table 2) were screened by electrophoresis from white muscle tissue extracts of 72 specimens. This involved the following main stages as outlined in Falk *et al,* (1996):

1.2.1.1 Sample Preparation

Between 1 - 2 g of white muscle was cut beneath the skin of upper flank of fish specimens. An equal amount of ice-cold distilled water was added to it in a test tube and homogenized by a mechanical macerator (ULTRA-TURRAX T25) into paste. The resulting paste was centrifuged at 8 000 x g and 4 °C for 30 minutes, after which the supernatant was poured into labeled 1.5 ml Eppendorf tubes and kept frozen for later use.

2.2.1.2 Potato Starch Gel Preparation

A 12.5 % buffered potato starch gel was cast in a Perspex gel mould (former) set on a glass plate. The gel was prepared by mixing thoroughly appropriate amount of hydrolyzed potato starch in a Buckner flask with continuous tris-citrate (CTC) buffer solution (Appendix LA). The mixture was then heated over a naked Bunsen flame with constant gentle swirling of the flask by hand. Heating was stopped after a first big bubble was observed and the content of the flask immediately degassed and poured into the gel mould and covered with a second glass plate. This was allowed to set at room temperature for three to four hours and then transferred into a refrigerator overnight.

2.2.1.3 Gel Loading and Running

A well-set gel after refrigeration was cut into two unequal parts using a scalpel. The smaller part (5 - 6 cm) was separated from the bigger part to allow for loading sample on inner surface of the

bigger gel. Sample strips made of 3 mm x 10 mm Whatman chromatography paper, soaked in the soluble protein extract of the fish muscle and blotted of excess liquid before being carefully arranged on inner surface of the bigger gel. On each loading event, samples of specimens from all four populations were represented on the same gel to allow for direct comparison of their allelic mobilities.

After loading of the gel, the smaller gel portion was pushed into place, after which the loaded gel was replaced in the mould as before and spacers were inserted between the smaller gel portion and the mould to ensure firm contact between the two gel parts. The loaded gel was then mounted on two joined buffer tanks, each containing electrode buffer. The ends of the two gel portions were connected to the electrode buffer using absorbing electrode wicks. In preparation to run the gel, the top of the gel was covered, first, with a transparent plastic sheet and then a glass plate. The assembled unit was placed in a refrigerator and connected to a power supply unit, by means of two platinum electrodes (ends of smaller and bigger gels connected to cathode and anode respectively). By setting a constant current at 50 mA with alternating voltage (140 -190 V), gels were run for 8 hours each.

2.2.1.4 Slicing, Staining and Scoring of Gels

After an electrophoresis run, the bigger gel portion was sliced into 2 mm thick slices after a snip at an edge was cut to indicate orientation and position of specimens on it. Each slice was put into a labeled staining tray according to the particular enzyme to be stained for. An appropriate staining mixture (enzyme substrate cum dye, Appendix IB) was prepared, stirred thoroughly with magnetic stirrer and poured onto the sliced gel for incubation at room temperature for 5-20 minutes. On appearance of protein bands (colored insoluble salts), gels were immediately washed with tap

water, followed by distilled water, after which bands were fixed by addition of fixing solution (Appendix IC) to prevent diffusion of the bands.

Summary of Staining Mechanism:

Enzyme + Substrate -> Colorless Product + Dye	—>	Colored Product
(soluble in sample)	(soluble in reagent)	(insoluble precipitate) (in reagent)		(insoluble precipitate)

After staining, photographs of the gels were taken with a 35 mm lens camera, scored graphically for each population on ruled sheets and the data analyzed.

2.2.1.5 Treatment of Electrophoretic Data

Electrophoretic data was recorded to represent relative positions of alleles (locus) from different individuals and populations. Characteristics used to estimate genetic variability within and between populations were mean number of alleles per locus, total number of alleles per population, mean number of alleles per population, effective number of alleles per population, heterozygosity and Hardy - Weinberg estimates, proportion of polymorphic loci, genetic identity and genetic distance. An individual was considered as either a homozygote or a heterozygote at a locus depending on whether it indicated one allele (one type of polypeptide from identical pair of alleles, i.e. a homozygote state), or different alleles at a locus (two types of polypeptides from different pairs of alleles, i.e. a heterozygote state).

Table 2: List of subunit structure and enzyme coding numbers of **9** enzymes screened in four **(4)** populations of *T. guineensis* (after Falk *et al,* **1996)**

Enzyme	Enzyme abbreviation	Enzyme Coding number	Subunit structure
Alcohol dehydrogenase	ADH	1.1.1.1	Monomeric
Isocitrate dehydrogenase	IDH	1.1.1.42	Monomeric
L-Lactose dehydrogenase	LDH	1.1.1.27	Dimeric
Malate dehydrogenase	MDH	1.1.1.37	Dimeric
Octanol dehydrogenase	ODH	1.1.1.73	Monomeric
Phosphoglucomutase	PGM	5.4.2.2	Monomeric
Phosphoglucose isomerase	PGI	5.3.1.9	Dimeric
Sorbitol dehydrogenase	SDH	1.1.1.14	Monomeric
Xanthine dehydrogenase	XDH	1.2.3.2	Monomeric

2.2.1.6 Estimation of Parameters

2.2.1.6.1 Allelic frequency

Allelic frequencies (F) were determined by the formula:

$$F = (2H + He)/2N \text{ (Ferguson, 1980)}$$

where, H = number of homozygotes for that allele

He = number of heterozygotes for that allele

N = number of individuals examined.

2.2.1.6.2 **Effective Number of Alleles** (Ef)

Effective number of alleles (Ef) per population represents the expected number of alleles for each locus. It was estimated as,

$$E_f = 1/S(X0^2 \text{ (Ferguson, 1980)}$$

where, *X,* = frequency of the ith allele at a locus.

2.2.1.6.3 Polymorphism per Locus

Proportion of polymorphic loci was assessed based on frequency of the most common allele. It may be estimated at various levels (e.g. 1 %, 5 % etc). Owing to low sample sizes (less than 30) of populations, both 1 % and 5 % frequency levels were estimated in the present study. At the 1 % level, the most common allele with frequency less than 0.99 (P < 99 %) was considered polymorphic at each locus, whiles at the 5 % level, the most common allele with frequency less

than 0.95 (P < 95 %) was considered polymorphic at each locus. Mean polymorphism per locus (P) was then estimated for each population as:

P = No. of polymorphic loci / No. of loci scored (Ferguson, 1980)

2.2.1.6.4 Heterozygosity and Hardy - Weinberg Distribution

Heterozygosity refers to mean frequency of heterozygotes per locus, or mean frequency of heterozygotes over all loci per individual. Heterozygosity per locus was expressed as an observed value (Ho), computed directly from observed genotype frequency (as a fraction of heterozygote individuals) or as an expected value (H_e), estimated as:

$$He = 1 - 2 (Xj)^2$$

where, Xi = the frequency of the ith allele at a locus.

Mean heterozygosity per locus (H_e) was estimated as

$$2 (H_{ei}) / L_T \text{ (Ferguson, 1980)}$$

where, H_ei = heterozygosity over all loci,

L_T = total number of loci screened

Estimated standard error about the mean heterozygosity per locus (ft_e) was derived from:

$$V [I(He-He)^2/L_T(L_T- 1)].$$

Expected Genotype frequencies were calculated from allelic frequency values using Hardy - Weinberg equation. By representing allelic frequencies as 'p' and 'q' for two alleles randomly segregating in a population, the expected genotype frequencies from the Hardy - Weinberg distribution have the ratios, p^2, 2pq, and q^2. The difference between the expected and observed genotype frequencies was tested for statistical significance using log likelihood N^2 test (G-test) for goodness of fit (Appendix **II),** such that;

$$G = 2 \, S \, \mathbf{H_0} \, \text{In} \, (\mathbf{H_0}/\mathbf{H_e}) \text{ (Sprent, 1990).}$$

where In = natural logarithm.

Number of degrees of freedom was determined as,

$$*/_2(n^2 - n) \text{ (Ferguson, 1980).}$$

where, n = number of alleles

2.2.1.7 Analyses of Molecular Variance (AMOVA)

The differences in allele frequencies of the populations studied were tested statistically by Kruskall-Wallis non-parametric test and Dunn's multiple comparisons test (Appendix IIIA). Genetic differences within and between populations were evaluated by analyzing molecular variance (AMOVA), using Wright's fixation index (F-statistic) by the formula:

$$F = 1 - Z^{n}_{(j} (H_0 \, y \, / \, H_e \, jj) \text{ (Weir and Cockerham, 1984).}$$

where H_0 y and H_e ij are respectively, the means of observed and expected heterozygosities at all loci. F - statistic indicates deviation (excesses or deficiencies of heterozygosities) from the Hardy - Weinberg expectation. Two parameters of F (i.e. FST and Fjs) were considered.

FST represents component of genetic variance among populations. It was calculated to measure the proportion of total variation that could be ascribed to differences between population allele frequencies. FST values were computed for all populations (denoted by FCT) and between pairs of populations (FST)- FB represents component of genetic variance within a population. It is also termed as 'inbreeding coefficient' since it gives an indication of loss of alleles resulting in heterozygote deficiencies. Values were calculated to determine the extent of heterozygosity (i.e. heterozygote deficiency or excess) within populations. Both components (FST and FB) were tested for significant differences between populations using Kruskall-Wallis non-parametric ANOVA test, and Dunn's non-parametric multiple comparison tests (GraphPad Software, 1993) (Appendices IIIB and IIIC).

2.2.1.8 Genetic Relationships Among Studied Populations

2.2.1.8.1 Genetic Identity (I)

Coefficient of genetic identity (I) between pairs of populations at a locus was estimated using the formula of Nei (1972) which estimates I as:

$$I = (\pounds X_i Y_i)/V(\pounds X_i^2 2;\ Y;^2)$$

where, *X,* and Yj are the frequencies of the ith allele in populations X and Y respectively. Values of genetic identity always lie within a range of 0.0 and 1.0. Genetic identity value of 1.0 indicates

absolute similarity between two taxa, whereas an identity value of 0.0 represents absolute dissimilarity. Mean genetic identity (1) over all loci studied was determined as:

$$I = I_{xy} / V(i_x i_y)$$

where, I_{xy}, I_x and I_y are the arithmetic mean over all loci of £X,Y_b SX;^{2} and SY,^{2} respectively. Mean genetic identities of paired populations were collated into a matrix, from which dendrograms were constructed for graphical representation of phenetic relationships among the studied populations.

2.2.1.8.2 Genetic Distance (D)

Genetic distance between two populations was determined from genetic identity (I), such that;

$$D = -\ln I \qquad \text{Nei(1972)}$$

where, In = natural logarithm.

Values of genetic distance between pairs of populations were also collated into a matrix.

2.2.1.8.3 Construction of Dendrograms

Construction of dendrograms to present estimated relationship among populations followed stepwise procedure described by Ferguson (1980), by agglomerative clustering of un-weighted pair group arithmetic average (UPGAA) (Sneath and Sokal, 1973), using the matrix of genetic identity.

To begin with the construction of dendrograms, population within the matrix were considered as operational taxonomic units (OTU). Two OTUs with the highest identity values were first selected and clustered. The two were then combined as a single OTU (as OTU 1/ 2 for OTU 1 and OTU 2) and then a new matrix of values was calculated. The similarity value of the combined OTU (i.e. OTU 1/ 2) to any other OTU was taken as the mean of that OTU's identity to that of OTU 1 and OTU 2. The whole process of selecting the highest identity value among all else in the new matrix and recalculation of identities into another new matrix was continued until all OTUs were clustered.

2.2.2 Procedure for Early life Culture Evaluation among Populations Studied

2.2.2.1 Monitoring and Evaluation of Reproductive Characteristics

2.2.2.1.1 Inter Spawn Period and Spawn Size among Fish Populations

To evaluate variability in the periods between spawns and spawn size among the populations, male and female fishes (broodstock weights ranged between 70 and 100 grams) from each population were paired in 1 x 1 m^2 fiber netting hapas of pore size 1.4 mm (diagonal), mounted in 3 x 1 m^2 out - door concrete tanks (Plate B). The tanks had been previously filled with tap water. A spawning groove was created within each hapa. The groove was made of 2 concrete blocks, placed 30 cm apart on sand - layered slate. This was created to boost courtship and offer substrate for oviposition, a natural requirement for spawning activities in the genus *Tilapia* (Trewavas, 1983; Campbel, 1987).

Mate B: Experimental culture units (Hapa — in — tank system)

Based on availability of both male and female fishes, number of pairs of fishes was four from the Volta at Kpong and three each from Okye at Akotogua and Volta at Aglorkpovie. Hapas were inspected *every* other day for evidence of mostly yolk - sac fry. Spawns (post larval fry) from population pairs were collected carefully with a scoop net and counted to determine spawn sizes. Fry were measured on a graduated petri - dish for total length and weighed with an electronic balance to nearest 0.0001 g. Periods between two spawns, over a period, for each pair of fish were recorded to estimate frequency of spawning.

2.2.2.1.2 Fry Survival

Due to mortalities, early life survival and growth assessments were evaluated among three of the four populations (i.e. Kpong, Aglorkpovie and Akotogua), while results on inter spawn periods and frequency of spawning were obtained for two populations only (i.e. Kpong and Akotogua).

Fry Stocking

Growth studies on fry of Kpong, Aglorkpovie and Akotogua populations were each conducted in two replicated 3 x 1 x 0.63 m^3 out - door concrete tanks filled with pipe-borne water to three-quarters its full capacity. Initially, fry were stocked at a density of 300 fry per tank (100 fry per m^2; 0.182 fish / litre) per population.

Feed and Feeding

Fry were fed with ARDEC formulated fish feed prepared from agro-industrial by-products. Feed was administered to fry in powdered form at 10 % fry body weight in three rations per day.

2.2.1.2 Growth Monitoring and Evaluation

Fifty (50) fry per population were sampled every two weeks for measurements of total length (to the nearest 1mm) and weight (to the nearest 0.00 lg) to monitor growth characteristics of fry for 12 weeks. Estimates of specific growth rate, mean daily weight gain, growth pattern condition factor and survival were made to assess growth characteristics of the fish populations. All graphs relating to the growth of fish were plotted by using Microsoft Excel Program.

Specific Growth Rate (SGR)

Specific growth rate (SGR) expressed as percentage body weight per day defines the instantaneous rate of weight or length increase over a time period (t) throughout the life of the fish. It reflects the pattern of growth, thus indicating how much flesh is deposited per unit time. SGR (% / day) was calculated by the formula:

$$SGR = 100 (\ln W_2 - \ln W,) / At \quad \text{(Ricker,1975)}$$

where, In W i and In W_2 = natural logarithms of the initial and final weights (in grams) of fish,

At = culture period (in days) for which fish was grown from Wi to achieve W_2.

Mean Daily Weight Gain (MDWG)

MDWG was calculated by the formula:

$$(W_2 - W,) / (W_2 + Wi)\ 0.5\ t \text{ (Ricker, 1975)}$$

where, Wi and W_2 = initial and final weights respectively,

0.5 = constant

t = period in days for growing fish from Wi to W_2

Fish Condition Factor (K)

Condition factor (K) (sometimes expressed as percentage), defines the physiological well being or fitness of fish. It is a function of weight and length such that,

$$K = W / L^b$$

where, W - weight in grams, L = total length in millimeters and b = regression coefficient (slope) of weight / length plots (Ricker, 1975).

Survival of Fish

Survival of fish expressed as a percentage, was determined at the end of the experiment as,

100 x (number of survivors at end of experiment) / (initial stocking size)

2.2.2.3 Water Quality Monitoring

Water quality of tanks was monitored throughout the culture period to ensure suitable environment for the fish. Major parameters monitored were:

- Temperature, measured by Celsius scale thermometer
- pH, measured with Suntex (SP-701) pH meter
- Dissolved oxygen, estimated by titration (Winkler method)
- Nitrite-nitrogen, estimated by diazotization method with Camspec Spectrophotometer
- Nitrate-nitrogen, by hadrazine reduction method with Camspec Spectrophotometer
- Phosphate-phosphorous, by stannous chloride method with Camspec spectrophotometer

2.2.2.4 DataAnalysis

Data collected on both fish and water quality were assembled and fed into a desktop computer from which statistical analysis were performed using GraphPad INSTAT software program (GraphPad Software, 1993). Statistical analyses were done through analysis of variance with Tukey - Kramer multiple range tests when sample sizes were more than 30 observations, else a Student-Newman-Keuls (t-test) multiple comparison test was adopted for sample sizes less than 30 observations (Appendices IVA-F and VA-G). Depending on the extremity of significant differences between populations, levels of significance ranged from 0.05 to 0.0001.

CHAPTER THREE

3.0 RESULTS

3.1 Population Genetic Characterization of *T. guineensis* based on Allozymes

Allozymes

A summary of observed allozyme patterns of the four populations of *T. guineensis* studied at 13 loci involving the nine (9) targeted enzymes is shown in Figure 2 and Plates D, E and F. For taxonomic comparison of the populations, each gene locus was considered as a taxonomic character and genotypes observed in individuals of a population as the range of character expression within the population.

At six of loci screened (Adh-1, Idh-1, Ldh-2, Odh-1, Pgm-1 and Xdh-1), individuals of all populations were fixed for one and the same allele (Plate D and Figure 2). Individuals of Aglorkpovie and Akotogua expressed non-variable characters at all the 13 loci screened (Figure 2). There were no observed private alleles at any of the studied loci for any one population.

3.1.1 Genic Variations at 13 Enzyme Loci in Populations of *T. guineensis*

The number of loci scored for each enzyme in the studied populations is presented in Table 3. The allelic frequencies, effective numbers of alleles, observed and expected heterozygosities at each locus and within a particular population are presented in Table 4, while the estimated levels of polymorphic loci within the studied populations are summarized in Table 5. Mean numbers of polymorphism, heterozygosities and levels of deviation from Hardy-Weinberg expectation within each population are summarized in Table 6.

Table 3: Enzyme systems screened and loci scored in Allozyme Electrophoresis of4 populations of *T. guineensis*

Enzyme	Enzyme abbreviation	Enzyme Coding number	No. of loci screened
Alcohol dehydrogenase	ADH	1.1.1.1	1
Isocitrate dehydrogenase	IDH	1.1.1.42	1
L-Lactose dehydrogenase	LDH	1.1.1.27	2
Malate dehydrogenase	MDH	1.1.1.37	3
Octanol dehydrogenase	ODH	1.1.1.73	1
Phosphoglucomutase	PGM	5.4.2.2	1
Phosphoglucose isomerase	PGI	5.3.1.9	2
Sorbitol dehydrogenase	SDH	1.1.1.14	1
Xanthine dehydrogenase	XDH	1.2.3.2	1
TOTAL			**13**

Alcohol dehydrogenase (ADH)

A single locus (ADH-1) was scored. A single allele was present in all individuals of the four populations studied (Figure 2 and Table 4).

Isocitrate dehydrogenase (IDH)

A single locus (IDH-1) with a single allele was scored for individuals of the studied populations. (Figure 2 and Table 4).

Figure 2: Allozyme patterns of 4 populations of *Tilapia guineensis* at 13 enzyme coding loci

Population

Locus Aglorkpovie Akotogua Kpong Okyereko

ADH-1 ....

IDH-1 ....

LDH-2 ....

LDH-1

MDH-3*

MDH-2

MDH-1

ODH-1

PGI-2*

PGI-1*

PGM-1

SDH-1

XDH-1

: Loci with hybrid bands. Hybrid bands lie in equidistant position between two alleles.

Lactate dehydrogenase (LDH)

Two loci (LDH-1 and LDH-2) were identified in all populations (Plate C). The first locus (LDH-1) revealed two alleles (a and b) for Okyereko population only. The faster allele 'b' was lowly represented with a frequency of 0.067, while the slower allele 'a' had very high occurrence rate of 0.933. The other three (Aglorkpovie, Akotogua and Kpong) populations were all homozygous for the slower allele 'a'. Individuals of all four populations were fixed for one allele at LDH-2 (Figure 2 and Table 4).

Malate dehydrogenase (MDH)

All four populations studied were polymorphic for malate dehydrogenase enzyme (Plate D). Three distinct loci (MDH-1, MDH-2 and MDH-3) were scored in individuals of all populations. Each of the first two loci (MDH-1 and MDH-2) revealed one allele 'a' in individuals of Aglorkpovie Kpong and Akotogua populations. Okyereko population however had a faster allele 'b' in addition to the slow allele 'a' (at frequencies of 0.067 and 0.933 respectively) for both MDH-1 and MDH-2 loci. No heterozygous were identified at these loci (Figure 2 and Table 4).

At MDH-3, two alleles ('a' and 'b') were common to all populations, but all individuals were heterozygotes for those alleles. Interaction between the two alleles in each individual generated a hybrid band, which lied in equidistant position from each of the alleles (Figure 2 and Table 4). The effective number of alleles estimated at this locus was 2 for all populations.

Octanol dehydrogenase (ODH)

One locus (ODH-1) was present in individuals of all four populations. A single allele was identified at the locus for the populations (Figure 2 and Table 4).

Phosphoglucomutase (PGM)

A unit locus (PGM) was scored. A single allele was commonly present in all the studied populations (Plate E, Figure 2 and Table 4).

Phosphoglucose isomerase (PGI)

Two loci (PGI-1 and PGI-2) were identified for all four populations. At both loci, Aglorkpovie and Akotogua populations exhibited homozygosities for a slow allele 'a' (Table 4). At both loci also, Okyereko and Kpong individuals revealed a faster allele 'b' with frequencies of 0.10 and 0.04 respectively. Both Okyereko and Kpong populations also showed the slow allele 'a' at frequencies of 0.90 and 0.96 respectively.

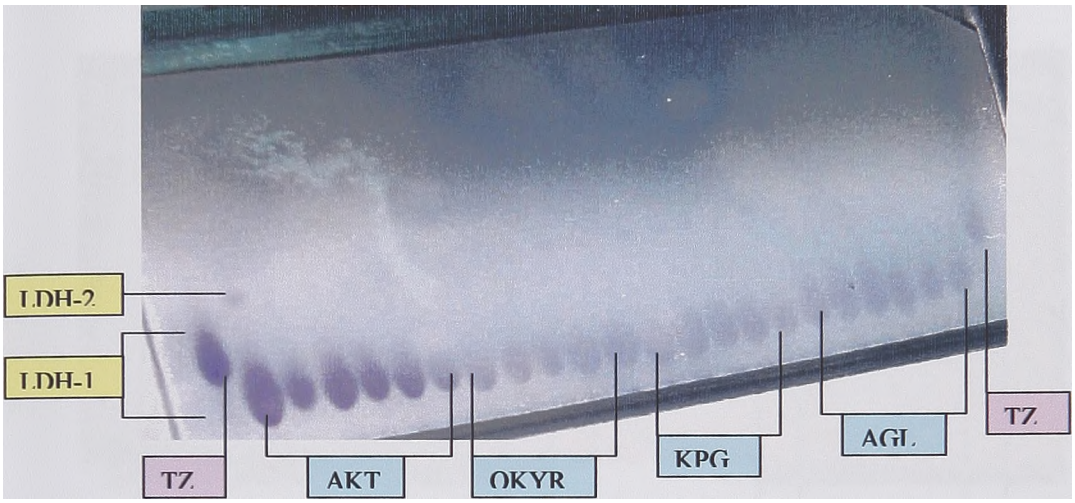
Sorbitol dehydrogenase (SDH)

A single locus (SDH-1) was scored for all populations. The locus for Aglorkpovie, Okyereko and Akotogua registered a single allele, making all individuals homozygotes for the allele (Table 4). The locus for Kpong population showed two alleles, a slow 'a' and a fast 'b', with frequencies of occurrence being respectively 0.04 and 0.96 respectively. No heterozygous were observed.

Xanthine dehydrogenase (XDH)

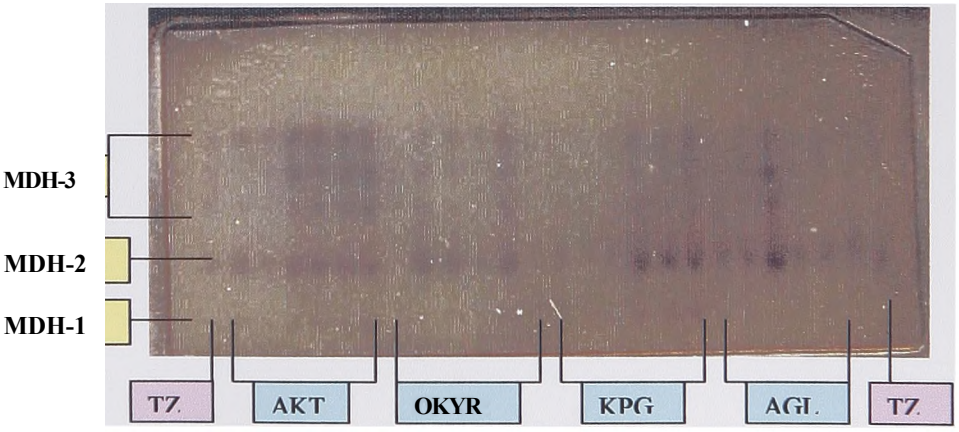
One locus (XDH-1) was recorded, with a single allele common to all studied populations of *T. guineensis* (Figure 2 and Table 4).

Plate C: Electrophoregram of Lactate dehydrogenase enzyme (with two loci; LDH-1 and LDH-2) of *I zillii* (TZ) (at terminals) and of representatives of four populations of *T. guineensis*



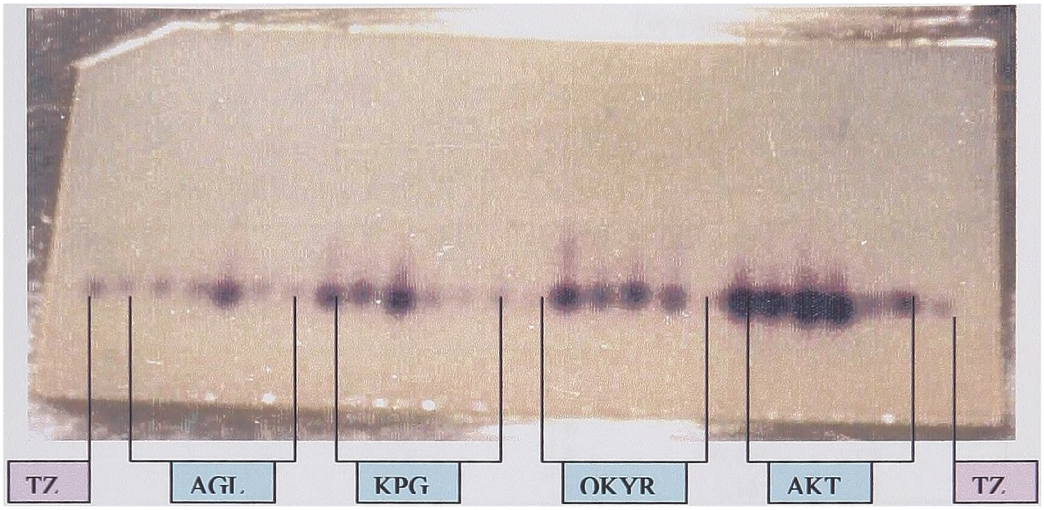
AGL = Aglorkpovie; OKYR = Okyereko; KPG = Kpong; AKT = Akotogua

Plate D: Electrophoregram of Malate dehydrogenase enzyme (with three loci; MDH-1, MDH-2 and MDH-3) of *T. zillii* (TZ) (at terminals) and of representatives of four populations of *T. guineensis*



AGL = Aglorkpovie; OKYR = Okyereko; KPG = Kpong; AKT = Akotogua

Plate E: Electrophoregram of Phosphoglucomutase enzyme of *T. zillii* (TZ) (at terminals) and of representatives of four populations of *T. guineensis*



AGL = Aglorkpovie; OKYR = Okyereko; KPG = Kpong; AKT = Akotogua

Table 4: Allelic frequency, effective number of alleles (e/), observed heterozygosity *(ho)* and expected heterozygosity *(he)* values in four populations of *T. guineensis* at 13 enzyme coding loci

		Population			
Locus	Allele	Aglorkpovie	Kpong	Okyereko	Akotogua
ADH-1	A	1.00	1.00	1.00	1.00
	ho	0	0	0	0
	he	0	0	0	0
	ef	1	1	1	1
IDH-1	A	1.00	1.00	1.00	1.00
	ho	0	0	0	0
	he	0	0	0	0
	er	1	1	1	1
LDH-1	A	0	0	0.067	0
	B	1.00	1.00	0.933	1.00
	ho	0	0	0	0
	he	0	0	0.125	0
	ef	1	1	1.14	1
LDH-2	A	1.00	1.00	1.00	1.00
	ho	0	0	0	0
	he	0	0	0	0
	ef	1	1	1	1
MDH-1	A	0	0	0.067	0
	B	1.00	1.00	0.933	1.00
	ho	0	0	0	0
	he	0	0	0.125	0
	ef	1	1	1.14	1
MDH-2	A	0	0	0.067	0
	B	1.00	1.00	0.933	1.00
	ho	0	0	0	0
	he	0	0	0.125	0
	e.f	1	1	1.14	1
MDH-3	A	0.5	0.5	0.5	0.5
	B	0.5	0.5	0.5	0.5
	ho	1	1	1	1
	he	0.5	0.5	0.5	0.5
	ef	2	2	2	2
ODH-1	A	1.00	1.00	1.00	1.00
	ho	0	0	0	0
	he	0	0	0	0
	Jj	1	1	1	1
PGI-1	A	0	0.04	0.10	0
	B	1	0.96	0.90	1.00
	ho	0	0.08	0.067	0
	he	0	0.077	0.18	0
	ef	1	1.08	1.22	1

Table 4 (Continued)

Locus	Allele	Aglorkpovie	Kpong	Okyereko	Akotogua
PGI-2	A	0	0.04	0.10	0
	B	1.00	0.96	0.90	1.00
	ho	0	0.08	0.067	0
	he	0	0.077	0.18	0
	ef	1	1.08	1.22	1
PGM-1	A	1.00	1.00	1.00	1.00
	ho	0	0	0	0
	he	0	0	0	0
	ef	1	1	1	1
SDH-1	A	1.00	0.96	1.00	1.00
	B	0	0.04	0	0
	ho	0	0	0	0
	he	0	0.077	0	0
	ef	1	1.08	1	1
XDH-1	A	1.00	1.00	1.00	1.00
	ho	0	0	0	0
	he	0	0	0	0
	ef	1	1	1	1

3.1.2 Polymorphism in Studied Populations of *T. guineensis*

Out of the 13 enzyme loci screened for the four populations of *T. guineensis,* seven (representing 53.8 %) were polymorphic, while six (representing 46.2 %) were monomorphic (Table 5). The polymorphic loci were LDH-1, MDH-1, MDH-2, MDH-3, PGI-1, PGI-2 and SDH-1. Only one locus (MDH-3) was polymorphic in all four populations. LDH-1, MDH-1 and MDH-2 loci were polymorphic in only Okyereko population, while SDH-1 locus was polymorphic in Kpong population only.

At 1 % frequency level, Aglorkpovie and Akotogua populations exhibited the same but lower mean loci polymorphism (P = 0.077). Okyereko showed the highest mean loci polymorphism (P = 0.462), followed by Kpong (P = 0.308). At 5 % level, P was 0.077 for Aglorkpovie, Akotogua and

Kpong, but 0.462 for Okyereko population. At both frequency levels (1 % and 5 %), Okyereko population indicated the highest polymorphism among the four populations.

Table 5: Distribution of Polymorphic* loci in four Populations of *T. guineensis*

	Population			
	Akotogua	Aglorkpovie	Kpong	Okyereko
n = 17		15	25	15
Locus				
ADH-1				
EDH-1				
LDH-1				++
LDH-2				
MDH-1				++
MDH-2				++
MDH-3	++	++	++	++
ODH-1				
PGI-1			+	++
PGI-2			+	++
SDH-1			+	

*: + = polymorphism at 1 % criterion
++ = „ at 5 % „
n = number of fish samples screened for a population

3.1.3 Heterozygosity, Wright Fixation Indices and Hardy-Weinberg Equilibrium

The observed and expected heterozygosities at individual enzyme loci screened for populations of *T. guineensis* are summarized in Table 4. Mean observed and expected heterozygosities and the within-population genetic diversity (Fi_S) index are presented in Table 6. The mean observed heterozygosity per population (H_0) was least in both Aglorkpovie and Akotogua populations ($H_0 \pm$

s.e = 0.077 ± 0.04). Okyereko population had 0.087 ± 0.076 while Kpong scored the highest mean observed heterozygosity (H_0 ± s.e = 0.089 ± 0.076).

Among all studied populations, the proportion of total variation that could be ascribed to differences among population allele frequencies (i.e. F_{CT}) was - 0.454. Levels of intra- (F_{is}) and inter- (F_{ST}) population variation at thirteen loci among all four populations are presented in Table

Analysis of variance and Student-Newman-Kuels multiple comparison test (Appendix III B) of Fis values within each population did not differ significantly from the other (P > 0.05). Pair-wise F_{ST} values between the populations (Table 8) indicated a least fixation index between Kpong and Okyereko (F_{ST} = - 0.166) while the highest was observed between Aglorkpovie and Akotogua (F_{ST} = - 1.026). Fixation index between Aglorkpovie and Okyereko (F_{ST} = - 0.233) was similar to that of Akotogua and Okyereko. Also, F_{ST} value of - 0.766 was similar to that between Aglorkpovie and Kpong and between Akotogua and Kpong.

Table 6: Indices of *T. guineensis* population genetic diversity (polymorphism, heterozygosities and effective number of alleles)at 13 loci and levels of deviation (Wright's Fixation Index) from Hardy - Weinberg expectation

						P		Ho		He		
Population	Water body	N	n	Na	Mean Ef	P(0.01)	P(0.05)	Ho	s.e	He	s.e	Fis
Aglorkpovie	Aglor lagoon	15	13	14	1.08	0.077	0.077	0.077	0.040	0.038	0.038	-1.026
Akotogua	Okye River	17	13	14	1.08	0.077	0.077	0.077	0.040	0.038	0.038	-1.026
Kpong	Volta River	25	13	17	1.10	0.308	0.077	0.089	0.076	0.056	0.038	-0.589
Okyereko	Ayensu River	15	13	19	1.14	0.462	0.462	0.087	0.076	0.095	0.040	-0.084

P (0.01), P (0.05) = polymorphism at 1 % and 5 % criteria respectively

H_0 H_e = means of observed and expected heterozygosities respectively

Ef = Effective number of alleles

s.e = standard error

N = number of fish samples

n = number of enzyme loci screened

F_{IS} = Wright's fixation index (F-statistic) that measures the within population deviation from Hardy-Weinberg equilibrium

Na = number of alleles scored

Table 7: Levels of intra - and inter -population variation at 13 loci in four populations of *T. guineensis.*

Locus	Fis	FST
Adh-1	1	1
Idh-1	1	1
Ldh-2	1	1
Ldh-1	1	1
Mdh-3	-1	-1
Mdh-2	1	1
Mdh-1	1	1
Odh-1	1	1
Pgi-2	0.647	0.428
Pgi-1	0.647	0.428
Pgm-1	1	1
Sdh-1	1	1
Xdh-1	1	1
Mean	0.869	0.758

Fis = Wright's F-statistic for analyzing heterozygosity deviation within populations, from Hardy-Weinberg expectation

F_{st} = Wright's F-statistic for analyzing heterozygosity deviation among populations, from Hardy-Weinberg expectation

Table 8: Pair - wise Matrix of Wright's Fixation Index (F_{ST}) values amongfour populations of *T. guineensis*

Population 1	2	3	4
1 - Aglorkpovie	-1.026	- 0.766	- 0.233
2 - Akotogua		- 0.766	-0.233
3 - Kpong			-0.166
4 - Okyereko			

3.1.4 Genotypic Distribution

The observed and expected genotypic distributions, the log likelihood Chi-square (G) values and probabilities (P) of deviation from the Hardy-Weinberg expectation for 13 enzyme loci scored are presented in Appendix II. The log likelihood Chi-square (G) values showed that observed genotypic distributions at LDH-1, MDH-1, MDH-2 and MDH-3 differed significantly from Hardy-Weinberg equilibrium (P varied from 0.05 to 0.001). Genotypic distribution at PGI-1 and PGI-2 did not deviate significantly from Hardy-Weinberg expectation ($P > 0.05$).

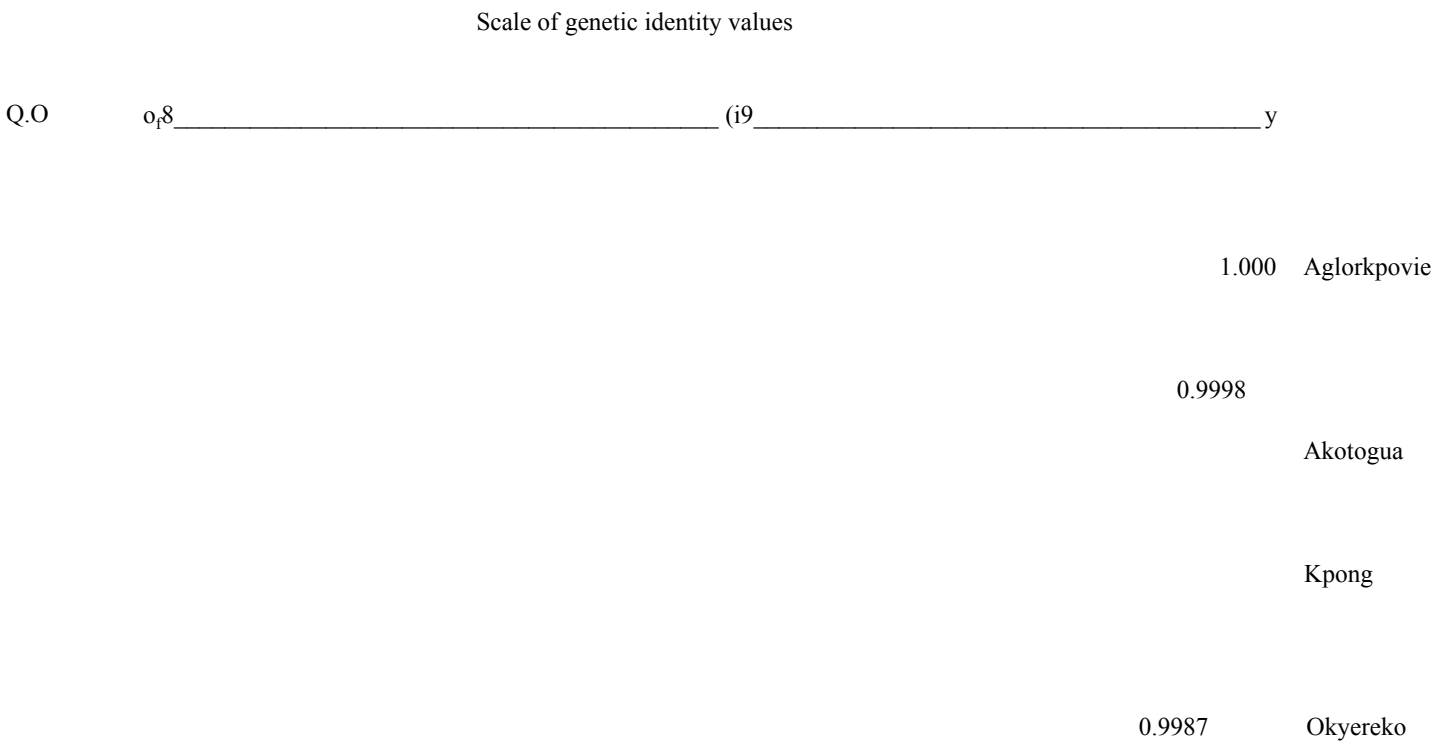
3.1.5 Genetic Relationships Among the four Populations

Values of genetic identity (I) and distance (D) between the four studied populations are presented in Table 9. Pair-wise matrix of genetic identity indicated absolute similarity coefficient of 1.000, and genetic distance of 0.000 respectively between Aglorkpovie and Akotogua populations (Table 9). The least genetic identity of 0.9985 and widest distance of 0.0015 respectively were found between Aglorkpovie and Okyereko and also between Akotogua and Okyereko. Both Aglokpovie and Akotogua were genetically closer to Kpong (I = 0.9998; D = 0.0002) than the closeness of Okyereko to Kpong (I = 0.9990; D = 0.0010) (Figure 3).

Table 9: Pair - wise Matrices of Genetic Identity (I) (above diagonal) and Genetic Distance (D) (below diagonal) of four Populations of *T. guineensis*

Population	1	2	3	4
1 - Aglorkpovie		1.0000	0.9998	0.9985
2 - Akotogua	0.0000		0.9998	0.9985
3 - Kpong	0.0002	0.0002		0.9990
4 - Okyereko	0.0015	0.0015	0.0010	

Figure 3: Phenetic relationships among populations of *Tilapia guineensis* based on genetic identities among populations



3.2 CHARACTERIZATION OF *T. guineensis* POPULATIONS BASED ON EARLY LIFE CULTURE CHARACTERISTICS

3.2.1 Reproductive Characteristics of Populations of *Tilapia guineensis*

Aspects of reproductive characteristics of populations of *T. guineensis* including: spawning frequency, spawn size and fry size (weight and length) at spawn were studied to contribute to culture performance evaluation of populations of the fish. Survival of fry to juvenile fish of the populations was also estimated to contribute to assessment of fingerling production capacity per population per year. Results obtained from the reproductive characteristics of two populations (Kpong and Akotogua) monitored for 150 days are presented in Table 10. In both populations, the first spawn after stocking of brood pairs, were not considered in the assessment of spawning activities. However, they provided starting points for assessment of populations' spawn frequencies and inter spawn periods. Spawn sizes per brood pair were determined by counting post-larval fry (free swimming) and not number of eggs per clutch, as some of the eggs could be attritional and unviable (Plate F). Each of four replicated pairs of *T. guineensis* originating from Akotogua spawned four times ($n = 4$) (at mean inter spawn period of 22 days), while replicate pairs of the fish from Kpong locality exhibited varied number of spawning during the monitoring period ($4 < n < 6$) at mean inter spawn period of 20 days.

The Kpong population had a spawn size range of 627 and 3232 (mean ± s.e. = 1895.5 ± 296.11), while Akotogua population had a range of 1120 and 4400 (mean ± s.e. = 2985.75 ± 707.53) (Table 10). Based on the present study, annual fry production per female is estimated as 54491 and 32954 for Akotogua and Kpong populations respectively.

Plate F: Spawn of female *T. guineensis,* showing scattered attrition eggs

There was variation in periods between successive spawns for both populations. Kpong population exhibited varied inter spawn periods (range = 15 to 27 days), while Akotogua population varied from 18 to 25 days. Mean inter spawn periods for Kpong and Akotogua populations were respectively 20 and 22 days.

Table 10: Inter spawning period, spawn sizes and fry survival of two populations of *T. guineensis* monitored in 1 m^2 hapas for 150 days

Population	Mean inter spawn period	Mean spawn size	Mean fry weight at spawn (mg)	Mean fry length at spawn (mm)	% Survival	Estimated annual fry production / female
Akotogua	20 ±2.517 [1]	2985.8 + 707.53	2.575 ±0.085	6.175 ±0.063	75.3	54491
n = 4 [+]	(17 - 25) *	(1120 - 4400)	(2.4 - 2.8)	(6 - 6.3)		
Kpong	22.1 ± 1.32	1895.5 ±296.11	2.633 ±0.088	6.067 ± 0.067	78.0	32954
n = 5	(15 -27)	(627 -3232)	(2.5 - 3)	(6.0 6.3)		

[1] Means of data observations are followed by standard errors

’ n = mean frequency of spawning

* Values in brackets are ranges of data observation

3.2.2 Early life Growth Characteristics

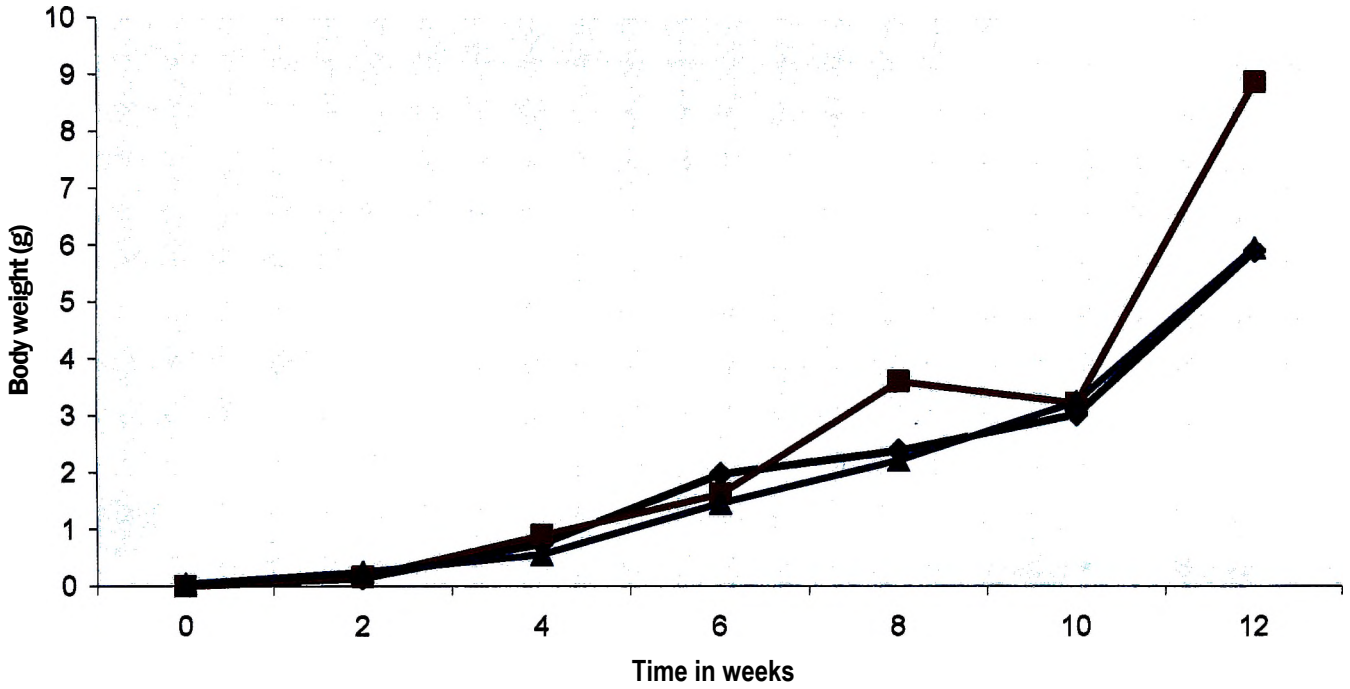
Growth [in terms of mean daily weight gain (MDWG) and specific growth rate (SGR) (both by weight), condition factor (K)] and percentage survival of three populations of *T. guineensis,* monitored in concrete tanks are shown in Table 11, Figures 5, 6 and 7a-c respectively. The early life growth trend and pattern of Akotogua population showed progressively higher performance after the sixth week of culture as compared to Kpong and Aglorkpovie populations (Figures 5 and 6). Initially, Aglorkpovie population exhibited slowest growth until it improved after the eighth week, exhibiting comparable growth with Kpong population.

By the 12^{th} week culture, growth (in terms of length and weight increases) differed among the studied populations as follows: Akotogua > Aglorkpovie > Kpong. Length increment in Akotogua population differed significantly from that of Aglorkpovie ($P < 0.05$) and Kpong ($P < 0.01$) populations (Table 11; Appendix VB). The difference in weight between Akotogua and Aglorkpovie was highly significant ($P < 0.001$), likewise the difference between Akotogua and Kpong ($P < 0.001$) (Appendix VC). However, between Kpong and Aglorkpovie there were no significant differences in both lengths and weights respectively ($P > 0.05$) (Appendices VB and VC).

Growth Rates (MDWG and SGR)

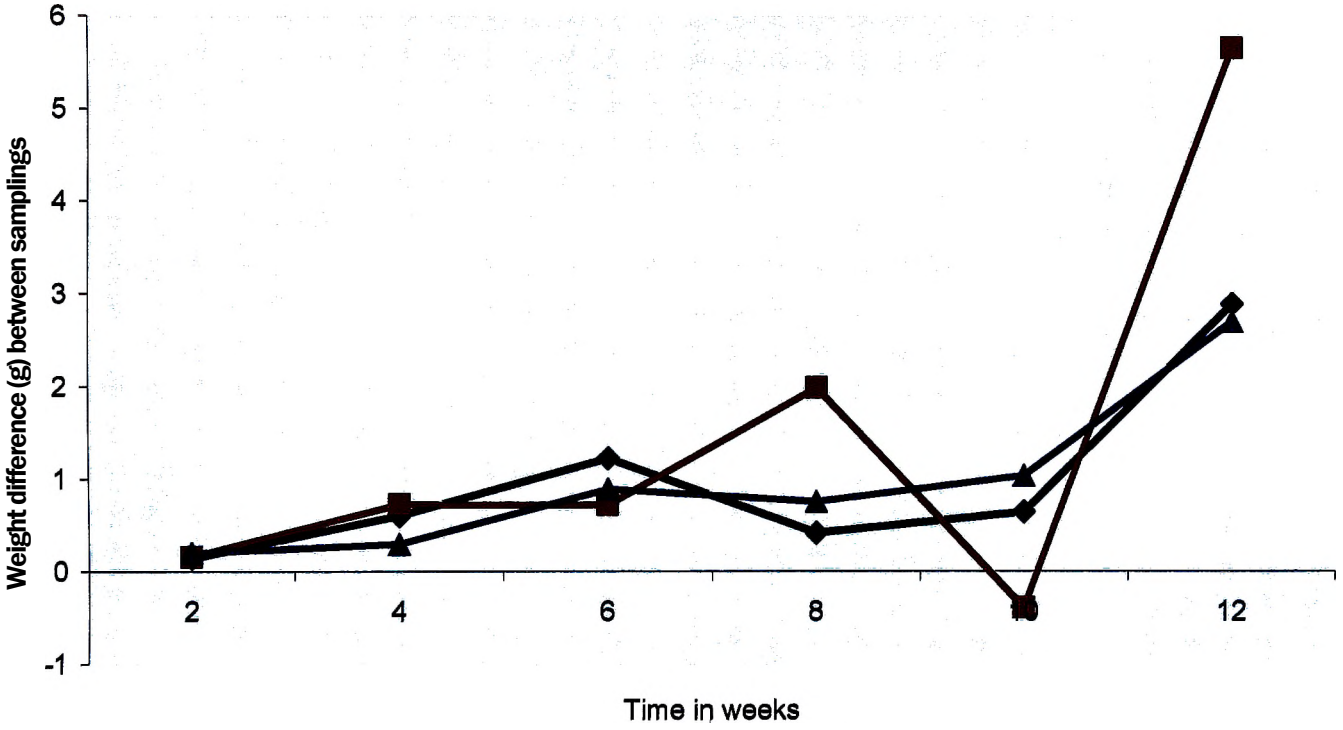
The rates at which flesh was deposited daily were respectively higher and comparable for Akotogua and Kpong populations compared to Aglokpovie population. SGR and MDWG for both Kpong and Akotogua populations did not respectively differ significantly ($P > 0.05$) (Appendices VD and VE) from those of Aglorkpovie population after performing a Student - Newman - Keuls multiple comparison tests (t - test) on the populations.

Figure 5: Early life growth trends of three populations of *T. guineensis* cultured in concrete tanks for 12 weeks



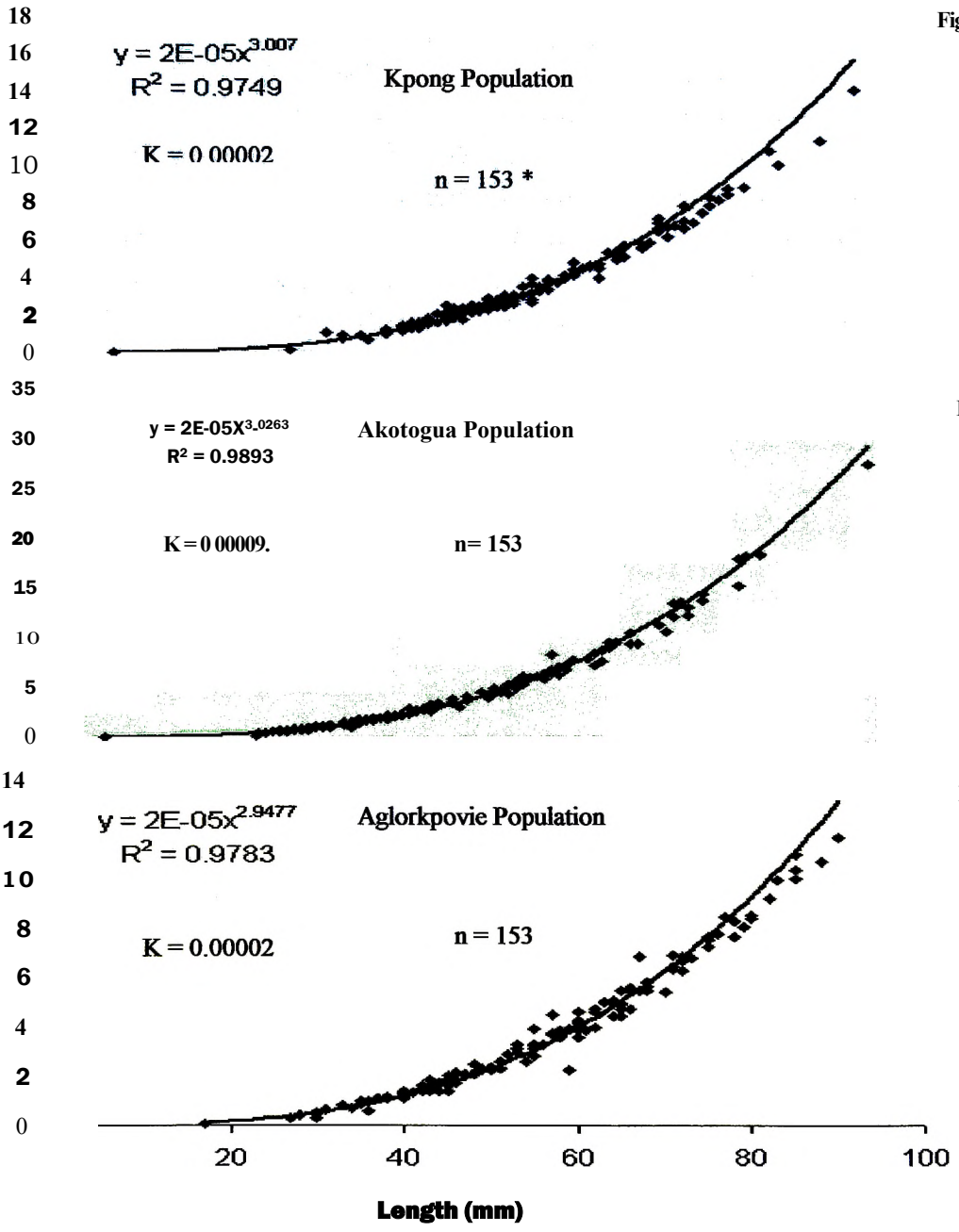
Kpong (Volta) Akotogua (Okye) -^Aglor lagoon

Figure 6: Growth patterns of fry of three populations of *T. guineensis*, cultured in concrete tanks for 12 weeks



•Kpong (Volta) Akotogua (Okye) -^Aglor lagoon

Figo 7: Length / weight relationships and mean condition factor (K) of three populations of *T. guineensis* fry, cultured for 12 weeks in concrete tanks



*n = number of fish samples observed

Table 11: Growth performance of three populations of *T. guineensis* monitored in concrete tanks for 12 weeks

Performance Parameter	Population		
	Aglorkpovie	Akotogua	Kpong
Initial mean weight (g)	0.0527	0.0053	0.0027
Final mean weight (g)	5.96[a*™] (0.35)	8.88 [b***] (0.70)	5.90[a ”*] (0.347)
Mean daily weight gain (g / d)	0.0234[3]	0.0238 [a]	0.0238 [a]
Specific growth rate (% g / d)	7.609[a]	11.387[a]	11.258 [a]
Initial mean length (mm)	17[a ***] (0.596)	6.8 (0.045)	6.2 [b***] (0.042)
Final mean length (mm)	68.36[a"] (1.481)	74.52 (1.983)	67.08 [a"] (1.43)
Final fish condition factor (g / mm[3])	1.78 x 10 ^ [a"'] (2.7 x 10'[7])	1.96 x 10* [b"'] (2.07 xlO "[7])	1.87xlO'[3 c"] (1.76 xlO'[7])
Survival (%)	64[a]	75.3 [a]	78 [a]

Values sharing different superscript letters in the same row differ significantly at levels defined by the least number of asterisks as follows;

* : significant level at (P < 0.05)

**: „ „ „(P<0.01)

***: „ „ „ **(P< 0.001)**

Values in brackets are standard errors about the mean

A further test between the three populations, Bartlett's test for homogeneity of variance (which assumes that all studied fish populations come from a common population with equal standard deviation) indicated a not quite significant difference (P = 0.088) among the standard deviations of the studied populations (Appendix V D).

Fish Condition Factor (K)

The early life fitness of fish populations observed over the entire culture period (12 weeks) is presented in Figures 7a-c and 8a-c. The length / weight relationship (Figure 7a-c) from which fish condition factor was derived fitted a perfect power curve for each of the three populations. Mean K over the entire growth period was 0.00002 in all three populations, 'b', as the slope of a power curve, was predominantly close to 3 in all populations ($0.9749 < R^2 < 0.9893$) (Figure 7a-c), which suggested that the fries of the three populations were growing isometrically (Ricker, 1975; Bolger and Connolly, 1989).

When calculated fish condition factor per sampling period was juxtaposed with fish growth, a similar distribution pattern was observed in all three populations. K showed variation with time, increasing progressively after two weeks of culture in all three populations, but peaked in the eighth week for Kpong and Akotogua populations (Figure 8a-c). After the peak, the condition factor of fish from Kpong and Akotogua decreased quite sharply until the twelfth week, but leveled after the eleventh week for Akotogua (Figures 8a and 8b respectively). In Aglorkpovie population however, K peaked in the sixth week, but decreased gently until the twelfth week (Figure 8a-c).

Figure 8: **Growth progression (G) and condition factor (K) of three populations of *T. guineensis* fry cultured for 12 weeks in concrete tanks**

Fig. 8a

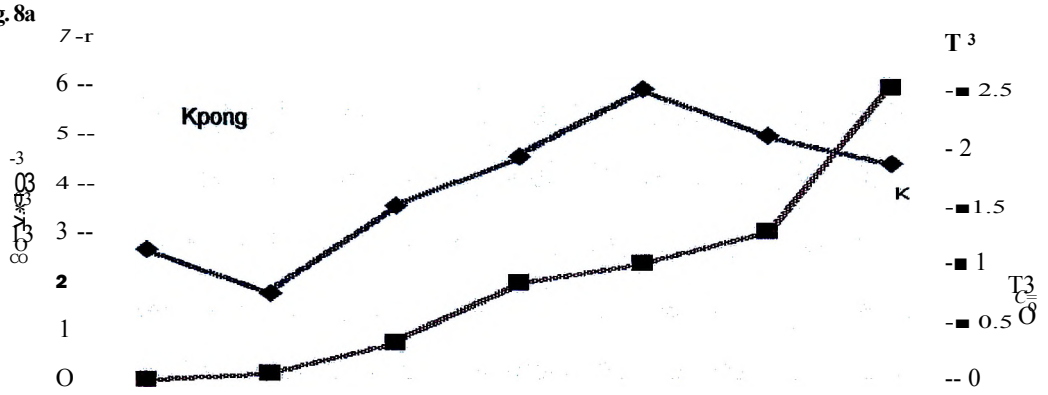


Fig. 8b

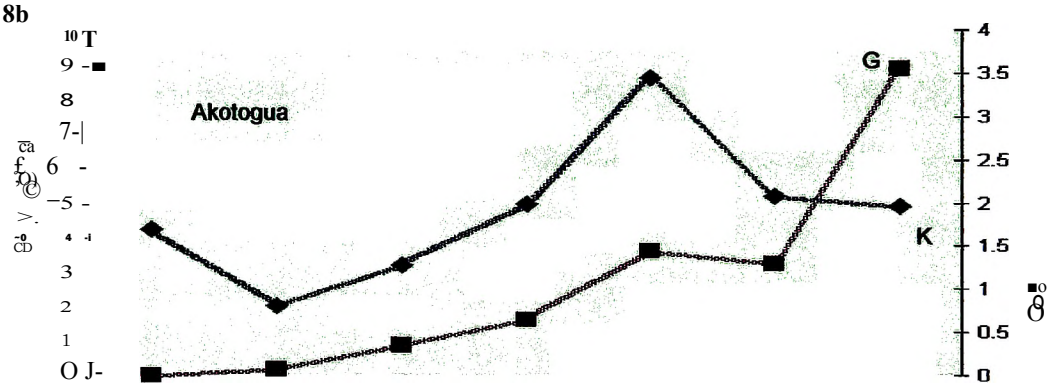
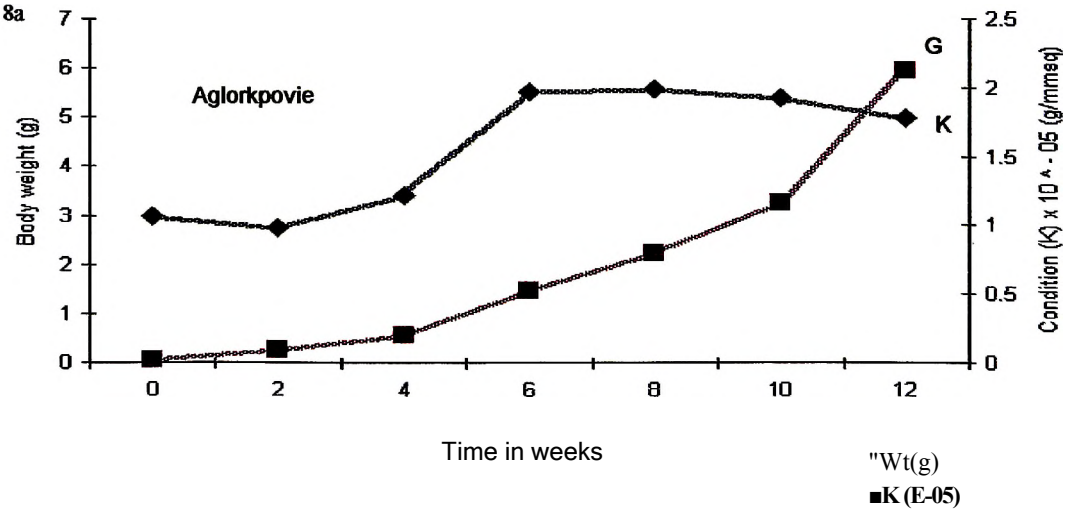


Fig. 8a



At the end of the culture period, the trend of condition factor of the populations was as follows: Akotogua > Kpong > Aglorkpovie. Akotogua fish condition differed significantly from that of Kpong at $P < 0.01$ and Aglorkpovie at $P < 0.001$. Fish condition of Kpong was significantly different from that of Aglorkpovie at $P < 0.01$ (Table 11 and Appendix V F).

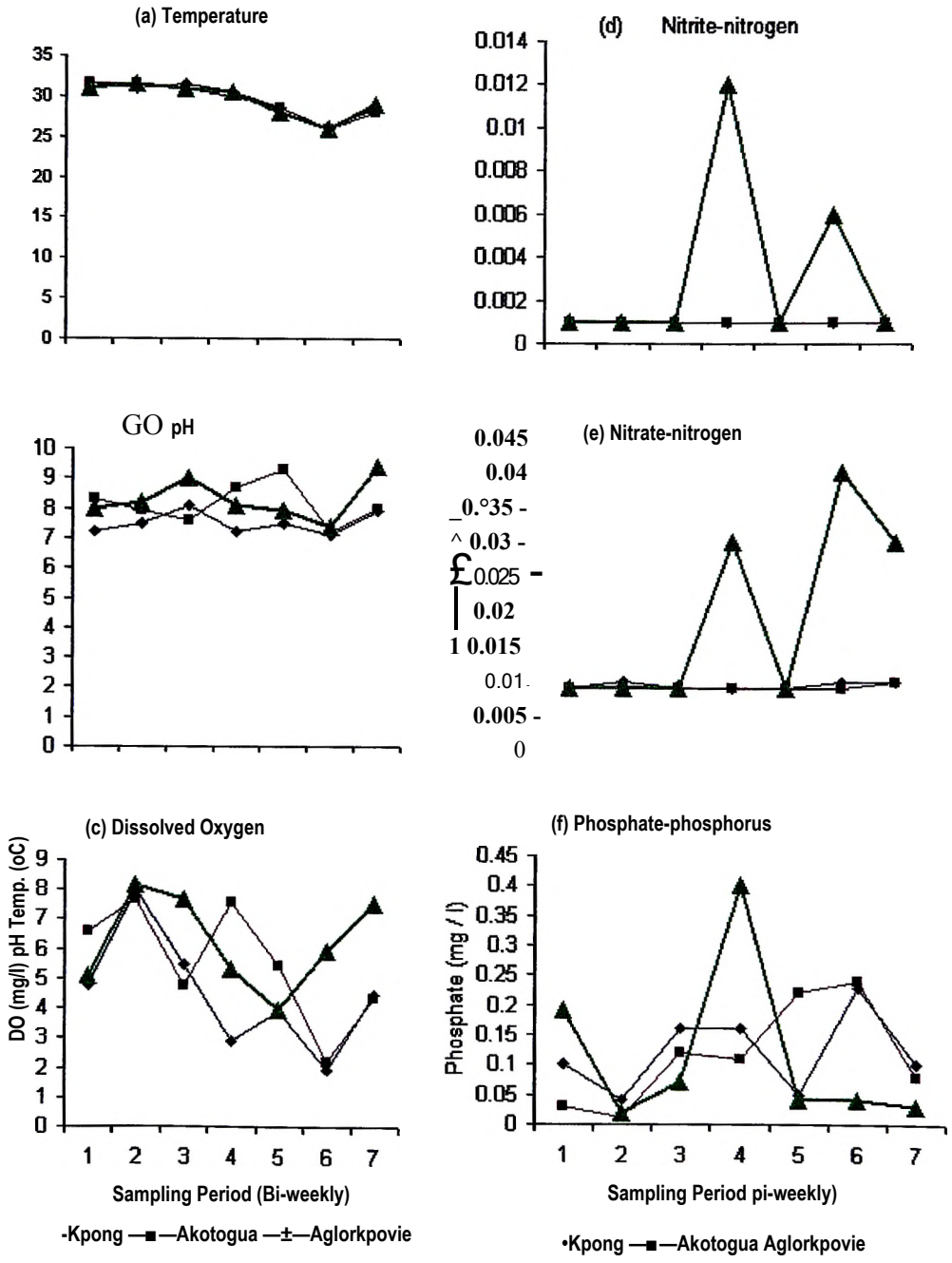
Fish Survival

Survival of fry for Akotogua and Kpong populations was similar (75.3 % and 78 % respectively) (Tables 10 and 11). Incidentally, Aglorkpovie population fry, being a 'sibling' stock of Kpong population rather had a lower survival rate over the period (64 %), but it was not significantly different from the others ($P > 0.05$) (Appendix V G).

3.3 WATER QUALITY ANALYSIS

Trends of water quality parameters monitored over the culture period are presented in Figure 9a-f. Of the six parameters monitored, only nitrate-nitrogen differed significantly ($P < 0.05$) between the culture tanks of Kpong and Aglorkpovie populations (Appendix IV A-F).

Figure 9: **Fluctuations in some physical and chemical characteristics (a- f) in the culture tanks of three populations of *7 guineensis* fry monitored for 12 weeks**



CHAPTER FOUR

4.0 DISCUSSION

4.1 Characterization of Populations of *T. guineensis* Based on Allozyme Genetic Study

Characterization of Ghanaian populations of *Tilapia guineensis* studied using allozyme electrophoresis and early life culture performance characteristics was carried out to contribute to the understanding and possible utilization of knowledge of population genetic structure in fish production and stocks assessment for identification of stock(s) most advantageous for culture. The present allozyme electrophoresis study of the species revealed variations in genetic characteristics of the studied populations with reference to the levels of polymorphism (P) at both 1 % and 5% criteria, allele diversity and heterozygosities (H). The differences in genetic characteristics were thus a reflection of their varied frequencies of alleles and effective number of alleles per locus of the populations.

The estimated proportion of total variation among the populations studied (i.e. F_{CT} = - 0.454) indicated that over 45% of the observed genetic variation was attributable to differences in population allele frequencies, but with excessive observed heterozygosity. However, the range of values of heterozygosities in the four populations was comparable to those reported *T. guineensis* populations in other parts of West Africa. For example, Pouyaud and Agnese (1996) found the range of heterozygosity values among Ivorian and Senegalese populations of *T. guineensis* to be between 0.015 and 0.132. The present heterozygosity values also compare well with results in similar studies on other tilapias (Me Andrew and Majumdar, 1983; Falk *et al,* 1999)

A relatively low genetic variability was found within the populations from Aglorkpovie (Ho = 0.077; p = 0.077) as compared to Kpong (Ho = 0.308; P = 0.089) and Okyereko (Ho = 0.462; P = 0.087). Low genetic diversity of the fish from Aglor lagoon compared with those from Kpong reservoir (both populations of Volta system) could be attributed to genetic drift, that is the random change in allele frequencies by mating error in the production of new generations (Ferguson, 1980; Ambali *et al,* 199; Desvignes *et al,* 2001; Dufresne *et al,* 2002). Apparently, the isolation of Aglor lagoon from the main Volta system, following construction of roads and farming activities might have let to some few founder individuals (from the Volta stock) colonizing the lagoon, a condition referred to as demographic bottleneck (Ferguson, 1980; Bouzat *et al,* 1998).

Bottlenecking of *T. guineensis* in Aglor lagoon appeared to have resulted in skewed distribution of population alleles such that only a limited part of allelic variation of the main Volta stock was found among the Aglorkpovie population compared to those of Kpong. Leberg (1992) and Bouzat *et al* (1998) observed that populations known to have experienced a reduction in demographic size often showed reduced genetic diversity.

The present estimated measure of deviations within the Aglorkpovie and Akotogua populations from the Hardy-Weinberg expectations (i.e. F is) was widest with heterozygote excesses. (F is = -1.026), compared to the measure of deviation within Kpong (. F is = -0.589) and Okyereko (F is = 0.084) populations (Table 6). The measure of deviation within Aglorkpovie population from Hardy- Weinberg equilibrium compared to that of Kpong supported a possible mating error towards production of generation of Aglorkpovie fish with low allele frequency.

Unsustainable fishing activities which has characterized lagoon fisheries in Ghana (Blay and Asabere-Ameyaw, 1993; Ofori -Danson *et al,* 1998), could also contribute to the presently

observed low genetic diversity status of *T. guineensis* in Aglor lagoon. This is because fishing is selective and could result in elimination of alleles that are potentially associated with possible fast growing fishes. The biochemical genetic structure of Akotogua populations was similar to that of Aglorkpovie population (characterized by 'low' genic diversity). *T. guineensis* from Akotogua may have been selected for low genetic diversity, perhaps owing to bottlenecking effect (Bouzat *et al,* 1998) due to isolation of new population during the creation of the Akotogua reservoir on the Okye River.

The relatively high observed heterozygosities (Ho) and polymorphisms (P) in Okyereko and Kpong populations (Table 6) suggested that present among some individuals of the two populations were several allozymes (different forms of enzymes [proteins], which are the products of alternate alleles segregating at a locus of a gene within a species). The occurrence of several allozymes within the two populations gives an indication of extensive assortative mating schemes among the sexually active individuals (Myers *et al,* 2001).

Among the study populations, genic diversities (based on heterozygosities and allele frequencies) were not significantly different from each other, with reference to the Kruskall —Wallis non-parametric ANOVA test and sequential Dunn's multiple comparison tests of population allele frequencies and Wright's fixation index (Fi_S) (Appendix III A). This was basically because the study did not reveal diagnostic alleles peculiar to any one population. The present scenario contrasts with findings of Pouyaud and Agnese (1996), Desvignes *et al* (2001) and Dufresne *et al* (2002) in which specific alleles were sometimes fixed for particular populations. The Results suggest that the population studied may not have been discretely isolated long enough for 'private alleles' to generate in populations. Population with polymorphic loci (Table 5) (with the exception

of PGI-1 and PGI -2 loci) showed significant deviations (at various levels of significance) in their genotypic distribution from Hardy- Weinberg equilibrium, usually with heterozygote deficiencies (Appendix II). In all individuals of the study populations only MDH-3 (in which all individuals were in heterodimeric state - A/B genotype) showed highly significant genotypic frequency deviations ($P < 0.001$) from expected Hardy-Weinberg's hypothesis. Thus twelve out of thirteen loci screened did not differ significantly at $P > 0.05$, which suggested that all four populations could be considered as panmictic. That is the four natural populations (Akotogua, Aglorkpovie, Okyereko and Kpong) of *T. guineensis* in Ghana appeared to consist of sexually out breeding individuals, whose assortative mating resulted in the observed allelic frequencies similar to those to be expected (Myers *et al* 2001).

Phenetic relationship among the study populations (Figure 3) indicated that all four populations share veiy basic and similar genetic characteristics. The 'very high' genetic identities (and thus 'very low' genetic distances) among the populations indicated that they are genetically close to each other. There was absolutely no genetic difference between Aglorkpovie populations. However, some level of genetic dissimilarities existed between some of the populations. The Kpong population was genetically closer to both Aglorkpovie and Akotogua populations, while the Okyereko population clustered with Kpong. The closeness of Aglorkpovie population to the Kpong population compared to similarity of Aglorkpovie and Okyereko populations supports the possibility of the Aglorkpovie and Kpong population sharing common genetic characteristics within the Volta system.

The generally closer biochemical genetic relationship among the *T. guineensis* populations in spite of habitat dissimilarities among the populations (example lagoon verses fresh water) suggested stable genotypic characteristics among the populations. The present observation however contrasts with conclusions of Lowe-McConnel (1987) that closer genetic relationships between fish populations correlate positively with habitat similarity or ecological homogeneity.

Characterization of Populations of *T. guineensis* Based on Early life Culture Performance characteristics

Characterization of populations of *T. guineensis* based on reproductive characteristics (spawn size, periods between spawns, fry size at spawn) and early life growth characteristics (growth rate, condition factor and survival) indicated various degrees of variations among the populations studied.

Generally, spawn sizes (fry numbers produced per spawn) varied through out the monitoring period for Akotogua (range = 1120 - 4400 per spawn) and Kpong (range = 627 - 3232 per spawn) populations. Such variations were commonly observed in most studied tilapias (Dadzie and Wangila, 19880; Rana, 1988; Msiska and Costa-Pierce, 1996; Coward and Bromage, 1998). Dadzie (1981) used hapas to rear fish of 30 - 66 g and found a range of 200 to 1532 fry / spawn with an average of 1202 per spawn. The Akotogua population showed relatively higher mean spawn size compared to Kpong population, but values were not significantly different ($P > 0.05$).

Inter-spawning periods averaged a three weekly duration for Akotogua and Kpong populations. The spawning periods of the two populations conform to reports on most studied tilapias (e.g. Dadzie, 1974; Babiker and Ibrahim, 1979; Legendre *et al,* 1990; Mackintosh and Little, 1995;

Coward and Bromage, 1998). Spawning was continuous throughout the five-month study period. Fish populations of Akotogua and Kpong did not breed spontaneously immediately after pairings, as other tilapias do in captivity (Lazard and Legendre, 1996; Coward and Bromage, 2000; Abban and Agyakwah -in press). Perhaps the delay towards spawning was necessary for courtship establishment. No significant difference was observed in the inter-spawn periods between the populations. Observed post-larval fry weights and lengths from both Akotogua and Kpong populations were very much comparable (Table 10) with no significant differences between the initial fry weights and lengths. The reproductive capacities of populations of Akotogua and Kpong were generally similar, implying that reproductive characteristics of both populations could be exploited in the improvement of *T. guineensis* culture.

Among wild and domesticated (intra-specific) fish populations, possible phenotypic variation in reproductive traits (such as spawn size and inter-spawn periods) has been attributed to age, genetics and environmental factors (including food availability and water quality characteristics) (Wootton, 1979; Legendre and Ecoutin, 1996). In studying potential factors responsible for differences in reproductive and growth characteristics, bi-weekly freshwater exchange in culture tanks was ensured to minimize possible influence of water quality on observed differences in reproductive characteristics. Monitored water quality parameters in culture tanks did not suggest significant differences in water quality in the different tanks except for nitrate-nitrogen. The difference in nitrate levels in the environments of the two populations did not however result in differences in spawning characteristics, growth and survival (Tables 10 and 11). All other studied parameters (temperature, oxygen, nitrite, phosphorous and pH) were statistically similar among culture tanks ($P > 0.05$) (Appendix IV A - F).

Of the three populations studied (Akotogua, Kpong and Aglorkpovie) for their early life growth performance evaluation, specific growth rate (SGR) and mean daily weight gain (MDWG) of populations from Akotogua (SGR = 11.387; MDWG =0.0238) and Kpong (SGR =11.258; MDWG = 0.0238) were similar and comparable to results (SGR = 9.2% g / day) reported by Legendre (1983) who monitored growth of fry of an Ivory Coast population of *T. guineensis* form 0.002g to 0.5g in circulating water tanks for 60 days. Dadzie (1981) also observed early life growth characteristics of the fish in ponds, and recorded SGR of 12.38% g / day.

Although SGR and MDWG were not significantly different among the populations ($P > 0.05$), the relatively higher SGR of Akotogua population led to final fish sizes of the Aglorkpovie and Kpong populations. A situation where growth rates between two populations do not differ significantly but result in significant differences in final weights or lengths is attributable to expression of mathematical function. Thus growth rate expresses a constant function with time. Hence between any two populations (e.g. Akotogua and Kpong) a growth rate difference of 1 unit is more likely to result in significant growth difference at a reasonable longer time interval. Therefore the significant difference in fish weight and length between Akotogua and the other two populations at the end of the experiment, in spite of insignificant differences in growth rates, was expected.

The significant variation in fish condition factor after 84 days of culture period was also reflective of growth rate, i.e. the higher tendency to achieve heavier weight at a given length. Akotogua population was significantly better in condition compared to *T. guineensis* populations of the Volta system (i.e. Kpong and Aglorkpovie populations). Within the Volta system, the Kpong population (freshwater reservoir) exhibited a relatively better condition and was significantly

different **(P < 0.01)** from that of the Aglorkpovie population (coastal lagoon). The slow growth rate, which characterized Aglorkpovie population, could have led to the relatively poor condition of the fish.

According to Bagenal **(1978),** spawns of substrate spawning tilapias are characterized by high numbers and high mortality rate due to vulnerability to predation pressure in natural waters compared to the mouth brooding tilapias. Under cultivation where predation could be controlled, Legendre *et al* **(1990)** observed high mortalities in batch spawns of Ivorian *T. guineensis,* but attributed low survival rate to larval first feeding on supplementary feed. In the current tank culture study, average fish survival for Aglorkpovie, Akotogua and Kpong populations studied contrasted with results obtained on the same species in a similar condition by Legendre *et al* **(1990)** where 45 - 56% survival was recorded.

1.3 General Conclusion

Biochemical genetic structure (based on heterozygosity, allelic frequency and polymorphism) of *Tilapia guineensis* populations of Akotogua and Aglorkpovie were characterized by 'low' genic diversity, while 'high' genic diversity was observed in both Kpong and Okyereko populations. Characterization of three populations (Akotogua, Aglorkpovie and Kpong) of *T. guineensis* based on early life culture characteristics (reproductive and growth performance characteristics) indicated that comparatively, Akotogua population (characterized with 'low' genic diversity) exhibited the capacity to produce large numbers of fry at spawn and 'high' early life growth performance (survival, growth rates and condition factor).

The association between population biochemical genetic diversity (heterozygosity, polymorphism and allelic frequency) and culture performance characteristics of Akotogua and Kpong populations was negatively related. For example, high genetic diversity of Kpong population did not commensurate with high culture performance characteristics of the fish, whereas low genetic diversity of Akotogua population conferred high culture performance characteristics on the fish.

The present negative association between fitness related traits (e.g. condition factor and growth rate) and genetic markers (such as alleles) of Akotogua and Kpong populations supports the neutral considerations of genetic markers (Dillon and Manzi, 1988; Shikano *et al* 2000; Desvignes, 2001). A positive association observed between allozymes and growth traits of fish from Aglorkpovie in which low genic diversity paralleled poor early life growth performance, suggested that high fishing intensity of the lagoon, as noted by Blay and Asabre-Ameyaw (1993) and Ofori-Danson *et al* (1998) could have influenced the overall genetic structure of the population in a negative way.

T. guineensis from Akotogua population appeared to be a better source of brood stock among the populations study for aquaculture and captive stock programs. The fish also has an advantage of being used for lagoon and estuarine aquaculture. Notwithstanding the present result of the study are by no means conclusive on reproductive and growth characteristics of the species. Further study on stages of growth during the entire life cycle of the fish of all populations available in Ghana is recommended to enable plausible selection of 'high' culture performing population(s) for enhanced fish production and development.

The present hypotheses that Ghanaian populations of *T. guineensis* could exhibit variations in their biochemical genetic structure and early life culture characteristics are accordingly not rejected. This implies that *T. guineensis* from each of the numerous and distinctive natural water bodies in Ghana could show discrimination on the basis of allozyme genetic characteristics and early life culture characteristics.

SUMMARY

Genetic characteristics of four populations of *Tilapia guineensis* from some Ghanaian inland water bodies were studied by two approaches: allozyme (starch gel) electrophoresis and early life culture performance characterization. The fish populations were sampled from three water systems: a reservoir on a tributary of Okye river at Akotogua; a coastal open lagoon of the Volta system (part of Keta lagoon) at Aglorkpovie; a freshwater reservoir also of the Volta system at Kpong and a reservoir on a tributary of Ayensu river at Okyereko, for the study. Observed heterozygosities (Ho) and polymorphism (P) of allozymes as indices of genetic diversity were relatively low in Akotogua (Ho = 0.0771; P = 0.077) and Aglorkpovie (Ho = 0.077; P = 0.077) populations, compared to those of Kpong (Ho = 0.308; P = 0.089) and Okyereko (Ho = 0.462; P = 0. 087) populations. Analysis of molecular variance (AMOVA) using Wright's Fixation index (F-statistic) did not indicate significant differences among the populations studied (P > 0. 05).

Evaluation of population early life culture performance characteristics (specific growth rate, condition factor and survival) within 84 days showed various levels of differences among the populations. Final mean length (FL) of 74.52 mm, weight gain (WG) of 8.88g and condition factor

(K) of 1.96 x $10'^{5}$ g / mm^3) estimated for Akotogua population were significantly higher compared to growth characteristics of Aglorkpovie (FL = 68.36 mm, WG = 5.96 g, K = 1.78 x 10^5 g / mm^3) and Kpong (FL = 67.08mm, WG = 5.90 g, K = 1.87 x $10''^{5}$ g / mm^3) populations.

Results suggested that heterozygosity and polymorphism are important in determining culture performance traits. Additionally, high genetic diversity did not confer high culture performance characteristics on the lie of the fish. Low genetic diversity and 'poor' culture performance observed in Aglorkpovie population could be attributable to unsustainable fishing activities, which characterized coastal lagoon fisheries in Ghana. The biochemical genetic structure and growth characteristics observed in Akotogua population identify it as a population with a potentially better culture performance that could be used for improvement of *T. guineensis* in aquaculture.

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APPENDIX I

(I A) CONTINUOUS TRIS CITRATE BUFFER

Distilled water added to make 1 litre

(IB) ENZYMES STAINING RECEIPES

for Alcohol dehydrogenase (ADH)

Nicotinamide adenine dinucleotide (NA)	15mg
Dimethylthiazol -di-phenyl-tetrazoliumbromide (MTT)	6mg
Tris-HCL (0.2M) pH 9.0	30mls
Iso-propanol	0.75mls
Phenazine methosulphate (PMS)	Trace

for Isocitrate dehydrogenase (IDH)

Sodium Isocitrate Acid	50mg
$MgCl_2$	lOmg
Nicotinamide adenine dinucleotide Phosphate (NADP)	4mg
MTT	7mg
PMS	Trace
Tris-HCL	30mls

for L-Lactose dehydrogenase (LDH)

NAD	l[5m]§
MTT	[7m]§
Sodium lactate solution	
PMS	Trace
Tris-HCl	30mls

for Malate dehydrogenase (MDH)

L-Malic Acid	15 Omg
MTT	[6m]S
NAD	[10m]S
Tris	60°mg
PMS	Trace
Water (H_20)	30mls

for Octanol dehydrogenase (ODH)

NAD	15mg
MTT	6mg
Octanol	0.75mls
Tris-HCL (0.2 M, pH 9)	30mls
PMS	Trace

for Phosphoglucomutase (PGM)

Na-G-$P0_4$	50mg

$MgCl_2$	70mg
NADP	3mg
MTT	7mg
Tris-HCL pH 8.0	30mls
G6PDH	50JJ.1
PMS	Trace

for Phosphoglucose isomerase (PGI)

Fructose -6-Phosphate	20mg
NADP	4mg
$MgCl_2$	20mg
MTT	7mg
6-GPDH	50|xl
PMS	Trace
Tris-HCL 0.2M	30mls

for Sorbitol dehydrogenase (SDH)

Sorbitol	150mg
NAD	15mg
$MgCl_2$	10mg
MTT	7mg
PMS	Trace
Tris-HCl	30mls

for Xanthine dehydrogenase (XDH)

Hypoxanthine	20mg
NAD	15mS
MTT	7m§
PMS	Trace
Tris-HCL pH 8.0	30mls

(IC) <u>FTXTNG SOLUTION</u>

Acetic acid (Glacial)	10%
Methanol	20%
Distilled water	*70%*

APPENDIX II

Observed (Obs) and expected (Exp) allozyme genotypic distributions, the log likelihood test (G) and probabilities (P) of deviations from Hardy-Weinberg expectations in 13 loci of four populations of *Tilapia guineensis.*

ADH-1

			Genotypic Distribution				
Population	Sample size		A/A	A/B	B/B	G	P
Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	17.00	0.00	0.00	0.00	>0.10
		Exp	17.00	0.00	0.00		
Kpong	25	Obs	25.00	0.00	0.00	0.00	>0.10
		Exp	25.00	0.00	0.00		
Okyereko	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		

IDH-1

Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	17.00	0.00	0.00	0.00	>0.10
		Exp	17.00	0.00	0.00		
Kpong	25	Obs	25.00	0.00	0.00	0.00	>0.10
		Exp	25.00	0.00	0.00		
Okyereko	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		

LDH-1

Population	Sample size		A/A	A/B	B/B	G	P
Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	0.00	0.00	17.00	0.00	>0.10
		Exp	0.00	0.00	17.00		
Kpong	25	Obs	25.00	0.00	0.00	0.00	>0.10
		Exp	25.00	0.00	0.00		
Okyereko	15	Obs	14.00	0.00	1.00	7.352	>0.10
		Exp	13.00	0.125	0.067		

LDH-2

Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	17.00	0.00	0.00	0.00	>0.10
		Exp	17.00	0.00	0.00		
Kpong	25	Obs	25.00	0.00	0.00	0.00	>0.10
		Exp	25.00	0.00	0.00		
Okyereko	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		

MDH-1

Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	17.00	0.00	0.00	0.00	>0.10
		Exp	17.00	0.00	0.00		
Kpong	25	Obs	25.00	0.00	0.00	0.00	>0.10
		Exp	25.00	0.00	0.00		
Okyereko	15	Obs	14.00	0.00	1.00	7.352	>0.10
		Exp	13.00	0.125	0.067		

MDH-2

Population	Sample size		A/A	A/B	B/B	G	P
Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	17.00	0.00	0.00	0.00	>0.10
		Exp	17.00	0.00	0.00		
Kpong	25	Obs	25.00	0.00	0.00	0.00	>0.10
		Exp	25.00	0.00	0.00		
Okyereko	15	Obs	14.00	0.00	1.00	7.352	>0.10
		Exp	13.00	0.125	0.067		

MDH-3

Aglorkpovie	15	Obs	0.00	15.00	0.00	20.75	<0.001
		Exp	3.75	7.50	3.75		
Akotogua	17	Obs	0.00	17.00	0.00	23.57	<0.001
		Exp	4.25	8.50	4.25		
Kpong	25	Obs	0.00	25.00	0.00	34.57	<0.001
		Exp	6.25	12.50	6.25		
Okyereko	15	Obs	0.00	15.00	0.00	20.79	<0.001
		Exp	3.75	7.50	3.75		

ODH -1

Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	17.00	0.00	0.00	0.00	>0.10
		Exp	17.00	0.00	0.00		
Kpong	25	Obs	25.00	0.00	0.00	0.00	>0.10
		Exp	25.00	0.00	0.00		
Okyereko	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		

PGI-1

Population	Sample size		A/A	A/B	B/B	G	P
Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	17.00	0.00	0.00	0.00	>0.10
		Exp	17.00	0.00	0.00		
Kpong	25	Obs	23.00	2.00	0.00	0.083	>0.10
		Exp	23.04	1.92	0.04		
Okyereko	15	Obs	13.00	1.00	1.00	3.566	>0.05
		Exp	12.15	2.70	0.15		

PGI-2

Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	17.00	0.00	0.00	0.00	>0.10
		Exp	17.00	0.00	0.00		
Kpong	25	Obs	23.00	2.00	0.00	0.083	>0.10
		Exp	23.04	1.92	0.04		
Okyereko	15	Obs	13.00	1.00	1.00	3.566	>0.05
		Exp	12.15	2.70	0.15		

PGM

Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	17.00	0.00	0.00	0.00	>0.10
		Exp	17.00	0.00	0.00		
Kpong	25	Obs	25.00	0.00	0.00	0.00	>0.10
		Exp	25.00	0.00	0.00		
Okyereko	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		

SDH-1

Population	**Sample size**		A/A	A/B	B/B	G	P
Aglorkpovie	15	Obs	0. 00	0.00	15.00	0.00	>0.10
		Exp	0. 00	0.00	15.00		
Akotogua	17	Obs	0. 00	0.00	17.00	0.00	>0.10
		Exp	0. 00	0.00	17.00		
Kpong	25	Obs	1.00	0.00	24.00	9.84	>0.10
		Exp	0.04	1.92	23.00		
Okyereko	15	Obs	0.00	0.00	15.00	0.00	>0.10
		Exp	0.00	0.00	15.00		

XDH -1

Aglorkpovie	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		
Akotogua	17	Obs	17.00	0.00	0.00	0.00	>0.10
		Exp	17.00	0.00	0.00		
Kpong	25	Obs	25.00	0.00	0.00	0.00	>0.10
		Exp	25.00	0.00	0.00		
Okyereko	15	Obs	15.00	0.00	0.00	0.00	>0.10
		Exp	15.00	0.00	0.00		

APPENDIX III

Analysis of variance (ANOVA) of genetic diversity indices among for populations of *T. guineensis*

(III A) ANOVA of allele frequencies among four populations of *T. guineensis*

Kruskal - Wallis Nonparametric ANOVA Test

Group	Number of Points	Sum of Ranks	Mean of Ranks
Aglorkpovie	20	832.00	41.600
Akotogua	20	832.00	41.600
Kpong	20	796.00	39.800
Okyereko	20	780.00	39.000

Kruskal-Wallis Statistic KW = 0.2207 (corrected for ties)
The exact P value calculation would have taken too long, so the chi-square approximate P value is shown instead.
The P value is 0.9742, considered not significant.
Variation among column medians is not significantly greater than expected by chance.

Dunn's Multiple Comparisons Test

Comparison		Mean Difference	P	value
Aglorkpovie	vs. Akotogua	0.000	ns	P>0.05
Aglorkpovie	vs. Kpong	1.800	ns	P>0.05
Aglorkpovie	vs. Okyereko	2.600	ns	P>0.05
Akotogua	vs. Kpong	1.800	ns	P>0.05
Akotogua	vs. Okyereko	2.600	ns	P>0.05
Kpong	vs. Okyereko	0.8000	ns	P>0.05

These tests are based on a Gaussian approximation. They are only accurate for large sample sizes.

Summary of Data

Group	Number of Points	Median	Minimum	Maximum
Aglorkpovie	20	1.000	0.000	1.000
Akotogua	20	1.000	0.000	1.000
Kpong	20	0.9600	0.000	1.000
Okyereko	20	0.9150	0.000	1.000

(III B) ANOVA of Wright fixation index (Fis) **amongfour populations of *T. guineensis***

One-way Analysis of Variance (ANOVA)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	3	0.2216	0.07386
Residuals (within columns)	48	15.962	0.3325
Total	51	16.184	

F = 0.2221
The P value is 0.8806, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Bartlett's test for homogeneity of variances

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 0.3523
The P value is 0.9499.
This test suggests that the difference among the SDs is not significant.

Student-Newman-Keuls Multiple Comparisons Test

Comparison		Mean Difference	q	E.....value
Kpong	vs Okyereko	-0.1026	0.6416	ns P>0.05
Kpong	vs Aglorkpovie	-0.1598	0.9994	ns P>0.05
Kpong	vs Akotogua	-0.1598	0.9994	ns P>0.05
Okyereko	vs Aglorkpovie	-0.05723	0.3578	ns P>0.05
Okyereko	vs Akotogua	-0.05723	0.3578	ns P>0.05
Aglorkpovie	vs Akotogua	0.000	0.000	ns P>0.05

Difference		Mean Difference	Lower 95% Cl	Upper 95% Cl
Kpong	- Okyereko	-0.1026	-0.5579	0.3526
Kpong	- Aglorkpovie	-.1598	-0.7076	0.3879
Kpong	- Akotogua	-.1598	-0.7627	0.4430
Okyereko	- Aglorkpovie	-0.05723	-0.5125	0.3980
Okyereko	- Akotogua	-0.05723	-0.6050	0.4905
Aglorkpovie	- Akotuga	0.000	-0.4552	0.4552

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Median
Aglorkpovie	13	0.8462	0.5547	0.1538	1.000
Akotogua	13	0.8462	0.5547	0.1538	1.000
Kpong	13	0.6863	0.6377	0.1769	1.000
Okyereko	13	0.7889	0.5551	0.1540	1.000

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Aglorkpovie	-1.000	1.000	0.5109	1.181
Akotogua	-1.000	1.000	0.5109	1.181
Kpong	-1.000	1.000	0.3009	1.072
okyereko	-1.000	1.000	0.4535	1.124

(III **C) ANOVA of Wright fixation index** (Fst) **among four populations of** *T. guineensis*

Kruskal - Wallis Nonparametric ANOVA Test

Group	Number of Points	Sum of Ranks	Mean of Ranks
Aglor / Akot	13	573.50	44.115
Aglor / Kpong	13	495.50	38.115
Aglor / Okyer	13	507.50	39.038
Akot / Kpong	13	495.50	38.115
Akot / Okyer	13	507.50	39.038
Kpong / Okyer	13	501.50	38.577

Kruskal - Wallis Statistic KW = 1.345 (corrected for ties)
The exact P value calculation would have taken too long, so the chi-square approximate P value is shown instead.
The P value is 0.9302, considered not significant.
Variation among column medians is not significantly greater than expected by chance.

Dunn's Multiple Comparisons Test

Comparison			Mean Difference	P	value
Aglor / Akot	vs.	Aglor / Kpong	6.000	ns	P>0. 05
Aglor / Akot	vs.	Aglor / Okyer.	5.077	ns	P>0. 05
Aglor / Akot	vs.	Akot / Kpong	6.000	ns	P>0. 05
Aglor / Akot	vs.	Ak/Ok	5.077	ns	P>0. 05
Aglor / Akot	vs.	Kp/Ok	5.538	ns	P>0. 05
Aglor / Kpong	vs.	Aglor / Okyer	-0.9231	ns	P>0. 05
Aglor / Kpong	vs.	Akot / Kpong	0.000	ns	P>0. 05
Aglor / Kpong	vs.	Ak/Ok	-0.9231	ns	P>0. 05
Aglor / Kpong	vs.	Kp/Ok	-0.4615	ns	P>0. 05
Aglor / Okyer.	vs.	Akot / Kpong	0.9231	ns	P>0. 05
Aglor / Okyer.	vs.	Ak/Ok	0.000	ns	P>0. 05
Aglor / Okyer.	vs.	Kp/Ok	0.4615	ns	P>0. 05
Akot / Kpong	vs.	Ak/Ok	-09231	ns	P>0. 05
Akot / Kpong	vs.	Kp/Ok	-0.4615	ns	P>0. 05
Ak/Ok	vs.	Kp/Ok	0.4615	ns	P>0. 05

These test are based on a Gaussian approximation. They are only accurate for large sample sizes.

Summary of Data

Group	Number of Points	Median	Minimum	Maximum
Aglor / Akot	13	1.000	-1.000	1.000
Aglor / Kpong	13	1.000	-1.000	1.000
Aglor / Okyer.	13	1.000	-1.000	1.000
Akot / Kpong	13	1.000	-1.000	1.000
Ak/Ok	13	1.000	-1.000	1.000
Kp/Ok	13	1.000	-1.000	1.000

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APPENDIX IV

Analysis of variance (ANOVA) of water quality parameters among culture tanks of four populations of *T. guineensis*

(IVA) ANOVA of NITRITE levels among culture tanks of four populations of *T. guineensis*

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments (between columns)	2	2.438E-05	1.219E-05
Residuals (within columns^	18	0.0001094	6.079E - 06
Total	20	0.0001338	

F = 2.005
The P value is 0.1636, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Bartlett's test for homogeneity of variances.

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett's test cannot be performed because at least one column's standard deviation is zero.

Student - Newman - Keuls Multiple Comparisons Test

Comparison			Mean Difference	q P	value
Akotogua	vs	Kpong	0.000	0.000 ns	P>0.05
Akotogua	vs	Aglorkpovie	-0.002286	2.453 ns	P>0.05
Kpong	vs	Aglorkpovie	-0.002286	2.453 ns	P>0.05

Difference			Means Difference	Lower 95% Cl	Upper 95% Cl
Akotogua	vs	Kpong	0.000	-0.0027690.	002769
Akotogua	vs	Aglorkpovie	-0.002286	-0.0056490.	001078
Kpong	vs	Aglorkpovie	-0.002286	-0.0050540.	0004830

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Median
Akotogua	7	0.0010000	0.000	0.000	0.0010000
Aglorkpovie	7	0.003286	0.004271	0.001614	0.0010000
Kpong	7	0.0010000	0.000	0.000	0.0010000

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	0.0010000	0010000	0.0010000	0.0010000
Aglorkpovie	0.0010000	0120000	-0.0006641	0.007236
Kpong	0.0010000	0010000	0.0010000	0.0010000

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(IV B) ANOVA of NITRATE levels among culture tanks of four populations of *T. guineensis*

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)	2	0.0004804	0.0002402
Residuals (within columns)	18	0.001084 .	6.024E - 05
Total	**20**	0.001565	

F = 3.987
The P value is 0.0369, considered significant.
Variation among column means is significantly greater than expected by chance.

Bartlett's test for homogeneity of variances.

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 57.533
The **P** value is < 0.0001.
This test suggests that the difference among the SDs is extremely significant.
Since ANOVA assumes populations with equal SDs, you should consider transforming your data (reciprocal or log) or selecting a nonparametric test.

Student-Newman-Keuls Multiple Comparisons Test

Comparison			Mean Difference	q	**P**	values
Akotogua	vs	Kpong	-0.0002857	0.09740	ns	P>0.05
Akotogua	vs	Aglorkpovie	-0.01029	3.506	ns	P>0.05
Kpong	vs	Aglorkpovie	-0.01000	3.409	*	P<0.05

Difference	Mean Difference	Lower 95% Cl	Upper 95% Cl
Akotogua - Kpong	-0.0002857	-0.0090010	.008430
Akotogua - Aglorkpovie	-0.01029	-0.020870.	0003013
Kpong - Aglorkpovie	.0.01000	-0.01872	-0.001285

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Median
Akotogua	7	0.009143	0.0003780	0.0001429	0.009000
Aglorkpovie	7	0.01943	0.01343	0.005075	0.009000
Kpong	7	0.009429	0.0005345	0.0002020	0.009000

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	0.009000	0.01000	0.008793	0.009492
Aglorkpovie	0.009000	0.04000	0.007010	0.03185
Kpong	0.009000	0.01000	0.008934	0.009923

*

(IV C) ANOVA of TEMPERATURE levels among culture tanks of four populations of *T. guineensis.*

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)	2	0.02381	0.01190
Residuals (within columns)	18	77.429	4.302
Total	20	77.452	

F = 0.002768
The P value is 0.9972, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Bartlett's test for homogeneity of variances.

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 0.02870
The P value is 0.9858.
This test suggests that the difference among the SDs is not significant.

Student-Newman -Keuls Multiple Comparisons Test

Comparison			Mean Difference	...q.....	P	value
Aglorkpovie	vs	Akotogua	-0.07143	0.9112	ns	P>0.05
Aglorkpovie	vs	Kpong	-0.07143	0.09112	ns	P>0.05
Akotogua	vs	Kpong	0.000	0.000	ns	P>0.05

Difference		Mean Difference	Lower 95% Cl	Upper 95% Cl
Aglorkpovie -	Akotogua	-0.07143	-2.400	2.258
Aglorkpovie -	Kpong	-0.07143	-2.901	2.758
Akotogua	Kpong	0.000	-2.329	2.329

Summary of Data

Group	Number °f Points	Mean	Standard Deviation	Standard Error of Mean	Median

Akotogua	7	29.643	2.155	0.8144	31.000
Aglorkpovie	7	29.571	2.009	0.7593	30.500
Kpong	7	29.643	2.056	0.7770	30.500

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	26.000	31.500	27.650	31.636
Aglorkpovie	26.000	31.500	27.713	31.429
Kpong	26.000	31.500	27.742	31.544

(IV D) ANOVA of DISSOLVED OXYGEN levels among culture tanks of four populations of *T. guineensis*

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)	2	10.727	5.363
Residuals (within columns)	18	60.976	3.388
Total	20	71.703	

F= 1.583
The P value is 02326, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Bartlett's test for homogeneity of variances.

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 0.3397
The P value is 0.8438
This test suggests that the difference among the SDs is not significant.

Student-Newman -Keuls Multiple Comparisons Test

Comparison		Mean Difference	q	P	value
Kpong vs	Akotogua	-1.037	1.491	ns	P>0.05
Kpong vs	Aglorkpovie	-1.740	2.501	ns	P>0.05
Akotogua vs	Aglorkpovie	-0.7029	1.010	ns	P>0.05

Difference		Mean Difference	Lower 95% Cl	Upper 95% C
Kpong vs	Akotogua	-1.037	-3.104	1.030
Kpong vs	Aglorkpovie	-1.740	-4.251	0.7706
Akotogua vs	Aglorkpovie	-0.7029	-2.770	1.364

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Median
Akotogua	7	5.529	1.965	0.7426	5.450
Aglorkpovie	7	6.231	1.574	0.5949	5.900
Kpong	7	4.491	1.956	0.7392	4.450

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	2.200	7.700	3.712	7.346
Aglorkpovie	3.950	8.150	4.776	7.687
Kpong	1.900	7.990	2.683	6.300

(IV E) ANOVA of pH levels among culture tanks of four populations of *T. guineensis*

One-way Analysis of Variance (ANOVA)

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between coiumns)	2	2.452	1 -226
Residuals (within columns)	.....18.....	.....6,606.....	0.3670
Total	20	9.058	

F= 3.341
The P value is 0.0583, considered not quite significant.
Variation among column means is not significantly greater than expected by chance.

Bartlett's test for homogeneity of variances

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 2.283
The P value is 0.3193
This test suggests that the difference among the SDs is not significant.

Student-Newman -Keuls Multiple Comparisons Test

Comparison			Mean Difference	q	P	value
Kpong	vs	Akotogua	-0.6429	2.808	ns	P>0.05
Kpong	vs	Aglorkpovie	-0.7857	3.432	ns	P>0.05
Akotogua	vs	Aglorkpovie	-0.1429	0.6239	ns	P>0.05

Difference		Mean Difference	Lower 95% Cl	Upper 95% Cl
Kpong vs	Akotogua	-0.6429	-1.323	0.03741
Kpong vs	Aglorkpovie	-0.7857	-1.612	0.04063
Akotogua vs	Aglorkpovie	0.1429	-0.8231	0.5374

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Media
Akotogua	7	8.143	0.6997	0.2644	8.000
Aglorkpovie	7	8.286	0.6842	0.2586	8.100
Kpong	7	7.500	0.3786	0.1431	7.500

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	7.200	9.300	7.496	8.790
Aglorkpovie	7.400	9.400	7.653	8.918
Kpong	7.100	8.100	7.150	7.850

(IV F) ANOVA of PHOSPHATE levels among culture tanks of four populations of *T. guineensis*

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)	2	0.0001810	9.048E-05
Residuals (within columns).....	18.....	.....0,1.899	0.01055
Total	20	0.1901	

F = 0.008575
The **P** value is 0.9915, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Bartlett's test for homogeneity of variances

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 3.041
The **P** value is 0.2186
This test suggests that the difference among the SDs is not significant.

Student-Newman -Keuls Multiple Comparisons Test

Comparison			Mean Difference	q	P	value
Aglorkpovie	vs	Akotogua	-0.002857	0.07359	ns	P>0.05
Aglorkpovie	vs	Kpong	-0.007143	0.1840	ns	P>0.05
Akotogua	vs	Kpong	-.004286	0.1104	ns	P>0.05

Difference			Mean Difference	Lower 95% Cl	Upper 95% Cl
Aglorkpovie	vs	Akotogua	-0.002857	-0.1182	0.1125
Aglorkpovie	vs	Kpong	-0.007143	0.1473	0.1330
Akotogua	vs	Kpong	-0.004286	-0.1196	0.1111

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Errorof Mean	Median
Akotogua	7	0.1157	0.8772	0.3316	0.1100
Aglorkpovie	7	0.1129	0.1392	0.05263	0.04000
Kpong	7	0.1200	0.06758	0.02554	0.1000

Group.....	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	0.01000	0.2400	0.03458	0.1968
Aglorkpovie	0.02000	0.4000	-0.01593	0.2416
Kpong	0.04000	0.2300	0.05750	0.1825

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APPENDIX V

Analysis of variance (ANOVA) of culture performance parameters among four populations of *T. guineensis.*

(V A) ANOVA of INITIAL STOCKING LENGTH among four populations of *T. guineensis.*

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)	2	736.80	368.40
Residuals (within columns)	27	32.240	1.194
Total	29	769.04	

F = 308.52
The P value is 0.0001, considered extremely significant.
Variation among column means is significantly greater than expected by chance.

Bartlett's test for homogeneity of variances.

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 67.984
The P value is 0.0001
This test suggests that the difference among the SDs is extremely significant.
Since ANOVA assumes populations with equal SDs, you should consider transforming your data (reciprocal or log) or selecting a nonparametric test.

Student-Newman -Keuls Multiple Comparisons Test

Comparison			Mean Difference	q	P value
Kpong	vs	Akotogua	-0.6000	1.736	ns P>0.05
Kpong	vs	Aglorkpovie	-10.800	31.254	*** PO.Ol
Akotogua	vs	Aglorkpovie	-10.200	29.518	*** PO.Ol

Difference			Mean Difference	Lower 95% Cl	Upper 95% Cl
Kpong	vs	Akotogua	-0.6000	-1.603	0.4033
Kpong	vs	Aglorkpovie	-10.800	-12.013	-9.587
Akotogua	vs	Aglorkpovie	-10.200	-11.203	-9.197

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Median
Akotogua	10	6.800	0.1247	0.03944	6.800
Aglorkpovie	10	17.000	1.886	0.5963	16.500
Kpong	10	6.200	0.1054	0.03333	6.200

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	6.500	6.900	6.711	6.889
Aglorkpovie	15.000	20.000	15.651	18.349
Kpong	6.000	6 .400	6.125	6.275

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(V B) ANOVA of FINAL LENGTH among four populations of *T. guineensis.*

Source of variation		Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)		2	1582.3	791.15
Residuals (within columns)		147	20022	136.20
Total	149		21604	

F= 5. 809
The P value is 0.0037, considered very significant.
Variation among column means is significantly greater than expected by chance.

Bartlett's test for homogeneity of variances.

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 6.602
The P value is 0.0368
This test suggests that the difference among the SDs is significant.
Since ANOVA assumes populations with equal SDs, you should consider transforming your data (reciprocal or log) or selecting a nonparametric test.

Tukey - Kramer Multiple Comparisons Test

If the value of q is greater than 3.353 then the P value is less than 0.05.

Comparison			Mean Difference	q	P	value
Akotogua	vs	Aglorkpovie	6.160	3.732	*	P<0.05
Akotogua	vs	Kpong	7.440	4.508	**	P<0.01
Aglorkpovie	vs	Kpong	1.280	0.7755	ns	P>0.05

Difference			Mean Difference	Lower 95% Cl	Upper 95% Cl
Akotogua	vs	Aglorkpovie	6.160	0.6260	11.694
Akotogua	vs	Kpong	7.440	1.906	12.974
Aglorkpovie	vs	Kpong	1.280	-4.254	6.814

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Median
Akotogua	50	74.520	14.025	1.983	75.000
Aglorkpovie	50	68.360	10.470	1.481	66.000
Kpong	50	67.080	10.113	1.430	67.000

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	47.000	113.00	70.530	78.510
Aglorkpovie	45.000	90.000	65.382	71.338
Kpong	49.000	93.000	64.203	69.957

* * *

(V C) ANOVA of FINAL MEAN WEIGHT among four populations of *T. guineensis*

One-way Analysis of Variance (ANOVA)

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)	2	289.88	144.94
Residuals .(within columns)	..... 147.	.....1795.1	12.212
Total	149	2085.0	

F= 11.869
The P value is 0.0001, considered extremely significant.
Variation among column means is significantly greater than expected by chance.

Bartlett's test for homogeneity of variances

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 34.056
The P value is 0.0001
This test suggests that the difference among the SDs is extremely significant.
Since ANOVA assumes populations with equal SDs, you should consider transforming your data (reciprocal or log) or selecting a nonparametric test.

Tukey-Kramer Multiple Comparisons Test

If the value of q is greater than 3.353 then the P value is less than 0.05.

Comparison			Mean Difference	q P	value	
Akotogua	vs	Aglorkpovie	2.921	5.911	***	P0.001
Akotogua	vs	Kpong	2.976	6.022	***	PO.Ol
Aglorkpovie	vs	Kpong	0.05520	0.1117	ns	P>0.05

Difference			Mean Difference	Lower 95% Cl	Upper 95% Cl
Akotogua	vs	Aglorkpovie	2.921	1.264	4.578
Akotogua	vs	Kpong	2.976	1.319	4.633
Aglorkpovie	vs	Kpong	0.05520	-1.602	1.712

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean Media
Akotogua	50	8.879	4.949	0.6998 8.065
Aglorkpovie	50	5.958	2.478	0.3504 5.450
Kpong	50	5.902	2.451	0.3466 5.600

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	2.060	27.270	7.471	10.286
Aglorkpovie	1.830	11.660	5.253	6.662
Kpong	2.380	14.000	5.205	6.600

(V D) ANOVA of SPECIFIC GROWTH RATE (SGR) among four populations of *T. guineensis*

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)	2	55.213	27.606
Residuals Cwithin columns')	15	744.67	49.645
Total	17	799.88	

F = 0.5561
The P value is 0.5848, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Bartlett's test for homogeneity of variances

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 4.866
The P value is 0.0878
This test suggests that the difference among the SDs is not quite significant.

Student-Newman -Keuls Multiple Comparisons Test

Comparison		Mean Difference	q	P	value
Aglorkpovie	vs Kpong	-3.649	1.269	ns	P>0.05
Aglorkpovie	vs Akotogua	-3.778	1.313	ns	P>0.05
Kpong	vs Akotogua	-0.1286	0.04470	ns	P>0.05

Difference		Mean Difference	Lower 95% Cl	Upper 95% C
Aglorkpovie	vs Kpong	-3.649	-12.319	5.020
Aglorkpovie	vs Akotogua	-3.778	-14.346	6.790
Kpong	vs Akotogua	-0.1286	-8.798	8.541

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Errorof Mean	Median
Aglorkpovie	6	7.609	2.975	1.214	6.603
Akotogua	6	11.387	7.706	3.146	9.983
Kpong	6	11.259	8.984	3.668	9.058

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Aglorkpovie	4.361	11.415	4.487	10.732
Akotogua	4.198	24.815	3.299	19.475
Kpong	3.813	28.453	1.829	20.688

(V E) ANOVA of MEAN DAILY WEIGHT GAIN among four populations of *T. guineensis*

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)	2	0.0005741	0.0002871
Residuals (within columns)	15	0.02654	0.001769
Total	17	0.02711	

F = 0.1623
The P value is 0.8517, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Bartlett's test for homogeneity of variances

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 2.147
The P value is 0.3418
This test suggests that the difference among the SDs is not significant.

Student-Newman -Keuls Multiple Comparisons Test

Comparison		Mean Difference	q	P	value
Aglorkpovie	vs Kpong	-0.01104	0.6429	ns	P>0.05
Aglorkpovie	vs Akotogua	-0.01274	0.7419	ns	P>0.05
Kpong	vs Akotogua	-0.001700	0.09899	ns	P>0.05

Difference		Mean Difference	Lower 95% Cl	Upper 95% C)
Aglorkpovie	vs Kpong	-0.01104	-0.062790	04071
Aglorkpovie	vs Akotogua	-0.01274	-0.075830	05035
Kpong	vs Akotogua	-0.001700	-0.053450	05005

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Median
Aglorkpovie	6	0.05145	0.02522	0.01030	0.04714
Akotogua	6	0.06419	0.04866	0.01987	0.06050
Kpong	6	0.06249	0.04799	0.01959	0.05519

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Aglorkpovie	0.02711	0.09479	0.02498	0.07793
Akotogua	-0.007940	0.1343	0.01312	0.1153
Kpong	0.01372	0.1376	0.01212	0.1129

*

(V F) ANOVA of CONDITION FACTOR (K) among four populations of *T. guineensis*

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)	2	8.679E-11	4.340E-11
Residuals (within columns)	147	3.975E-10	2.704E-12
Total	149	4.843E-10	

F= 16.047
The P value is 0.0001, considered extremely significant.
Variation among column means is significantly greater than expected by chance.

Bartlett's test for homogeneity of variances

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 9.182
The P value is 0.0101
This test suggests that the difference among the SDs is significant.
Since ANOVA assumes populations with equal SDs, you should consider transforming your data (reciprocal or log) or selecting a nonparametric test.

Tukey-Kramer Multiple Comparisons Test

If the value of q is grater than 3.353 than the p value is less than 0.05

Comparison			Mean Difference	q	P value	
Akotogua	vs	Aglorkpovie	1.862E-06	8.006	***	P0.001
Akotogua	vs	Kpong	9.900E-07	4.257	**	P<0.01
Aglorkpovie	vs	Kpong	-8.720E-07	3.750	*	P<0.05

Difference			Mean Difference	Lower 95% Cl	Upper 95% Cl
Akotogua	vs	Aglorkpovie	1.862E-06	1.082E-06	2.642E06
Akotogua	vs	Kpong	9.900E-07	2.102E-07	1.770E-06
Aglorkpovie	vs	Kpong	-8.720E-07	-1.652E-06	-9.22E-08

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Errorof Mean	Median
Akotogua	50	1.964E-05	1.672E-06	2.364E-07	1.960E-05
Aglorkpovie	50	1.778E-05	1.939E-06	2.743E-07	1.760E-05
Kpong	50	1.865E-05	1.248E-06	1.765E-07	1.870E-05

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	1.680E-05	2.600E-05	1.916E-05	2.012E-05
Aglorkpovie	1.100E-05	2.430E-05	1.723E-05	1.833E-05
Kpong	1.600E-05	2.210E-05	1.829E-05	1.901E-05

(V G) ANOVA of SURVIVAL among four populations of *T. guineensis*

One-way Analysis of Variance (ANOVA)

Source of variation	Degrees of freedom	Sum of Squares	Mean Square
Treatments (between columns)	2	5216.9	2608.4
Residuals (within columns)	18	24508	1361.5
Total	20	29725	

F = 1.916
The P value is 0.1761, considered not significant.
Variation among column means is not significantly greater than expected by chance.

Bartlett's test for homogeneity of variances

ANOVA assumes that all columns come from populations with equal SDs. The following calculations test that assumption.

Bartlett statistic (corrected) = 4.081
The P value is 0.1300
This test suggests that the difference among the SDs is not significant.

Student-Newman -Keuls Multiple Comparisons Test

Comparison		Mean Difference	q	P	value
Aglorkpovie	vs Akotogua	-31.000	2.223	ns	P>0.05
Aglorkpovie	vs Kpong	-35.429	2.540	ns	P>0.05
Akotogua	vs Kpong	-4.429	0.3175	ns	P>0.05

Difference		Mean Difference	Lower 95% Cl	Upper 95% Cl
Aglorkpovie	vs Akotogua	-31.000	-72.435	10.435
Aglorkpovie	vs Kpong	-35.429	-85.762	14.904
Akotogua	vs Kpong	-4.429	-45.864	37.007

Summary of Data

Group	Number of Points	Mean	Standard Deviation	Standard Error of Mean	Median
Akotogua	7	262.21	26.473	10.006	256.00
Aglorkpovie	7	231.21	52.478	19.835	228.00
Kpong	7	266.64	25.098	9.486	272.00

Group	Minimum	Maximum	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Akotogua	228.00	300.00	237.73	286.70
Aglorkpovie	167.50	300.00	182.68	279.75
Kpong	237.00	300.00	243.43	289.86