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Evaluation of the Performance of *Bacillus Subtilis* for Assay of Beta-Lactams in Broiler Chicken Meat

**Mwangi, W. W.**, **Shitandi, A.** and **Ngure, R.**

*Department of Biochemistry and Molecular Biology, Egerton University, P.O. Box 536 Egerton Kenya*

**Abstract**

The uncontrolled and unrestricted use of antimicrobials may lead to undesirable drug residues in the treated animals and their products. However, in the Kenyan poultry industry there is lack of affordable, easy to perform antibiotic residues screening methods. The aim of this study was to evaluate the performance of *Bacillus subtilis* as test organism for assay of beta-lactam antibiotics in chicken meat. Microbiological detection was achieved by agar well diffusion. The test bacteria grew optimally at a pH range of 6-7.3. Zone diameters decreased significantly (p<0.001) when *B. subtilis* was used to detect penicillin G (PEN G) at decreasing pH from 7.3, 7.0 to 6. There was significant (p<0.001) difference in the detection of PEN G in both kidney and liver samples at the different pH values although high antibiotic concentrations produced zones that were not significantly different. It was concluded that the pH of the growth media influences the growth of test organism. Although PEN G was reliably detected below the MRLs, of 50ng/g in both liver and kidney by *B. subtilis*, the pH of the growth media and type of organ affected both the detection and the limits of detection (LODs) of this antibiotic. *Bacillus subtilis* plate at pH 7.3 can be used effectively for routine screening for residues of PEN G in chicken kidney and liver tissues. The *B. subtilis* plate had LOD of 0.1ng/ml in both liver and kidney tissues. This screening test is technically simple and can be carried out in any laboratory.

**Keywords:** *Bacillus subtilis*, agar well diffusion, chicken, PEN G, Kenya

**Introduction**

A wide range of antibiotics are usually administered via feed or drinking water for the prevention and treatment of infectious diseases in poultry. They have also been used to enhance feed efficiency, promote growth and improve productivity (Mitema *et al.*, 2001; Hermes, 2003; Gaudin *et al.*, 2004; Bergwett, 2005). In particular, broiler chicken are grown actively often with
antibiotics to attain maximum weight within a short period of time (Nonga et al., 2009).

The uncontrolled and unlimited use of these antibiotics may however lead to undesirable residues in the treated animals and their products. Drug residues are as a result of the failure of the elimination of the drug from the edible portions of the animal because withdrawal period was not observed. These residues may have adverse effects on both the animals and human beings such as drug resistance and hypersensitivity that could be life threatening (Oslon and Sanders, 1975; Lee et al., 2001; McCracken et al., 2005).

To protect the public against possible health risks caused by such hazards, regulations regarding veterinary use of drugs including withholding periods after antibiotics therapy and maximum residue limits (MRLs) have been formulated (WHO/FAO, 1998; WHO/FAO, 1999) and followed in developed countries (Lee et al., 2001; Donoghue, 2003). However, such regulations are not usually adhered to, especially in developing countries where routine monitoring of drug residues in food is not done (Shitandi and Sternesjo, 2001).

Microbiological agar diffusion tests are widely used to screen for antimicrobial residues in animal tissues. These methods rely on their ability to inhibit growth of sensitive bacteria (Koenen-Dierick and De Beer, 1998; Myllyniemi et al., 1999; Myllyniemi et al., 2000; Popelka et al., 2005). Usually these assays utilize the genus Bacillus because of its high sensitivity to the majority of antibiotics (Popelka et al., 2003; Popelka et al., 2005). In the Kenyan poultry industry, for example, there is lack of cheap, easy to perform antibiotic residues screening methods with the capability for a high sample throughput and which can be used to rapidly sift large numbers of samples for suspect or potential non-compliant results. In this study the performance of a sensitive bacteria B. subtilis in the detection of pencillin G (PEN G) in poultry meat was evaluated. The screening test and its reagents including optimal conditions for this antibiotic were also documented.

**Materials and Methods**

**Bacterial Suspensions**

*Bacillus subtilis* BGA (E. Merck, Darmstadt, Federal Republic of Germany) was used as spore suspension. Five batches of Mueller Hinton agar at pH 5, 6, 7, 7.3 and 8 were prepared for use. Mueller Hinton agar was prepared as per the manufacturer’s instruction and the pH adjusted appropriately using.
0.1M HCl or 0.1M NaOH. After autoclaving at a pressure of 15 psi at a temperature of 121°C for 15 minutes, it was cooled to 55°C. The spore suspension of \textit{B. subtilis} was inoculated into the molten agar and mixed thoroughly to ensure uniform distribution. Approximately $10^5$ spores per ml were determined by spectrophotometry and inoculated. Sterile Petri plates (diameter 90mm) were filled with 15ml of the inoculated media and incubated at 30°C for 18-24 hours to determine the effect of pH on bacterial growth.

\textbf{Test Plates}

Three different plates were used for antibiotic detection: plate I=Mueller Hinton agar (MHA) pH 6, plate II=MHA pH 7 and plate III=MHA pH 7.3. The plates were seeded with \textit{B. subtilis}. The test plates were coded such that the first two letters represented the test organism, the third letter the antibiotic, the fourth letter poultry organ to be used while the fifth number represented the pH e.g. BSPL7 representing plate II with PEN G spiked chicken liver fluid. The sterile Petri dishes (diameter 90 mm) were filled with 15ml of the prepared and seeded media. After solidification, the media was used immediately or stored at 2-5°C for a maximum of 5 days.

Seven holes with diameter of 10mm were punched into the agar layer and filled with 100µl of the artificially spiked kidney or liver fluid at different concentration of the antibiotic, an antibiotic-free negative control and a positive reference standard fortified with the established MRL for PEN G. The holes were distanced at least 30mm from each other. After a pre-diffusion period of about 1 hour, at room temperature, the plates were incubated at 30°C for 13-18 hours. The samples and the standards were run in triplicates. The diameters of the zones of inhibition, from the edges of the wells, were measured and an inhibition zone of $\geq 2$mm was considered as positive. These diameters were measured minus the diameter of the punch hole which was 10mm (Omija \textit{et al.}, 1994; Aila \textit{et al.}, 2009; Nonga \textit{et al.}, 2009).

\textbf{Stock Antibiotic Solutions}

Stock solution of 0.05g/l of PEN G was prepared in distilled water. \textit{In vitro} sensitivity test was carried out by serial dilutions of the stock solution. Dilutions were made in distilled water and/or kidney/liver supernatants. PEN G was obtained from Sigma Chemical Company (St. Louis, MO, USA).
Control Samples

50 µg/l of PEN G, which is MRL concentration for liver and kidney, of PEN G were used as positive control in the search for the limits of detection of each drug in the two tissues. Liver or kidney tissues that were free of any antimicrobial drugs were used as the negative control.

Preparation of Fortified Liver and Kidney

One hundred frozen samples consisting of 50 livers and 50 kidneys from chicken that had not been treated with antibiotics were homogenized with distilled water at a ratio of 1:2 (tissue: distilled water) and the homogenates centrifuged for 5 min at 3000g to eliminate tissue debris. Supernatants from these extracts were used to dilute stock solutions to produce working solutions standards of 0.0125 - 0.083µg/ml of PEN G. These are dilutions of the established MRLs of PEN G for the two tissues (Shitandi and Kihumbu, 2004; Aila et al., 2009).

Determination of Limits of Detection

To verify the detection limits, the spiked supernatants were added to the holes on the agar such that each hole containing a replicate of each drug concentration received 100µl of test solution. Each concentration was analyzed in three replicates. The plates were allowed to stand for 1 h to allow the supernatant to diffuse into the media. They were then incubated at 30°C for 13-18h. The zones of inhibition were measured using a Vanier caliper. A regression line of log concentration (µg/ml/well) vs inhibition zone diameters was used to calculate the LOD (Koenen-Dierick et al., 1995). This was done using GraphPad Prism 5 statistical software (GraphPad Software, Inc. 2009).

Data Analysis

Experimental treatments were arranged in a randomized complete block design. The data was analyzed using GraphPad Prism 5 statistical software (GraphPad Software, Inc. 2009). Two way analysis of variance (ANOVA) was applied to test for the significant differences in mean inhibition zones among the pH and concentrations combinations. Bonferroni post-hoc tests were carried out to compare replicate means. Comparisons were considered significantly different at p values <0.001. Regression analysis of concentration and the inhibition zone diameters was used to determine the LODs.
Results

The five batches of Mueller Hinton agar prepared showed different growth of *B. subtilis* when $10^5$ cfu/ml was used. No growth was observed at pH 5 and 8 while growth was observed at pH values of 6, 7 and 7.3. There was maximal growth after 14-16 hours at 30°C.

Poultry liver and kidney samples with known concentrations of PEN G were analysed and the influence of the type of organ and pH on the sensitivity of the organism was also examined. Tests were carried out for the presence of PEN G residues that ranged from 0.25 to 1.4 times the MRL for poultry tissues. Concentrations of PEN G of 0.0125 - 0.083µg/ml in liver and kidney were tested.

Positive results showed inhibition activities around the well while negative results showed no inhibition. Negative controls showed no inhibition of test organism. The zones of inhibition are presented as mean ±SE in table 1.

<table>
<thead>
<tr>
<th>PEN G concentration (µg/ml)</th>
<th>Kidney Mean zones of inhibition (mm)</th>
<th>Liver Mean zones of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 6.0</td>
<td>pH 7.0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.0125</td>
<td>0</td>
<td>12.9±0.1</td>
</tr>
<tr>
<td>0.0167</td>
<td>0</td>
<td>13.8±0.1</td>
</tr>
<tr>
<td>0.025</td>
<td>0.6±0.1</td>
<td>14.8±0.1</td>
</tr>
<tr>
<td>0.05</td>
<td>3.1±0.1</td>
<td>15.5±0.1</td>
</tr>
<tr>
<td>0.0625</td>
<td>4.7±0.1</td>
<td>16.7±0.1</td>
</tr>
<tr>
<td>0.083</td>
<td>6.3±0.1</td>
<td>17.6±0.1</td>
</tr>
</tbody>
</table>

Change in pH had an effect on drug detection based on the size of the inhibition zone. Two way ANOVA showed that the effect of the pH accounted for 48.93% of the total variance (p<0.0001) observed in liver supernatants while the concentration of PEN G in the liver supernatants accounted for 41.01% of the total variance (p<0.0001). The interaction between concentration and media pH contributed 9.25% of the total variance (p<0.0001). Zone diameters decreased significantly (p<0.001) when *B. subtilis* was used to detect PEN G at decreasing pH from 7.3, 7.0 to 6. Bonferroni posttests showed that there was significant (p<0.001) difference in the inhibition zones measured in BSPL6 and BSPL7, BSPL6 and BSPL7.3, BSPL7 and BSPL7.3, BSPK6 and BSPK7, BSPK6 and BSPK7.3, BSPK7 and BSPK7.3. There was no significant (p>0.05) difference in mean
inhibition zones in the plates compared at 0 µg/ml concentration (Table 1). In the kidney supernatants, media pH accounted for 55.01% of the total variance (p<0.0001). The source of variation from concentration and interaction was 34.96% (p<0.0001) and 9.49% (p<0.0001) respectively. Bonferroni posttests showed that there was no significant (p>0.05) difference in the detection of PEN G in both kidney and liver samples at the different pH values. However, 0.025 µg/ml, 0.05 µg/ml, 0.0625 µg/ml and 0.083 µg/ml produced inhibition zones that were significantly (p<0.001) different when BSPL6 and BSPK6 were compared (Table 1).

The test organism was able to detect PEN G at the legally acceptable levels. PEN G spiked liver and kidney produced large zones at pH 7 and 7.3. The zones were however much reduced at pH 6 on the same plate. Table 2 shows the mean inhibition zones obtained from liver and kidney spiked with the appropriate MRL concentrations.

Table 2: Mean inhibition zones (mm) in the control samples at MRL at pH 6, 7 and 7.3

<table>
<thead>
<tr>
<th>Plate</th>
<th>Antibiotic</th>
<th>MRL (µg/kg)</th>
<th>Mean inhibition zone (mm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pH 6</td>
<td>pH 7</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>PEN G</td>
<td>50¹</td>
<td>3.1 (0.9)</td>
<td>15.5 (0.5)</td>
</tr>
<tr>
<td></td>
<td>PEN G</td>
<td>50²</td>
<td>4.7 (1.0)</td>
<td>15.6 (0.5)</td>
</tr>
</tbody>
</table>

¹Kidney MRL, ²liver MRL for chicken, ³SD in brackets

The detection levels were very low which is indicative of high sensitivity of the test organism. However, plate I and II had the lowest LODs. The LODs were 1.5-6 times below the MRLs. The LODs are shown in table 3.

Table 3: Limits of detection for PEN G at different pH

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Antibiotic</th>
<th>¹MRL (ng/g)</th>
<th>Limits of detection (ng/ml)</th>
<th>B. subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>PEN G</td>
<td>50</td>
<td>pH 6</td>
<td>pH 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>PEN G</td>
<td>50</td>
<td>pH 6</td>
<td>pH 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29.3</td>
<td>0.1</td>
</tr>
</tbody>
</table>

¹Chicken tissues MRLs

Discussion

The growth of B. subtilis is greatly influenced by culture pH. In this study, no bacterial growth was observed at pH 5 and 8. These findings are in accordance with previous studies which reported reduction in bacterial growth at extreme pH values which prevent growth by affecting enzymes
involved in metabolism (Cheong, 2006). Low inocula number of approximately $10^5$ spores per ml could also have led to this observation.

The pH of the test medium is an important factor influencing the detection limits of most antibiotics. The pH influences the permeability of bacteria to antibiotics, the stability and activity of enzymes which inactivate antibiotics and the stability and kinetics of certain antibiotics (Todt and McGroarty, 1992; Corkill et al., 1994; Lakaye et al., 2002). The activity of PEN G decreased on a B. subtilis plate when pH was decreased as has been reported previously (Schwartz, 1965). B. subtilis has penicillinase whose activity is enhanced at low pH. Additionally, it has been shown that penicillin acts as an inducer of this enzyme (Pollock, 1950; Pollock, 1961; Knox and Smith, 1962). It has also been reported that the mechanisms of the effect of pH on antimicrobial activity are inconsistent from drug to drug and also based on the sensitivity or resistance of the test microorganisms to various antibiotics (Amsterdam, 1996; Karraouan et al., 2009). PEN G was detected below the MRLs in all the plates with plates II and III having the lowest LODs in both kidney and liver (Table 3). Plate III gave the largest zones of inhibition for PEN G (Table 1) for both kidney and liver and the LODs were also optimal, meaning that this plate was the most sensitive to residues of PEN G. However these results were at variance with those reported by Popelka et al., (2005). This difference could be attributed to the different sample preparation and matrix effects.

Incurred samples obtained from routine monitoring programs have been used to evaluate the performance of a method (Currie et al., 1998; Myllyniemi et al., 1999; Myllyniemi et al., 2000; Myllyniemi et al., 2001; Okerman et al., 2004). However, such an approach is very much limited by the availability of these samples. It is also impossible to produce incurred samples from different animal species with a specified concentration of specific residue. This has resulted in validation of most microbiological methods using antibiotic standard solutions and hence potential matrix effects are neglected. It is generally expected that the presence of matrix components has a negative effect on the sensitivity of an assay (Okerman et al., 1998; Pikkemaat et al., 2007). However, Myllyniemi et al., (2000) showed that incurred kidney samples containing PEN G or oxytetracycline (OTC) at their MRLs caused wider inhibition zones compared to standard solutions of corresponding concentration. Fortifying extracted matrix fluid with the analyte or analytes at the required concentration, as was done in this case, may give more realistic results although tissue binding is not taken into account (Cantwell and O’Keeffe, 2006; Pikkemaat et al., 2009).
Kidney and liver tissues are commonly used for screening slaughter animals for the presence of antibiotic residues (Hassan et al., 2007; Shahid et al., 2007; Pikkemaat et al., 2008; Pikkemaat et al., 2009). Although chicken liver and kidney have equal MRLs for PEN G, inhibition zones produced by kidney and liver spiked with PEN G were at variance. However, the LODs were generally comparable in both liver and kidney. The presence of naturally occurring growth inhibiting compounds in kidney causes non-specific inhibition (Pikkemaat et al., 2008). This may have led to the variability in sizes of inhibition zones produced by PEN G in kidney and liver. However, some high molecular weight naturally occurring growth inhibiting compounds in kidney, such as lysozymes, were removed by the centrifugation step (Pikkemaat et al., 2008).

Penicillin G was microbiologically detected in both liver and kidney samples in this study. This was in line with previous observations which indicated that *B. subtilis* detect penicillins and tetracyclines as well as other antibiotic families but it is most sensitive for β-lactams detection (Okerman et al., 1998; De Wasch et al., 1998; Jevinova et al., 2003; Okerman et al., 2004; Popelka et al., 2005; Karraouan et al., 2009).

The results from this study showed that PEN G residues could be detected microbiologically in poultry tissues by *B. subtilis*. Plate III is efficient for assay of PENG residues in chicken liver or kidney. This plate gave largest inhibition zones and optimal LOD for this antibiotic.

**Conclusion**

Penicillin G can reliably be detected below the MRLs by *B. subtilis* in broiler chicken meat. Chicken kidneys are small and are not removed with other offals during slaughter. Analysis of chicken liver using microbial agar well diffusion plate test is sufficient for the detection of level antibiotic residues in chicken meat. This approach offers the advantage of not requiring specialized microbiological facilities or expertise in order to perform the analysis. It is technically simple and can be performed in any laboratory as compared to other methods.

**References**

Evaluation of the Performance of Bacillus subtilis for Assay of Beta-Lactams


GraphPad Prism 5. (2009). GraphPad Software, Inc.


oxytetracycline, enrofloxacin and ciprofloxacin residues in bovine and porcine tissues. Food Addit. Contam. 17, 991-1000.


Estimation of Growth Parameters of Indigenous Chicken Populations Intensively Reared in Kenya

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Abstract

This study compared body weights of indigenous chicken (IC) ecotypes from 0 to 34 weeks of age and modeled the growth curves to estimate the associated parameters and their relationship using four growth models, namely: Von-Bertalanffy, Gompertz, Logistic, and Brody. A total of 17,748 age-weight data collected biweekly on 522 birds belonging to five ecotypes, namely, Bomet (BE), Bondo (OE), Kakamega (KE), Narok (NE) and West Pokot (WE) were evaluated. Over the growth period, OE was the heaviest from week 2. However, from week 12 onwards, there were no significant differences in body weight between the ecotypes. The fitted parameters for growth demonstrate ecotype differences \( (P<0.05) \) in the asymptotic weights \( (A) \), maturity index \( (k) \) and scaling parameters \( (b) \) in all the models. However, \( A \) were not different \( (P>0.05) \) among BE, NE and WE. The correlations between \( A \) and \( k \) were negative for all ecotypes in all models, and ranged from -0.63 to -0.99. The age at constant degree of maturity in all the models differed \( (P<0.05) \) among the ecotypes. Estimates from the Brody model indicated that IC attained maturity when older. The study revealed differences in growth curve parameters and patterns among the ecotypes, which indicate genetic influences. Such differences among the ecotypes give the chance to choose the best parental lines for practical IC breeding and development of commercial stock through selection and/ or crossbreeding.

Key words: ecotypes, growth curves, indigenous chicken, Kenya.

Introduction

Indigenous chicken (IC) production in Kenya has potential to contribute to improved rural livelihoods through enhancing their productivity and competitiveness. Their production performance presents evidence of a high between and within ecotype variation to form basis for genetic improvement.
on productivity. Their annual egg production ranges from 20 to 100 eggs, mature live weight from 0.7 to 2.1 kg for females and 1.2 to 3.2 kg for males (Guèye, 1998; Ndegwa and Kimani, 1996; Tadelle et al., 2003). The knowledge on growth processes measured in body mass change overtime is important to improve meat production of IC.

Growth curve models provide a visual assessment of growth as a function of time, and prediction of body weight at a specific age. From dimensional perspective, growth is an increase in size (height, length, weight) with age (Reeds and Fiorotto, 1990). There are several growth models for estimating growth curve parameters in chicken by fitting longitudinal experimental data. Of these models, the most often used which yields mathematical growth parameters that have biological meaning are nonlinear functions, including Logistic, Gompertz, Brody and Von-Bertalanffy models (Fitzhugh, 1976; Karkach, 2006). The growth parameters like the initial body weight, the mature body weight, the rate of maturing and daily gain are important fragmentary aspects of each growing point and can describe the shape of a growth curve (Fitzhugh, 1976; Tsukahara et al., 2008).

Estimation of growth parameters of IC using nonlinear models provides useful biological information for predicting growth rates, feed requirements and responses to selection. This provides sound biological basis for designing genetic and feeding interventions targeted at improving productivity, efficiency and capability of whole growth process (Lambe et al., 2006). Kenya has a large diversity of IC ecotypes but their growth curve patterns are largely unknown to inform the design of appropriate genetic improvement strategies. The objectives of this study were to compare live body weights of IC ecotypes at different ages from 0 to 34 weeks of age and to model the growth curves to estimate the associated parameters and their relationships using four growth models.

Materials and Methods

Data Source

Growth data were obtained from an experimental flock of IC population kept for breeding and nutrition research purposes at Naivasha National Animal Husbandry Research Centre of the Kenya Agricultural Research Institute. Initially the flocks were established through collection of eggs from unselected, random mating populations of IC from the rural farmers. Eggs were collected from Bomet, Bondo, Kakamega, Narok and West Pokot, regions, which were chosen because there has been minimum exotic chicken
genetic dilution in these regions. Five hundred eggs were collected from each area and each of the area represents an ecotype sample. In this study, therefore, five ecotypes were evaluated, namely: Bomet (BE), Bondo (OE), Kakamega (KE), Narok (NE) and West Pokot (WE) ecotypes.

Management of Experimental Birds

Eggs were simultaneously incubated but separated according to ecotype within the incubator. At hatching, each chick was weighed (Hatching weight-HW) and wing tagged with an identification number. Brooding was from hatching (day old) to 6 weeks. Brooding of chicks was done in deep litter brooders using infra-red electric bulbs and separated into ecotypes. The population density was 12 birds/m². At the beginning of the 7th week, chicks were transferred to randomly selected deep litter rearing pens within the same house. Sex was determined by phenotypic appearance. The birds were fed *ad libitum* on commercial formulated feeds for hybrids; on a starter diet from 0 to 6 weeks of age, a growing diet from the 7th to 20th week and a laying diet thereafter. Fresh clean water was provided *ad libitum* daily. Disinfection of brooding and rearing pens were done procedurally. All birds were vaccinated against Marek’s, Gumboro, Fowl Typhoid, Newcastle and Infectious Bronchitis as per recommendations of the veterinarian. Any other incidence of disease condition was treated promptly by a resident veterinarian. Age-weight data was collected bi-weekly for each bird until 34 weeks of age. A total of 17,748 body weight records from 522 birds (149 BE, 139 KE, 72 NE, 86 OE and 76 WE,) produced from 3 hatchings were available for analysis.

Statistical Analyses and Growth Models

The PROC GLM of SAS (1998) was used for analysis of variance of body weight at each age. The fitted model accounted for the fixed-effects of ecotype, sex, hatch and interaction between ecotype and sex. In the analysis of body weight at hatch, egg weight was fitted as a covariate, while in all other ages; body weight at hatch was fitted as a covariate. Least squares means were separated using the probability differences option. The model fitted is as shown in equation 1:

\[
Y_{ijkl} = \mu + H_i + E_j + S_k + ES_{jk} + e_{ijkl}\tag{1}
\]

where \(Y_{ijkl}\) is the body weight of the \(l\)th bird at a particular age, \(\mu\) the overall mean,

- \(H_i\) the fixed effect of \(i\)th hatch \((i=1, 2, 3\) batches\),
- \(E_j\) the fixed effect of \(j\)th ecotype \((BE, KE, NE, OE,\) and \(WE)\),
- \(S_k\) the random effect of hatch and ecotype,
- \(e_{ijkl}\) the error term.
S_k the fixed effect of k^{th} sex (k = male or female), ES_{jk} the interaction between ecotype and sex; and e_{ijkl} the error term associated with each body weight at a particular age.

Four nonlinear growth curve models were fitted to the longitudinal growth data set for each individual bird using the PROC NLIN of SAS (1998) to describe the growth pattern of each ecotype. The models fitted were Brody, Logistic, Gompertz, and Von-Bertalanffy (Fitzhugh, 1976) as shown in equations 2-5:

\[ \text{Brody: } y_t = A \left(1 - b e^{-kt}\right) \]  
\[ \text{Gompertz: } y_t = A e^{-be^{-kt}} \]  
\[ \text{Logistic: } y_t = A \left(1 + be^{-kt}\right)^{-2} \]  
\[ \text{Von-Bertalanffy: } y_t = A \left(1 - be^{-kt}\right)^{g} \]

where \( y_t \) is the observed live weight at age \( t \),

\( A \) the asymptotic or mature weight, parameter \( b \) the scaling parameter, \( k \) the maturity index and \( t \) the age in weeks. The models were evaluated using the goodness of fit criteria determined by \( R^2 \), where the highest \( R^2 \) values indicate best fit of the model to the data. The estimated parameters were fitted into the function to model body weights, absolute growth rate (AGR) and relative growth rate (RGR) at particular ages for each bird. Analysis of variance for the estimated body weights and associated parameters for each equation were performed according to equation (1) above. The least squares means for each model in all the ecotypes were then plotted against age to obtain growth curve patterns. The degree of maturity (\( U \)) was calculated from the definition by Fitzhugh and Taylor (1971) as depicted in equation 6:

\[ U = \frac{y_t}{A} \]  

The age at constant maturity (\( tu \)) were calculated by logarithmically transforming original equations for the models (Tsukahara et al., 2008) and estimated parameters \( b \) and \( k \) used were as shown in equations 7, 8, 9 and 10:

\[ \text{Brody: } tu = -\log \left(1 - \frac{U}{b}\right) \]

(7)
\[
\text{Gompertz: } tu = \frac{-\log\left(\frac{-\log U}{b}\right)}{k} \tag{8}
\]
\[
\text{Logistic: } tu = \frac{-\log\left(\frac{1}{U} - 1\right)}{k} \tag{9}
\]
\[
\text{Von-Bertalanffy: } tu = \frac{-\log\left(1 - \frac{U^3}{b}\right)}{k} \tag{10}
\]

where \(tu\) is the age in weeks, and \(b\) and \(k\) were the estimated values derived from each of the models.

## Results

### Performance of Ecotypes, Goodness of Fit and Parameter Estimation

Table 1 presents the least square means of observed live body weights of IC ecotypes at different ages from one-day old to 34 weeks of age. The KE chicks were heaviest and the WE chicks lightest at hatching. Over the growth period, the OE were the heaviest from week 2. However, from week 12 onwards, there were no significant difference in body weight between the ecotypes. The least square means of the growth curve parameters (\(A\), \(b\) and \(k\)) and their \(R^2\) for the four growth models by ecotypes are presented in Table 2. The \(R^2\) ranged from 0.99 to 1.0 for all the ecotypes in the four models. Generally, the goodness of fit was highest in the Von-Bertalanffy and Gompertz models and lowest in the Brody model, indicated by \(R^2 > 0.99\). This demonstrates the adequacy of these models in fitting and describing the growth data. A comparison of \(R^2\) between ecotypes showed that they were lowest for the OE for Gompertz, Brody and Von-Bertalanffy models.
Table 1: Least square means for observed live body weights of IC ecotypes at different ages from 0 to 34 weeks

<table>
<thead>
<tr>
<th>BWt</th>
<th>Age (days)</th>
<th>Ecotypes</th>
<th>Overall means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BE</td>
<td>KE</td>
</tr>
<tr>
<td>BW0</td>
<td>1.0</td>
<td>32.62±0.31a</td>
<td>38.06±0.35a</td>
</tr>
<tr>
<td>BW1</td>
<td>13.7±0.76</td>
<td>79.20±1.82a</td>
<td>84.25±2.58a</td>
</tr>
<tr>
<td>BW2</td>
<td>28.8±1.95</td>
<td>197.25±4.97a</td>
<td>223.99±7.06a</td>
</tr>
<tr>
<td>BW4</td>
<td>41.5±0.87</td>
<td>373.76±4.90a</td>
<td>404.67±7.08b</td>
</tr>
<tr>
<td>BW6</td>
<td>56.0±0.70</td>
<td>577.81±14.96a</td>
<td>601.91±21.28b</td>
</tr>
<tr>
<td>BW8</td>
<td>70.2±1.80</td>
<td>744.90±21.05a</td>
<td>832.51±29.93b</td>
</tr>
<tr>
<td>BW10</td>
<td>83.6±1.58</td>
<td>887.32±26.02a</td>
<td>952.39±37.01a</td>
</tr>
<tr>
<td>BW12</td>
<td>98.2±1.38</td>
<td>1117.56±39.02a</td>
<td>1115.20±55.50b</td>
</tr>
<tr>
<td>BW14</td>
<td>112.4±0.85</td>
<td>1215.64±39.05a</td>
<td>1312.97±55.54b</td>
</tr>
<tr>
<td>BW16</td>
<td>120.0±1.31</td>
<td>1357.82±44.76b</td>
<td>1409.66±63.66b</td>
</tr>
<tr>
<td>BW18</td>
<td>140.1±1.48</td>
<td>1438.82±50.03a</td>
<td>1577.03±71.15a</td>
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<tr>
<td>BW20</td>
<td>153.8±1.51</td>
<td>1501.95±55.19a</td>
<td>1715.82±78.49b</td>
</tr>
<tr>
<td>BW22</td>
<td>168.1±1.37</td>
<td>1584.99±59.18a</td>
<td>1710.86±84.18b</td>
</tr>
<tr>
<td>BW24</td>
<td>181.2±1.05</td>
<td>1637.23±55.61a</td>
<td>1864.01±79.09b</td>
</tr>
<tr>
<td>BW26</td>
<td>195.2±1.23</td>
<td>1692.79±60.83a</td>
<td>1873.03±86.54a</td>
</tr>
<tr>
<td>BW28</td>
<td>210.0±1.98</td>
<td>1780.81±59.97a</td>
<td>1941.04±85.29a</td>
</tr>
<tr>
<td>BW30</td>
<td>223.8±0.45</td>
<td>1804.93±62.00a</td>
<td>1892.79±88.18a</td>
</tr>
<tr>
<td>BW32</td>
<td>238.3±0.50</td>
<td>1848.76±66.30a</td>
<td>1913.12±94.30a</td>
</tr>
</tbody>
</table>

BWt = live body weight (g) at age t in weeks.
BE= Bomet ecotype; KE = Kakamega ecotype; NE= Narok ecotype ; OE = Bondo ecotype; WE= West Pokot ecotype.
abc = Means in a row with one or more letter superscripts in common are not significantly different (P≤0.05).

The fitted parameters for the nonlinear models for growth (Table 2) demonstrate ecotype differences (P<0.05) in the asymptotic weights (A), maturity index (k) and scaling parameters (b) in all the models. However, A were not different (P ≥ 0.05) among the BE, NE and WE. The highest estimates of A are those from the Brody function followed by the Von-Bertalanffy, Gompertz and Logistic models for all the ecotypes.
Table 2: The least squares means of growth curve parameters and $R^2$ for the four growth models by ecotype

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>Observed body weight</th>
<th>Model</th>
<th>Parameters</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>b</td>
</tr>
<tr>
<td>BE</td>
<td>1848.76</td>
<td></td>
<td>1881.79±27.49a</td>
<td>1.27±0.049a</td>
</tr>
<tr>
<td>KE</td>
<td>1913.12</td>
<td></td>
<td>2025.35±29.86a</td>
<td>1.30±0.051a</td>
</tr>
<tr>
<td>NE</td>
<td>1880.61</td>
<td></td>
<td>2030.90±30.71a</td>
<td>1.32±0.044a</td>
</tr>
<tr>
<td>OE</td>
<td>1972.68</td>
<td></td>
<td>2090.04±33.79a</td>
<td>1.34±0.062a</td>
</tr>
<tr>
<td>WE</td>
<td>1874.09</td>
<td></td>
<td>1948.78±30.02a</td>
<td>1.33±0.060a</td>
</tr>
<tr>
<td>Ecotype</td>
<td></td>
<td>Logistic</td>
<td>A</td>
<td>b</td>
</tr>
<tr>
<td>BE</td>
<td>1848.76</td>
<td></td>
<td>1781.80±37.25a</td>
<td>13.18±2.19a</td>
</tr>
<tr>
<td>KE</td>
<td>1913.12</td>
<td></td>
<td>1928.09±33.46b</td>
<td>13.83±1.951a</td>
</tr>
<tr>
<td>NE</td>
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<td>1904.28±34.56a</td>
<td>15.05±2.013a</td>
</tr>
<tr>
<td>OE</td>
<td>1972.68</td>
<td></td>
<td>1997.87±34.28a</td>
<td>14.66±2.186a</td>
</tr>
<tr>
<td>WE</td>
<td>1874.09</td>
<td></td>
<td>1860.85±35.77a</td>
<td>14.37±2.447a</td>
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<tr>
<td>Ecotype</td>
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<td>Brody</td>
<td>A</td>
<td>b</td>
</tr>
<tr>
<td>BE</td>
<td>1848.76</td>
<td></td>
<td>2591.65±211.16a</td>
<td>1.04±0.020a</td>
</tr>
<tr>
<td>KE</td>
<td>1913.12</td>
<td></td>
<td>2739.57±263.99c</td>
<td>1.05±0.026a</td>
</tr>
<tr>
<td>NE</td>
<td>1880.61</td>
<td></td>
<td>3114.42±407.48a</td>
<td>1.04±0.021b</td>
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<tr>
<td>OE</td>
<td>1972.68</td>
<td></td>
<td>2787.73±282.09a</td>
<td>1.05±0.030a</td>
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<tr>
<td>WE</td>
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<td>2591.09±229.56a</td>
<td>1.05±0.027a</td>
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<tr>
<td>Ecotype</td>
<td></td>
<td>Von-Bertallanfy</td>
<td>A</td>
<td>b</td>
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<tr>
<td>BE</td>
<td>1848.76</td>
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<td>1951.92±40.34a</td>
<td>0.82±0.0417a</td>
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<tr>
<td>KE</td>
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<td>2093.91±37.53a</td>
<td>0.81±0.033a</td>
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<tr>
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<td></td>
<td>2172.89±40.34a</td>
<td>0.81±0.029a</td>
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<tr>
<td>OE</td>
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<td></td>
<td>2154.14±44.16a</td>
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<tr>
<td>WE</td>
<td>1874.09</td>
<td></td>
<td>2008.99±33.88a</td>
<td>0.84±0.037a</td>
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</tbody>
</table>

$^{a}$BE= Bomet ecotype; KE = Kakamega ecotype; NE= Narok ecotype; OE = Bondo ecotype; WE = West Pokot ecotype.

$^{b}$A = asymptotic size body weight (g); b = scaling parameter; k = maturity index

$^{abc}$= Means in a column with one or more letter superscripts in common are not significantly different (P≤0.05).

Correlations between Growth Curve Parameters

Table 3 shows the correlations between $A$ and $k$ by ecotypes for Gompertz, Logistic, Brody and Von-Bertalanffy models. The correlations between $A$ and $k$ were negative for all ecotypes in all models. The coefficients ranged from -0.63 to -0.99 with the highest values estimated by the Brody model and the lowest by the Logistic model for all the ecotypes.
Table 3: Correlation coefficient between A and k by ecotype for the Gompertz, Logistic, Brody and Von-Bertalanffy models

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>Gompertz</th>
<th>Logistic</th>
<th>Brody</th>
<th>Von-Bertalanffy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BE</td>
<td>-0.83</td>
<td>-0.65</td>
<td>-0.98</td>
<td>-0.91</td>
</tr>
<tr>
<td>KE</td>
<td>-0.82</td>
<td>-0.65</td>
<td>-0.98</td>
<td>-0.88</td>
</tr>
<tr>
<td>NE</td>
<td>-0.85</td>
<td>-0.68</td>
<td>-0.99</td>
<td>-0.91</td>
</tr>
<tr>
<td>OE</td>
<td>-0.81</td>
<td>-0.64</td>
<td>-0.98</td>
<td>-0.87</td>
</tr>
<tr>
<td>WE</td>
<td>-0.81</td>
<td>-0.63</td>
<td>-0.98</td>
<td>-0.87</td>
</tr>
</tbody>
</table>

aBE = Bomet ecotype; KE = Kakamega ecotype; NE = Narok ecotype; OE = Bondo ecotype; WE = West Pokot ecotype.

Variation in Age at Constant Degree of Maturity

Estimates of age (in weeks) at 0.67 ($t_{0.67}$) and 0.90 ($t_{0.90}$) degree of maturity in the models are presented in Table 4. The age at constant degree of maturity ($t_u$) in all the models differed ($P<0.05$) among the ecotypes. Estimates from the Brody model indicated that animals attained maturity when older. For example, the hen NE reached 90% maturity when 47.08 weeks old in the Brody model and when 24.25 weeks old in the Logistic model. Figure 2 shows the degree of maturity for all the ecotypes from hatch to 34 weeks of age. All ecotypes showed similar maturation pattern in the attainment of degree of maturity, but KE attained maturity at younger age whereas NE attained maturity when older compared to the other ecotypes from week 4 to 26. The KE, OE and WE ecotypes had degrees of maturity close to each other at all ages and were younger than BE and NE at degree of maturity.

Table 4: Estimated variation in age at constant degree of maturity ($t_{0.67}$ and $t_{0.90}$) for the Gompertz, Logistic, Brody and Von-Bertalanffy models

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>$t_{0.67}$</th>
<th>$t_{0.90}$</th>
<th>$t_{0.67}$</th>
<th>$t_{0.90}$</th>
<th>$t_{0.67}$</th>
<th>$t_{0.90}$</th>
<th>$t_{0.67}$</th>
<th>$t_{0.90}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BE</td>
<td>16.61</td>
<td>26.62</td>
<td>15.63</td>
<td>22.45</td>
<td>28.42</td>
<td>38.18</td>
<td>19.42</td>
<td>32.36</td>
</tr>
<tr>
<td>KE</td>
<td>16.53</td>
<td>26.47</td>
<td>15.70</td>
<td>22.70</td>
<td>27.59</td>
<td>37.06</td>
<td>17.48</td>
<td>29.67</td>
</tr>
<tr>
<td>NE</td>
<td>18.70</td>
<td>29.05</td>
<td>16.90</td>
<td>24.25</td>
<td>35.05</td>
<td>47.08</td>
<td>19.42</td>
<td>32.36</td>
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<tr>
<td>OE</td>
<td>16.21</td>
<td>25.76</td>
<td>15.43</td>
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<td>WE</td>
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<td>21.97</td>
<td>26.00</td>
<td>34.93</td>
<td>16.49</td>
<td>29.67</td>
</tr>
</tbody>
</table>

aBE = Bomet ecotype; KE = Kakamega ecotype; NE = Narok ecotype; OE = Bondo ecotype; WE = West Pokot ecotype.
Figure 2: Degree of maturity from hatch to 34 weeks of age using the four growth models: Bomet ecotype (——); Bondo ecotype (— — —); Kakamega ecotype (— — —); Narok ecotype (——); West Pokot ecotype (— — —).
Discussion

Performance of Ecotypes and Goodness of Fit

Under the intensive management practices applied, the IC ecotypes showed variations in growth consistent with results from Ethiopia (Dessie and Ogle, 2001), Tanzania (Msoffe et al., 2001), Zimbabwe (Mcainsh et al., 2004), Bangladesh (Bhuiyan et al., 2005) and Botswana (Badubi et al., 2006). The foregoing studies have demonstrated presence of several ecotypes, breeds and strains of IC that are well adapted to specific environmental conditions.

The observed mean hatching weight (29.85 to 38.08 g) were 5.55 to 11.58 g heavier than those reported under intensive production system in Nigeria (24.3 to 26.5 g) (Adedokun and Sonaiya, 2001), Ethiopia (28 to 30.8 g) (Dessie and Ogle, 2001; Tadelle et al., 2003) and Tanzania (26 to 30 g) (Msoffe et al., 2004). The wide variations in hatching weights (29.85 to 38.08 g) of chicks follow the egg weight pattern in the parental population (Teketel, 1986), attributed to positive correlation with egg weights and age of hens at lay (Saatci et al., 2005; Ojedapo et al., 2008; Magothe et al., 2010). Consistent with the present results, 1 g egg weight difference results in 1.5 g body weight differences at hatch (Tadelle et al., 2003).

The Kenya’s IC ecotype populations seem generally heavier compared to other African IC populations. For instance, the 8th week body weight (542.80 to 616.09 g) estimates in Kenyan IC ecotypes are about 2 to 5 times heavier than reported by Adedokun and Sonaiya (2001) for Nigerian (242 to 311g) and Msoffe et al. (2004) for Tanzanian (118 to 358 g) IC populations. At 20 weeks, Kenya’s IC populations had body weights (1438.82 to 1527.34 g) that were about 2 (1.74 to 2.24) times heavier than reported in Nigeria (948 to 1096g) (Adedokun and Sonaiya, 2001), Tanzania (Msoffe et al., 2004) (741 to 1089 g) and Ethiopia (643 to 877 g) (Tadelle et al., 2003) IC populations. The weight differences in the first 8 weeks of age may be attributable to maternal effects arising from the egg size (Nwosu, 1979).

The observed differences between and within the IC ecotypes may be attributed to genetic diversity of these populations, age and size of hens used to produce the experimental birds and the prevailing wide variations in diurnal ambient temperatures, which for present experimental birds ranged from 8 to 26°C (NAHRC, 2000) and altitude (Zhang et al., 2008). The presence of variations in body weight among ecotypes of IC population established in this study may be the result of geographical isolation as well as periods of natural and artificial selections. Previous studies in Senegal

(Missohou et al., 1998); Ethiopia (Halima et al., 2006) and findings by Hauser et al. (1995) state that ecological subdivision allows the establishment of population traits with possible changes in genetic effect.

The $R^2$ values were high ($R^2 > 0.98$) for all growth models, indicating a significant relationship between age and weight in all the ecotypes. Based on $R^2$ alone, all the models in this study seemed to appropriately describe the association between ages and live weight, which is in agreement with observations made by Sengul and Kiraz (2005).

**Correlations between Growth Curve Parameters**

The negative correlations coefficients (-0.63 to -0.99) between $A$ and $k$ for all the ecotypes in all the models were consistent with the analyses of Taylor and Fitzhugh (1971). The IC breeding program should focus on both the large body frame and early maturity at the same time to improve flock productivity and increase returns for enhanced commercial competitiveness.

**Variation in Age at Constant Degree of Maturity**

The optimum timing of age at first breeding for females and approximate maturity provides interesting information for producers (Tsukahara et al., 2008). It would be useful to have more data on the weight at first estrus as a proportion of mature weight in various breeds in order to prevent premature breeding and stunted growth (Devendra and Burns, 1983). Ages at constant degree of maturity ($t_{0.67}$ and $t_{0.90}$) were different among the ecotypes. Variations in age at a constant degree of maturity were in harmony with reports in other countries (Mcainsh et al., 2004; Bhuiyan et al., 2005; Badubi et al., 2006). The KE, WE and OE attained degree of maturity closer to each other at all ages and were younger than BE and NE and, therefore, may start breeding earlier.

**Conclusions**

The study revealed differences in growth curve parameters and patterns among the ecotypes, which indicate genetic influences. Such differences among the ecotypes give the chance to choose the best parental lines for practical indigenous chicken breeding, develop breeding strategies and developing a commercial stock through selection and/or crossing programs within the indigenous chicken ecotypes. Von-Bertalanffy, Logistic and Gompertz models fits and describes indigenous chicken growth data adequately ($R^2 > 0.98$).
Acknowledgement

We are grateful to the International Foundation for Science (IFS) and the Kenya Agricultural Productivity Project (KAPP) for funding this study, and the National Animal Husbandry Research Centre (NAHRC) and Egerton University for provision of facilities for the study.

References


Figure 1: Estimated body weight at various ages using the four growth models: Bomet ecotype ( ); Bondo ecotype ( ); Kakamega ecotype ( ); Narok ecotype ( ); West Pokot ecotype ( )
Spatial and Temporal Variations of Zooplankton in Relation to Some Environmental Factors in Lake Baringo, Kenya

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Abstract

The zooplankton community of Lake Baringo, a shallow and turbid lake in the eastern arm of the Great Rift Valley in Kenya, was studied between April 2008 and November 2009. This study focused on the spatial and temporal distribution, composition and abundance of zooplankton in the lake. Physico-chemical variables measurements and triplicate zooplankton samples were taken monthly from five stations. The zooplankton community comprised 31 species. Only two Copepod species, Thermocyclops consimilis and Thermodiaptomus galebi were recorded in the orders Cyclopoida and Calanoida respectively. Cladocera was represented by 8 species, with Diaphanosoma excisum being the most common in all sampling stations. Rotifera, with five families, had a total of 21 species. Spatially, species diversity ranged from 0.649 in C2 to 0.695 in C1 while temporally these were 0.36 to 0.87 in September 2009 and June 2008 respectively. The mean zooplankton abundance per station varied from 27.46±4.16 individuals l\(^{-1}\) in December, 2008 to 120.13±17.50 individuals l\(^{-1}\) in November 2009. Among sampling sites, C3 had the highest abundance (79.09±7.95 individuals l\(^{-1}\)) and the lowest abundance was recorded at C1 (56.37±6.58 individuals l\(^{-1}\)). There was significant difference between the sampling stations (\(P<0.001\)) and among the sampling months (\(P<0.001\)). A number of environmental parameters were correlated with the abundance of different species of zooplankton.

Key words: Lake Baringo, Zooplankton, Distribution, Abundance, Environmental factors

Introduction

Zooplankton play a prime role in the functioning and productivity of aquatic systems and make up a major portion of the abundance and biomass of these ecosystems (Gannon & Stemberger, 1978). In addition to their numerical contribution, zooplankton occupy a significant intermediate position in aquatic food chains and are important in energy transfer and have the potential to regulate the structure of phytoplankton and zooplankton prey assemblages. The organisms form a significant component of the diets of many fish and have also been used as indicators of water quality (Ogari & Dadzie, 1988; Hoxmeier & Wahl, 2004).

The structure of zooplankton communities is influenced by climatic and physico-chemical parameters as well as biological interactions. Some species are therefore found in a wide range of environmental conditions, while others are limited by many physico-chemical factors (Gannon & Stemberger, 1978; Neves et al., 2003). While Calanoida are generally abundant in oligotrophic environments, cyclopoids and cladocerans dominate in eutrophic waters (Margaleff, 1983; Wetzel, 1990). A complex set of related factors have been implicated for the seasonality in population dynamics and species succession. These include physical and chemical variables (Angino et al. 1973), food (Behn & Boumans, 2001; Abdel-Aziz & Gharib, 2006) and predation (Jeppesen et al. 2005). Comparisons have also been made between the abundance and biomass of micro- and macrozooplankton (Pace et al. 1998, Sprules et al. 1988) to algal chlorophyll a (Canfield & Jones, 1996).

Many lakes in the world are shallow and are thus susceptible to substantial sediment resuspension during windy conditions (Wetzel & Likens, 1990). Besides the allochthonous materials from the catchment, the high turbidity in Lake Baringo has been attributed to the resuspension of the sediment daily by winds in the lake (Oduor, 2000). The suspended solids influence the Secchi depth, euphotic zone and light attenuation coefficient. Resuspended sediments exert many direct and indirect effects on food webs. It may also decrease or increase primary productivity depending on the relative extent to which it attenuates light (Hart, 1992). Elevated turbidity also reduces the feeding success of fish that locate prey visually (Gardner, 1981).

Despite the importance of zooplankton in freshwater ecosystems, little is known about the diversity, distribution and abundance of zooplankton in Lake Baringo. The present study aims at evaluating the effect of environmental factors on spatial and temporal zooplankton community structure in the lake. Lake Baringo, like other lakes with high drainage ratios
are expected to have a Secchi depth of 4 – 5m, but the lake has much lower values (~ 50cm), indicating high turbidity, probably because of its closed endorrrheic nature and catchment degradation. Nutrients inflow and retention rate are also expected to be high. This study hypothesized that zooplankton dynamics would be governed not by hydrographic and nutrient factors but rather by physico-chemical and hydrological factors such as temperature, pH, conductivity and seasonal runoff.

Materials and Methods

Study Area

Lake Baringo is located in Baringo District of Rift Valley Province in Kenya. It is one of the lakes in the eastern arm of the Great Rift Valley between latitude 0˚30` N and 0˚45` N and longitude 36˚ 00` E and 36˚ 10` E and lies approximately 60Km north of the equator at an altitude of 975 m above sea level (Figure 1). The lake has a surface area of approximately 130
Figure 1: Map of Lake Baringo showing the sampling stations S2, C1, C2, C3 and S2
Km$^2$ and a catchment area of 6,820Km$^2$. Its depth varies with an average of about 3m and a deepest point of about 7m. The lake became a Ramsar site on the 10$^{th}$ January 2002. It is a critical habitat and refuge for more than 500 species of birds and fauna, some of the migratory aquatic bird species being significant regionally and globally.

The lake is part of the Great Rift Valley system with the Tugen Hills, an uplifted fault block of volcanic and metamorphic rocks, lying west of the lake at an altitude of 300-1000m Above Sea level while Laikipia escarpment lies to the east. The lake has several small islands with the largest being Ol Kokwe. A group of hot springs discharge along the shoreline at the northeastern corner of the island. The lake is fed by several rivers, Molo, Perkerra and Ol Arabel, and has no obvious outlet; the waters are assumed to seep through lake sediments into the faulted volcanic bedrock. Most of the rivers and streams enter the lake at the southern and eastern shores where they form swamps harbouring different types of macrophytes dominated by emergent *Typha domingensis* and submerged *Ceratophyllum demersum*. However, damming of some of these rivers has reduced the amount of water reaching the lake. The climate of the area is arid to semi-arid with two seasons; the dry season is from September to February while there are rains between March and August. Rainfall ranges from about 600mm on the east and south of the lake to 1500mm on the western escarpment of the Rift Valley. Soil erosion and the subsequent deposition of the eroded materials in waterways and water bodies is one of the most serious environmental problems facing Lake Baringo. The erosion has caused land damages and deposited the silt into Lake Baringo causing serious turbidity and siltation.

Samples were obtained monthly from five stations in the lake representing different habitats between April 2008 and November 2009. Stations S2 and C3 have river influence and have adjacent swampy shorelines while C1 and N2 have no river influence and have rocky adjacent shorelines. Station C2 occurs at the centre of the lake and has intermediary characteristics to the other stations. Global positioning system (GPS) navigational unit (Garmin II model) was used to track sampling positions. A surveyor II model hydrolab was used for the measurements of temperature, dissolved oxygen, pH, conductivity and redox potential at 0.5m depths. Turbidity was measured with a HACH 2100P turbidimeter while a 20cm diameter black and white Secchi disc was used to determine transparency. Maximum depth was determined using a marked rope with weight at the end.

Water samples for nutrients and chlorophyll-\(a\) analyses were collected using a 4 litre Van Dorn sampler and placed in plastic bottles. These were kept in a
cooler box at 4°C and transported to the laboratory. In the laboratory, samples were filtered using 0.45μm pore size filter papers to remove phytoplankton. The levels of soluble reactive phosphorus (PO₄-P), total phosphorus (TP), nitrate-nitrogen (NO₃-N), ammonium-nitrogen (NH₄-N), silica and nutrients were determined according to standard methods (APHA, 1995). These were analysed within three days after sampling.

Triplicate zooplankton samples were collected using a conical net with a mesh size of 60μm and a diameter of 30cm at the opening. The net was lowered close to the bottom of the lake without disturbing the sediment and hauled vertically to the surface and the depth noted from the marked rope. The net was rigged with a weight suspended from the receptacle to ensure the hauls are vertical. The net was washed after each haul to rinse off any zooplankton, which could remain in the net. After collection, the material retained in the net was kept in 400ml plastic bottles and fixed in 4% formalin. Zooplankters were counted in sub samples of 1-3ml, depending on their density, using a plastic pipette and a gridded counting chamber under an optical microscope (x25). The effect of surface tension on the specimens was reduced by addition of a few drops of liquid detergent while visibility was improved by dyeing with Lugol’s solution.

The number of individuals per litre of lake water (D) was determined using the formula:

\[ D = \frac{N}{V}, \quad \text{where} \]

\[ N = \frac{\text{number in sub-sample} \times \text{Volume of sample}}{\text{sub-sample volume}} \]

\[ V = \text{volume of lake water filtered} = \pi r^2 d, \quad \text{where} \]

\[ r = \text{radius of mouth of net (15cm)} \]
\[ d = \text{depth of haul} \]

Abundance was expressed as individuals per litre of lake water. Besides samples from the established sampling stations, qualitative samples were also collected from a variety of different habitats using a hand net fixed to a wooden handle. The additional habitats included macrophytic areas and rocky shores. Zooplankton were identified using relevant taxonomic literature. Korovchinsky (1992) and Smirnov (1996) were used in Cladocera identification while Koste (1978), Koste & Shiel (1987) and Segers (1995) were used for the identification of Rotifera. Species diversity was calculated using Shannon-Weiner diversity index, \( H' \) (Shannon & Weiner, 1963).

The abundance data was standardized as number of individuals per litre of water (individuals l⁻¹). To compare the zooplankton abundances and
environmental parameters, Kolmogorov-Smirnov and Lilliefors tests were first applied to check the normality of distribution and the homogeneity of variances. Due to the heteroscedasticity of the zooplankton data (K-S \( P < 0.01 \); Lilliefors \( P < 0.01 \)), it was transformed using log \((x + 1)\) transformations to avoid violations of linearity assumptions and one way ANOVA was then used to determine significant differences between spatial (over all stations) and temporal (over all seasons) distribution of zooplankton abundance using STATISTICA 8.0 computer package.

**Results**

**Physico-chemical Parameters**

Variations in the physico-chemical variables are shown in Table 1. There was little variation in the values of physico-chemical parameters between sampling stations (two-factor ANOVA \( P = 0.164 \)). However, a significant difference between months was evident (\( P = 0.024 \)). The deepest and the lowest sites in the lake were N2 (5.15 ± 0.14 m) and C3 (3.69 ± 0.14 m), respectively, with a clear increasing trend from south (S2) to north (N2). Water transparency increased from south to north with the deepest Secchi depth of 31.5 ± 0.14 cm at N2 and the shallowest of 25.68 ± 1.20 cm at S2 (Table1).

**Table 1: Mean (± SE) values of some physico-chemical and biological parameters measured at the sampling stations (n = 20)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>S2</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>N2</th>
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<tr>
<td>Depth (m)</td>
<td>4.10±0.08</td>
<td>4.56±0.09</td>
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<td>Secchi (cm)</td>
<td>25.68±0.79</td>
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<td>Turbidity (NTU)</td>
<td>90.94±2.62</td>
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<td>87.0±3.02</td>
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<td>Temperature (°C)</td>
<td>26.18±0.17</td>
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<td>8.70±0.03</td>
<td>8.70±0.03</td>
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<td>8.67±0.03</td>
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<td>Conductivity (μScm⁻¹)</td>
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<td>647.42±10.93</td>
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<td>70.72±1.86</td>
<td>68.40±9.99</td>
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<td>196.93±6.19</td>
<td>194.03±6.69</td>
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<td>DO (mg l⁻¹)</td>
<td>6.42±0.09</td>
<td>6.94±1.06</td>
<td>7.12±0.11</td>
<td>6.68±0.10</td>
<td>7.03±0.10</td>
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<td>Chl a (mgm⁻³)</td>
<td>16.84±1.69</td>
<td>13.27±0.75</td>
<td>15.35±0.84</td>
<td>16.26±1.21</td>
<td>13.96±1.08</td>
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<td>Silicates (mg l⁻¹)</td>
<td>27.72±0.58</td>
<td>27.86±0.49</td>
<td>27.87±0.56</td>
<td>26.90±0.4</td>
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<td>SRP (μg l⁻¹)</td>
<td>14.85±1.46</td>
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<td>NH₄ (μg l⁻¹)</td>
<td>63.87±9.38</td>
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<td>NO₃ (μg l⁻¹)</td>
<td>6.33±0.52</td>
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<td>5.94±0.42</td>
<td>5.37±0.4</td>
<td>5.36±0.42</td>
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</table>

Water temperatures were generally high with the mean temperature ranging from 26.18 ± 0.17°C at site S2 to 28.44 ± 0.22°C at site C1. Values of pH ranged between 8.67 ± 0.03 at site C3 and 8.72 ± 0.03 at C2 while dissolved oxygen concentration ranged between 6.42 ± 0.09 mg.l⁻¹ at S2 and 7.12 ± 0.11 mg.l⁻¹ at C2. Conductivity values were generally high but with little variation between stations.

Soluble reactive phosphates, silicates and ammonia exhibited erratic changes in concentration while nitrates concentrations showed little variation throughout the sampling period (Figure 2). The average values of nutrients fluctuated between minima of 22.44 mg.l⁻¹, 4.46 µg.l⁻¹ and 15.06 µg.l⁻¹ and maxima of 33.58 mg.l⁻¹, 46.14 µg.l⁻¹ and 134.4 µg.l⁻¹ for silicates, soluble reactive phosphates and ammonium respectively.

![Figure 2: Chemical variables (± SE) between April 2008 & November 2009](image)

The lowest and highest concentrations of Chlorophyll a of 4.86 (± 0.72) and 30.7 (± 5.31) mg.m⁻³ were realized in June 2009 and November 2009 respectively (Figure 3). Other peaks occurred in the months of July 2008, February 2009 and July 2009. While the July 2008 peak was followed by a gradual decline up to December, in July 2009 and August there was a decrease, followed by immediate increase from September to November.
Zooplankton Species Composition and Distribution

A total of 31 species of zooplankton were recorded in Lake Baringo between April 2008 and November 2009 (Table 2). Only two species of Copepoda were recorded, *Thermodiaptomus galebi* (Diaptomidae) and *Thermocyclops consimilis* (Cyclopidae) in the orders Calanoida and Cyclopoida, respectively. Cladocerans were represented by five families dominated by Chydoridae with three species. Others included Daphnidae, Sididae, Macrothricidae and Moinidae. Rotifera, which was the most speciose, was dominated by Lecanidae with 12 species followed by Brachionidae with 6 species. Other families represented were Euchlanidae, Filinidae and Mytilinidae. The list of rotifers may, however, not be complete considering the mesh size of the net used. Because of the turbidity use of smaller mesh size had the risk of clogging. During the study period, zooplankton was dominated by euplanktonic organisms. However, littoral and periphytic rotifers (*Lecane* spp and *Mytilina ventralis*) and cladoceran species (*Macrothrix spinosa*, *Alona* spp and *Chydorus* sp) occurred in the lake pelagial in low numbers. The latter were common in the qualitative samples from the swampy areas in the southern and eastern parts of the lake.

Table 2: List of zooplankton species recorded in Lake Baringo between April 2008 and November 2009

<table>
<thead>
<tr>
<th>Copepoda</th>
<th>Cladocera</th>
<th>Rotifera</th>
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<tbody>
<tr>
<td>Cyclopoida</td>
<td>Daphnidae</td>
<td>Brachionidae</td>
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<tr>
<td>Cyclopidae</td>
<td>Ceriodaphnia cornuta</td>
<td>Brachionus angularis</td>
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<tr>
<td><em>Thermocyclops consimilis</em></td>
<td>Daphnia barbata</td>
<td><em>B. calyciflorus</em></td>
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<tr>
<td>Calanoida</td>
<td>Sididae</td>
<td><em>B. falcatus</em></td>
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<tr>
<td>Diaptomidae</td>
<td>Diaphanosoma excisum</td>
<td><em>B. patulus</em></td>
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Figure 3: Mean values (± SE) of Chlorophyll a during the sampling period
Copepoda dominated zooplankton in abundance in all the study sites and throughout the study period. Among the sampling stations the group formed 60-72% of the total zooplankton (Figure 3a) while in the sampling period this proportion was 44-87% (Figure 3b). Copepods were in turn dominated by the juvenile stages, nauplii, with a minimum and maximum proportion of 37.3 and 90.4% in August 2008 and September 2009, respectively. The proportion of Calanoida decreased from 7.4% in April 2008 to 0.04% in October 2008 after which none was recorded in the samples.

Figure 3a: Percentage composition of the major zooplankton groups at stations sampled

Cladocerans were dominated by *D. excisum* which appeared in all samples throughout the study period. The species dominated in 80% of the sampling period with a dominance ratio of between 26 and 96% in the months of November 2008 and August 2009, respectively (Figure 4). Other common species included *M. micrura, C. cornuta, D. barbata* and *M. spinosa*.

*Figure 3b: Percentage composition of the major groups of zooplankton in Lake Baringo during the study period*

*Figure 4: Percentage composition of different Cladocera species in Lake Baringo between April 2008 and November 2009*
Rotifera were dominated by *F. opoliensis* and *K. tropica*. The former dominated in all the months sampled, with a dominance ratio of between 6 and 85%, except in May 2008 when the latter dominated. Other species recorded included *B. angularis*, *B. calyciflorus*, *B. falcatus*, *B. patulus*, *Hexarthra* sp and *Polyarthra* sp (Figure 5). Proportion of *F. opoliensis* was highest (85%) in March 2009 followed by 83% in the month of October 2009. The species, however, had the lowest relative composition of 6% in May 2008 when *K. tropica* had the highest proportion of 44%. *B. patulus* was observed between April and September 2008 after which the organism was absent in the samples.

**Zooplankton Abundance and Diversity**

Zooplankton abundance ranged from 17 individuals l\(^{-1}\) at station C2 in December 2008 to 163 individuals l\(^{-1}\) at N2 in November 2009 over the period of study. Among sampling sites C3 had the highest mean abundance (79.1±8.0 individuals l\(^{-1}\)) and the lowest abundance was recorded at C1 (56.4±6.6 individuals l\(^{-1}\)) (Figure 6a). The mean zooplankton abundance per station varied from 27.46±4.16 individuals l\(^{-1}\) to 120.13±17.50 individuals l\(^{-1}\) in December 2008 and November 2009 respectively (Figure 6b).
Two-way analysis of variance on log (x+1) transformed abundance data revealed that there was significant difference among the sampling months ($P<0.001$), and between the sampling stations ($P<0.001$). Further analyses using Duncan’s multiple range test grouped similar stations and months with respect to densities. Stations C2 and N2 ($P = 0.472$) and S2 and C3 ($P = 0.472$) formed the two group of stations while several groups of months were also formed.

Lake depth was negatively correlated with all zooplankton species except *D. barbata* ($r = 0.20$) and *M. micrura* ($r = 0.14$) (Table 3). Turbidity was positively correlated to all the zooplankton except *D. barbata* and *B. patulus*. Conductivity was positively correlated with all the organisms except *M. micrura, D. barbata, B. calyciflorus* and *B. patulus* while Chlorophyll a was only negatively correlated to *D. barbata* and *B. patulus*.
Table 3: Pearson’s correlation coefficients (r) between some abiotic and biotic factors

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<td>-0.18</td>
<td>0.48</td>
<td>-0.32</td>
<td>0.60</td>
<td>0.35</td>
<td>-0.75</td>
<td>0.95</td>
<td>1</td>
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</tr>
<tr>
<td>DO</td>
<td>-0.63</td>
<td>-0.82</td>
<td>-0.55</td>
<td>-0.81</td>
<td>0.30</td>
<td>-0.82</td>
<td>-0.76</td>
<td>-0.57</td>
<td>0.20</td>
<td>-0.37</td>
<td>-0.97</td>
<td>0.66</td>
<td>-0.71</td>
<td>-0.46</td>
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<tr>
<td>Chl a</td>
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<td>0.54</td>
<td>0.39</td>
<td>0.91</td>
<td>-0.66</td>
<td>0.95</td>
<td>0.22</td>
<td>0.53</td>
<td>-0.61</td>
<td>0.68</td>
<td>0.70</td>
<td>-0.64</td>
<td>0.94</td>
<td>0.88</td>
<td>-0.72</td>
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</tr>
</tbody>
</table>


Mean zooplankton diversity was found, in some instances, to be inversely related to zooplankton abundance. Mean (± SE) zooplankton diversity was highest at C1 followed by N2 (Figure 7a) while temporally this was highest in June 2008 and lowest in September 2009 (Figure 7b). Diversity decreased steadily from April 2008 to March 2009 after which there was an increase in April 2009 followed with another constant decline to September then a rise to November.
Figure 7: Mean zooplankton diversity by station (a) and by month (b)

Discussion

The high water temperatures recorded in the study were mainly due to the high intensity of solar radiation the lake being situated in an arid area. The difference in temperatures at different stations was due to the different times of sampling with stations sampled early in the morning recording lower temperatures. Besides the belief that there is an underwater outlet in the

northern part of the lake, the steady decrease of depth at the rate of 12 cm per
month between May and November 2009 could also be attributed to the high
temperatures which led to loss of lake water through evaporation. With
increased evaporation of water from the lake, the decrease in water
transparency was expected due to increased concentration of insoluble
particles. The same reason could be given to the steady increase in
conductivity. The frequent peaks of nutrients were probably caused by their
flushing into the lake after rains in the catchment.

The high turbidity recorded in the lake had also been recorded by other
investigators (Wahlberg et al., 2003) and was attributed to the resuspension
of the sediment by wind action (Oduor, 2000). The active resuspension of
bottom sediments into the water column is a common feature of shallow
lakes which may have negative or positive impact on distribution and
abundance of zooplankton (Ghidini et al., 2009). The suspended solids in
turn influence the Secchi depth, euphotic zone and light attenuation
coefficient. Brown-water lakes have some physical and chemical features
different from those of clear water lakes, which can affect the growth and
distribution of plankton organisms. The latter absorbs solar radiation and
results in a steep thermal stratification and high thermal stability, in
particular in small and sheltered lakes, and an increased extinction of light
(Eloranta, 1999). Turbidity indirectly affects the level of dissolved oxygen
by limiting photosynthesis by reducing light penetration in water.

Zooplankton community of Lake Baringo was characterized by both
planktonic and macrophytic-loving species with fairly low species richness,
especially for Copepoda. The highest species diversity was recorded at C1, a
station adjacent to a shelter bay, with least influence of the rivers and wind.
Tiwari & Vijayyalakshimi (1993) attributed high diversity to calmer and
more stable waters. The low species richness of copepoda has also been
reported in the Ethiopian Rift Valley Lakes Abiata and Langano (Wodajo &
Belay 1984) and Kivu (Sarmento et al. 2009). The low abundance and
diversity found in this study might be explained by the unfavourable
conditions such as the low penetration of light due to high turbidity of water
and presence of planktivorous fish species. Higher abundances and species
richness in shallow lakes than in the larger African Great lakes pelagic
environments had earlier been reported by Lehman (1996). Green (1967)
showed that rotifers are more species rich and abundant in the lake littoral
regions than in the open waters which is corroborated by results of our
studies where most of the rotifer species, especially *Lecane* spp, were
realized in the qualitative samples from the littoral swampy areas. Besides
their small size which them difficult to locate by predators, their life history

characteristics of parthenogenesis and eutely results in short maturation times and rapid rates of population growth under suitable conditions (Bennett & Borass, 1989).

The occurrence of macrophytic species may be attributed to the presence of swamps in the southern and eastern parts of the lake. Occurrences of such species have previously been associated with algal blooms in Lake Turkana (Ferguson 1982). Gehrs (1974) had earlier also observed that horizontal distribution of the animals may be influenced by aquatic macrophytes. The latter have been reported to affect the distribution and abundance of zooplankton by providing refugia and food (Carpenter & Lodge, 1986; Cronin et al., 2006).

The dominance of cyclopoids in the lake in this study corroborates the findings of earlier investigations in African lakes (Mavuti & Litterick, 1991, Ndawula, 1994). Drenner et al. (1978) attributed the dominance of cyclopoid copepods in turbid habitats to their efficiency at escaping from fish predators. In the present study, while nauplii and cyclopoids were recorded throughout the sampling period, no calanoids were recorded after September 2008 a phenomenon which we attributed to the increasing conductivity. Conductivity was negatively correlated to Calanoida (r = -0.251) and B. patulus (r = -0.320).

In the present study, D. barbata was found to be strongly correlated to turbidity (r = 0.634). Hart (1992) described the species as a typical turbid water species. The occurrence of the large bodied D. barbata in Lake Baringo where several species of fish occur is probably because of the high turbidity. This has also been reported in the turbid Lake Chilwa in Malawi where the cladoceran dominated the zooplankton community despite the high densities of cichlid fishes (Kalk, 1979). Geddes (1984) also reported that fish predators in turbid habitats may be non-selective thus allowing the persistence of large zooplankton species.

The two stations, S2 and C3, which had the highest zooplankton densities, were at the vicinity of river/ stream mouths confirming earlier reports of increases of abundance in such habitats in other water bodies (Green, 1971; Gophen, 1972). The inflowing water brings into the lake particulate organic matter and nutrients. Peak zooplankton densities in our study coincided with those of nutrients and Chl a, findings supported by the positive correlation between Chl a and density of most zooplankton species. These increase may be due to greater availability of food in form of phytoplankton in the lake due to increase of nutrients brought in from inflowing river waters. Green
(1971) also attributed peak zooplankton abundance during rains to the nutrient influx thus increase in phytoplankton production.

**Recommendation**

Considering the possible effects of frequent resuspension of sediments and high turbidity on some zooplankton taxa, care should be taken to protect Lake Baringo from catchment nutrient loads as this could easily lead to rapid eutrophication and consequent ecological problems.

**Acknowledgements**

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**References**


Computational Pool-Testing Strategy

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Abstract

Pooling sample for the purpose of testing has a long history dating back to the Second World War with its first application being screening of pooled urine samples for the presence of syphilis. Recently, pooling has been used in epidemiological studies for screening of human immunodeficiency virus HIV/AIDS antibody to help curb the spread of the virus. Pooling reduces the cost but also – and more importantly – offers a feasible way to lower the misclassifications associated with labeling samples when imperfect tests are used in inspection. Computer intensive pool-testing strategy is discussed in this study with a view to digitalizing the procedure. Moments of the number of tests as in the traditional pool-testing strategy have been digitized via MATLAB code and the same has been done for the case of misclassifications associated with this strategy. Moments are used in the computation of the cost or efficiency associated with pool-testing procedure. The digitalization provides insight into the merits and demerits of the pool-testing procedure supported by empirical evidence.

Keywords: Pool; Pooling; Specificity; Sensitivity; Tests; Misclassifications.

Introduction

The idea of pool testing originated during World War II as an economical method of testing blood samples of army inductees in order to detect the presence of infection (Dorfman, 1943). A pool test is a test performed on a pool of more than one item in which a negative reading indicates the group contains no diseased items and a positive reading indicates the presence of at least one diseased. The basic idea is to put specimens from individuals for
example; urine, sera, plasma, etc., to form a pool and then test the pool rather than testing each individual for evidence of a disease. The objectives of pool testing are two-fold: classification of the units of a population as either diseased or non-diseased (Dorfman, 1943) and estimation of the prevalence of a disease in a population (Sobel and Elashoff, 1975). For both objectives, pool testing has proved to reduce the cost of testing when the prevalence rate is low because if a pool tests negative, it implies all its constituent members are non-diseased and hence it is not necessary to test each member of the pool. A procedure for classifying the population of interest into diseased and non-diseased when each unit $i$ of the population has a different probability $p_i$ of being diseased which is called a generalized binomial group test (GBGT) problem has been studied (Hwang, 1975). In the case where all the units have the same probability $p$ of being diseased, the generalized binomial group test problem reduces to a binary pool testing problem. An efficient dynamic programming algorithm for obtaining an optimal Dorfman procedure has been developed (Hwang, 1975). An optimal pool testing procedure in this case implies a procedure which minimizes the expected number of tests. There is an upper bound on the size of a pool test which if incorporated into the Dorfman procedure can in effect reduce the amount of computation (Hwang, 1975). Similarly, pool testing model in the presence of dilution effect i.e. a pool containing a few diseased items may possibly be misidentified as a pool containing no such items, especially when the size of the pool is large has been studied (Hwang, 1976).

In recent years, there has been renewed interest in pool testing strategies of biological specimens because of the application in HIV/AIDS epidemiology (Kline et al., 1989). The cost effectiveness of pooling algorithm for the objective of identifying individuals with the trait has also been studied (Johnson et al., 1992). In their procedure, each individual pool that test positive was divided into two equal groups, which were tested, groups that tested positive were further subdivided and tested and so on. This work has been extended by considering pooling algorithms when there are errors and showed that some of these algorithms can reduce the error rates of the screening procedures (the false positives and false negatives) compared to individual testing (Litvak et al., 1994). Hierarchical pooling studies which involve testing pools and then sequentially subdividing and testing the positive pools have also been considered (Nyongesa, 2004). Pool testing with re-testing i.e. re-testing of both pools classified as positive and negative has been studied (Nyongesa, 2005). Indeed it was observed that re-testing improves the sensitivity and specificity of the pool-testing algorithm. Computational statistics has been used in pool testing to compute the statistical measures when perfect tests are used (Maheswaran et al., 2008).

Extension and generalization of this procedure when imperfect tests are used has been considered (Nyongesa and Syaywa, 2011; Nyongesa and Syaywa, 2010).

Studies have been carried out to estimate the probability $p$ of an arbitrary unit being diseased. A class of nested halving procedures in estimating the prevalence rate $p$ has been considered (Sobel and Elashoff 1975). The maximum likelihood estimator (MLE) of the proportion of infected units in a population using pools is upwardly biased estimator of the population proportion (Nyongesa, 2011). The bias of the MLE has been investigated when testing pools of different sizes (Hepworth and Watson, 2008). They were able to suggest methods for bias correction. Estimation of the prevalence rate based on a pool-testing scheme with re-testing has been studied and it has been shown that re-testing improves the efficiency of the estimator (Nyongesa, 2011).

Pool testing has been applied in many areas (Sobel and Groll, 1966). Pool testing can be applied in industries (Mundel, 1984). Recently, pool testing has been applied in screening the population for the presence of HIV antibody (Kline et al., 1989; Monzon et al., 1992). Pool testing has been used in screening HIV antibody to help curb the further spread of the virus (Litvak et al., 1994). It has been established that pooling offers a feasible way to lower the error rates associated with labeling samples when screening low risk HIV population. For instance, given the limited precision of the available test kits, it has been shown that screening pooled sera can be used to reduce the probability that a sample labeled negative in fact has antibodies since each test has a certain sensitivity and specificity.

In this study, we discuss the computation of statistical measures based on pool testing strategy via computer package MATLAB. We consider this procedure when the tests in use are imperfect i.e. the specificity and sensitivity are less than 100%. To the authors’ knowledge, no article has appeared in the literature of pool-testing based on Dorfman design that has discussed the procedure in the computational aspect. The rest of the paper is arranged as follows: Section 2.0 formulates the problem while the pool-testing strategy is discussed in Section 3.0. Misclassifications in the proposed group testing design are discussed in Section 4.0. Section 5.0 provides the discussion and conclusion to the present study.
Problem Formulation

Consider a large sample of a population of interest say of size $N$. We wish to establish the number of diseased individuals in the population $N$. For efficiency and cost effectiveness, pool the sample $N$ into $n$ independent pools each of equal sizes say $k$. The procedure is described diagrammatically below.

**Figure 1: Diagrammatic description of the pool testing strategy**

In this testing procedure, the sample of interest $N$ is divided into $n$ distinct pools each of size $k$. The $n$ constructed pools are subjected to testing one-at-a-time as shown in (Figure 1). The outcome of each pool is binary, that is, is either positive or negative. In pool-testing strategy, pools that test positive on the test then constituent members are subjected to testing in order to find diseased ones while if the test result is negative, a single test is sufficient. The figure shows the $n$ constructed pools and the test result on the $i^{th}$ pool, for $i=1, 2, ..., n$. The analysis in this study will require the following indicator variables:

Let

\[
T_i = \begin{cases} 
1; & \text{if the } i^{th} \text{ pool tests positive on the test kit} \\
0; & \text{otherwise} 
\end{cases}
\]

\[
D_i = \begin{cases} 
1; & \text{if the } i^{th} \text{ pool is positive} \\
0; & \text{otherwise} 
\end{cases}
\]

\[
T_{ij} = \begin{cases} 
1; & \text{if the } j^{th} \text{ individual in an } i^{th} \text{ pool tests positive on the test kit} \\
0; & \text{otherwise} 
\end{cases}
\]

and

\[
\delta_{ij} = \begin{cases} 
1; & \text{if the } j^{th} \text{ individual in the } i^{th} \text{ pool is positive with probability } p \\
0; & \text{otherwise} 
\end{cases}
\]
The indicator functions provided above are essential in the subsequent developments. The observations of the constituent members of the \( i \)th pool will be represented by \( (\delta_{i1}, \delta_{i2},...,\delta_{ij},...,\delta_{ik}) \) or simply \( \{\delta_{ij}\}^k_{j=1} \). Clearly,
\[ \Pr(D_i = 0) = \Pr(\delta_{i1} = 0, \delta_{i2} = 0,...,\delta_{ij} = 0,...,\delta_{ik} = 0) \]
by definition. For analysis purposes, we shall assume that the constituent member of a pool act independently of each other, hence
\[ \Pr(D_i = 0) = (1 - p)^k. \] (1)

Equation (1) is critical in this study and \( p \) is the prevalence rate and \( p \in [0,1] \). Notice that in practical experiments such as HIV/AIDS testing among others, we observe the readings on the test kits (Kline et al., 1989). Therefore we require the probabilities \( \Pr(T_i = 1) \) and \( \Pr(T_{ij} = 1) \). To obtain the \( \Pr(T_i = 1) \), we require the application of the law of total probability (Ross, 1997), thus
\[ \Pr(T_i = 1) = \eta \left[ 1 - (1 - p)^k \right] + (1 - \phi)(1 - p)^k \]
and we shall denote it by \( \pi \) i.e.
\[ \pi = \eta \left[ 1 - (1 - p)^k \right] + (1 - \phi)(1 - p)^k, \] (2)
where \( \eta \) is the sensitivity of the test kit and \( \phi \) the specificity of the test kit.

By sensitivity, we mean the probability of correctly classifying a diseased pool or diseased individual while \( \phi \) is specificity of the test kits and specificity here means the probability of correctly classifying a non-diseased pool or non-diseased individual. Ideally, we have introduced the error element in our model. The error component will be assumed to be based on the manufacturers’ specifications and will remain constant in the entire experiment. That is, sensitivity and specificity will remain constant at group level and individual level. Similarly, for individual testing, Equation (2) reduces to
\[ \Pr(T_{ij} = 1) = p\eta + (1 - \phi)(1 - p). \] (3)

Now, we constructed \( n \) pools as illustrated in (Figure 1) with the purpose of testing in order to detect the diseased ones. Suppose \( X \) out of \( n \) pools test positive on the test then, we know that an \( i \)th pool tests positive on the test with probability \( \Pr(T_i = 1) \) for \( i = 1, 2,\ldots, n \). Therefore,
\[ X \sim \text{binomial} \left( n, \pi \right) \] (4)
Next, we consider the computation of pool testing based on Model (4).

**Pool Testing Strategy**

In this study we propose a computer intensive pool-testing strategy. With the advent of the digital computers in early 1980’s, computational statistics has evolved (Martinez and Martinez, 2002; L’Ecuyer, 2004). In a similar format we wish to develop a computational pool-testing model based on Dorfman’s ideas. The next section discusses the moments of the number of tests in the testing scheme.

**The Number of Tests and Moments**

Note that Model (4) is of major interest in this study. Suppose \( X \) pools test positive on the test, the next stage is to perform testing of individuals in these pools. The overall number of tests in the testing scheme is

\[
Z = 1 + n + kX,
\]

where \( n \) is the number of pools and \( k \) is the pool size

It follows easily that,

\[
E[Z] = 1 + n + kn\pi. \tag{6}
\]

In the field experiments, the sensitivity and specificity of the test kits are normally provided by the manufacturers’ specifications and in the absence of this, we can estimate the sensitivity and specificity by testing known positive pools and known negative pools respectively. Therefore, for given \( \eta \) and \( \phi \), we have a MATLAB code (1) for computing (6) and similarly, the variance of the number of test is

\[
Var(Z) = k^2 n\pi (1-\pi) \tag{7}
\]

from which, the standard deviation is given by \( \sqrt{k^2 n\pi (1-\pi)} \). The skewness of the number of tests is

\[
\gamma_1 = \frac{1-2\pi}{\left\{n\pi(1-\pi)\right\}^{1/2}} \tag{8}
\]

and its kurtosis is given by,

\[
\gamma_2 = \frac{6\pi^2 - 6\pi + 1}{\left\{n\pi(1-\pi)\right\}^2} + 3. \tag{9}
\]

We shall utilize Equations (6), (7), (8) and (9) to generate the mean, standard deviation, skewness and kurtosis in the proposed pool-testing strategy via
MATLAB code (1). The formulas provided will be used in the simulation from the sample of interest in computing the central moments of the number of tests involved in the proposed pool-testing procedure. Ideally, the sample will be seeded with known infection then the mean, variance, standard deviation, skewness and kurtosis of the number of tests that are required to classify the sample when pool-testing strategy is employed will be digitized via MATLAB code (1) as provided in the Appendix.

**Table 1: Various characteristics for pool testing strategy with 1000 runs, \( N=100, \ k=10, \ \eta = \phi = 99\% \)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>( p=0.010 )</th>
<th>( p=0.050 )</th>
<th>( p=0.100 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu )</td>
<td>1.916</td>
<td>1.339</td>
<td>0.609</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>3.077</td>
<td>2.318</td>
<td>0.328</td>
</tr>
<tr>
<td>( \gamma_1 )</td>
<td>3.940</td>
<td>2.318</td>
<td>0.328</td>
</tr>
<tr>
<td>( \gamma_2 )</td>
<td>2.889</td>
<td>2.318</td>
<td>0.328</td>
</tr>
<tr>
<td>( \mu )</td>
<td>10.000</td>
<td>10.000</td>
<td>10.000</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \gamma_1 )</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \gamma_2 )</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \mu )</td>
<td>10.400</td>
<td>9.640</td>
<td>9.640</td>
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<tr>
<td>( \sigma )</td>
<td>3.377</td>
<td>3.377</td>
<td>3.377</td>
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<tr>
<td>( \gamma_1 )</td>
<td>39.700</td>
<td>14.626</td>
<td>14.626</td>
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<tr>
<td>( \gamma_2 )</td>
<td>0.140</td>
<td>2.978</td>
<td>2.978</td>
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<td>( \mu )</td>
<td>21.400</td>
<td>9.640</td>
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<td>( \sigma )</td>
<td>3.377</td>
<td>3.377</td>
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<tr>
<td>( \gamma_1 )</td>
<td>50.700</td>
<td>14.626</td>
<td>14.626</td>
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<td>3.377</td>
<td>3.377</td>
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<td>49.300</td>
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<td>( \gamma_2 )</td>
<td>0.140</td>
<td>2.978</td>
<td>2.978</td>
</tr>
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</table>

**Table 2: Various characteristics for pool testing strategy with 1000 runs, \( N=500, \ k=20, \ \eta = \phi = 99\% \)**

<table>
<thead>
<tr>
<th>Characteristics</th>
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<tbody>
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<td>( \mu )</td>
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<td>3.130</td>
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<td>( \gamma_1 )</td>
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<td>( \gamma_2 )</td>
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<td>2.903</td>
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<td>( \mu )</td>
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<td>5.361</td>
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</tr>
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<td>-</td>
</tr>
<tr>
<td>( \gamma_2 )</td>
<td>3.100</td>
<td>2.990</td>
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</tr>
<tr>
<td>( \mu )</td>
<td>50.003</td>
<td>16.791</td>
<td>25.700</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>6.988</td>
<td>1.647</td>
<td>15.630</td>
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<td>-0.120</td>
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<tr>
<td>( \gamma_2 )</td>
<td>2.964</td>
<td>2.977</td>
<td>2.935</td>
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Table 3: Various characteristics for pool testing strategy with 1000 runs, \( N = 100 \), \( k = 10 \), \( \eta = \phi = 95\% \)

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<th>( p = 0.050 )</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>( \mu )</td>
<td>( \sigma )</td>
<td>( \gamma_1 )</td>
<td>( \gamma_2 )</td>
<td>( \mu )</td>
<td>( \sigma )</td>
</tr>
<tr>
<td>Number of diseased individuals</td>
<td>5.966</td>
<td>2.409</td>
<td>0.455</td>
<td>3.279</td>
<td>9.485</td>
<td>2.903</td>
</tr>
<tr>
<td>Number of diseased groups</td>
<td>1.402</td>
<td>1.105</td>
<td>0.743</td>
<td>3.531</td>
<td>4.119</td>
<td>1.543</td>
</tr>
<tr>
<td>Number of group tests</td>
<td>10,000</td>
<td>-</td>
<td>-</td>
<td>10,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total number of tests</td>
<td>14,020</td>
<td>11,050</td>
<td>0.743</td>
<td>3.531</td>
<td>41,190</td>
<td>15,425</td>
</tr>
<tr>
<td>Percentage savings</td>
<td>74,980</td>
<td>11,050</td>
<td>0.743</td>
<td>3.531</td>
<td>47,810</td>
<td>15,425</td>
</tr>
</tbody>
</table>

Table 4: Various characteristics for pool testing strategy with 1000 runs, \( N = 500 \), \( k = 20 \), \( \eta = \phi = 95\% \)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>( p = 0.010 )</th>
<th></th>
<th>( p = 0.050 )</th>
<th></th>
<th>( p = 0.100 )</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \mu )</td>
<td>( \sigma )</td>
<td>( \gamma_1 )</td>
<td>( \gamma_2 )</td>
<td>( \mu )</td>
<td>( \sigma )</td>
</tr>
<tr>
<td>Number of diseased individuals</td>
<td>29.693</td>
<td>5.209</td>
<td>0.212</td>
<td>3.023</td>
<td>47.178</td>
<td>6.523</td>
</tr>
<tr>
<td>Number of diseased groups</td>
<td>5.397</td>
<td>2.122</td>
<td>0.363</td>
<td>3.127</td>
<td>15.640</td>
<td>2.463</td>
</tr>
<tr>
<td>Number of group tests</td>
<td>25,000</td>
<td>-</td>
<td>-</td>
<td>25,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total number of tests</td>
<td>107,940</td>
<td>42.440</td>
<td>0.363</td>
<td>3.127</td>
<td>312,800</td>
<td>49.360</td>
</tr>
<tr>
<td>Total testing cost</td>
<td>26,788</td>
<td>42.440</td>
<td>0.363</td>
<td>3.127</td>
<td>67,760</td>
<td>49.360</td>
</tr>
<tr>
<td>Percentage savings</td>
<td>73,212</td>
<td>42.440</td>
<td>0.363</td>
<td>3.127</td>
<td>52,240</td>
<td>49.360</td>
</tr>
</tbody>
</table>

Remark 1: In all the above tables we have;
\[ \mu = \text{mean}, \sigma = s \text{ tandard deviation}, \gamma_1 = \text{skewness}, \gamma_2 = \text{kurtosis} \]

The MATLAB code (1) was used to generate the (Tables 1, 2, 3, and 4) by varying the sensitivity and specificity of the test kits. In the tables we have used the word total testing cost to imply the overall cost of performing the procedure. Next, we consider the misclassifications in the pool-testing strategy.

**Misclassifications**

Notice that we allowed inspection errors in our design as this is true in real life problem since the test kits in use are not 100\% perfect in some situations. In the event of this, two possible misclassifications can arise in practice; false- positive and false- negatives. A false- positive refers to a non- diseased item being classified as diseased whereas a false- negative means that a diseased item is classified as non-diseased. First, we derive the probability of
correctly classifying a diseased individual herein referred to as sensitivity of the testing procedure, Sensitivity= Pr(T_i = 1, T_j = 1 | \delta_j = 1) and by the assumption of independence in the tests used, we have the sensitivity of the testing procedure as

\[ \text{Sensitivity} = \eta^2. \]  

(10)

The probability of the model at hand yielding false positive is

\[ f_p = 1 - \eta^2. \]  

(11)

We know that \( \eta < 1 \) in practice, this implies that \( \eta^2 < \eta \), hence pool-testing scheme lowers the sensitivity in general. We derive the specificity of the testing procedure.

Specificity= Pr(T_i = 1, T_j = 0 | \delta_j = 0) + Pr(T_i = 0 | \delta_j = 0)

\[ = (\eta \phi + (1 - \phi))(1 - (1 - p)^{k-1}) + \{(1 - \phi)\phi + \phi\}(1 - p)^{k-1}. \]  

(12)

One minus the specificity of the testing scheme yields the probability of false negative as

\[ f_n = 1 - \left[ (\eta \phi + (1 - \phi))(1 - (1 - p)^{k-1}) + \{(1 - \phi)\phi + \phi\}(1 - p)^{k-1} \right]. \]  

(13)

Upon utilizing Equation (11) and (13), we have a MATLAB code (2) for computing the false-positive and false-negative. The pool size in Equation (12) appears to be \( k - 1 \) since the \( j^{th} \) sample is known to be non negative and sampling without replacement was used in this case.
Table 5: Number of false positives in the pool testing strategy for different pool sizes

\( \eta = \phi = 99\% \)

<table>
<thead>
<tr>
<th>Probability, ( p )</th>
<th>( N=100, k=10 )</th>
<th>( N=500, k=20 )</th>
<th>( N=1000, k=20 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu )</td>
<td>( \sigma )</td>
<td>( \mu )</td>
<td>( \sigma )</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>0.010</td>
<td>0.039</td>
<td>0.196</td>
<td>0.202</td>
</tr>
<tr>
<td>0.020</td>
<td>0.059</td>
<td>0.240</td>
<td>0.292</td>
</tr>
<tr>
<td>0.030</td>
<td>0.078</td>
<td>0.277</td>
<td>0.394</td>
</tr>
<tr>
<td>0.040</td>
<td>0.010</td>
<td>0.312</td>
<td>0.489</td>
</tr>
<tr>
<td>0.050</td>
<td>0.115</td>
<td>0.337</td>
<td>0.586</td>
</tr>
<tr>
<td>0.100</td>
<td>0.214</td>
<td>0.458</td>
<td>1.068</td>
</tr>
<tr>
<td>0.150</td>
<td>0.315</td>
<td>0.555</td>
<td>1.553</td>
</tr>
</tbody>
</table>

Table 6: Number of false positives in the pool testing strategy for different pool sizes

\( \eta = \phi = 95\% \)

<table>
<thead>
<tr>
<th>Probability, ( p )</th>
<th>( N=100, k=10 )</th>
<th>( N=500, k=20 )</th>
<th>( N=1000, k=20 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu )</td>
<td>( \sigma )</td>
<td>( \mu )</td>
<td>( \sigma )</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>0.010</td>
<td>0.585</td>
<td>0.726</td>
<td>2.868</td>
</tr>
<tr>
<td>0.020</td>
<td>0.666</td>
<td>0.776</td>
<td>3.307</td>
</tr>
<tr>
<td>0.030</td>
<td>0.759</td>
<td>0.828</td>
<td>3.772</td>
</tr>
<tr>
<td>0.040</td>
<td>0.856</td>
<td>0.879</td>
<td>4.207</td>
</tr>
<tr>
<td>0.050</td>
<td>0.930</td>
<td>0.916</td>
<td>4.641</td>
</tr>
<tr>
<td>0.100</td>
<td>1.374</td>
<td>1.114</td>
<td>6.834</td>
</tr>
<tr>
<td>0.150</td>
<td>1.807</td>
<td>1.277</td>
<td>8.996</td>
</tr>
</tbody>
</table>

Table 7: Number of false negatives in the pool testing strategy for different pool sizes

\( \eta = \phi = 99\% \)

<table>
<thead>
<tr>
<th>Probability, ( p )</th>
<th>( N=100, k=10 )</th>
<th>( N=500, k=20 )</th>
<th>( N=1000, k=20 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu )</td>
<td>( \sigma )</td>
<td>( \mu )</td>
<td>( \sigma )</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>0.010</td>
<td>0.093</td>
<td>0.305</td>
<td>0.884</td>
</tr>
<tr>
<td>0.020</td>
<td>0.168</td>
<td>0.409</td>
<td>1.565</td>
</tr>
<tr>
<td>0.030</td>
<td>0.236</td>
<td>0.485</td>
<td>2.116</td>
</tr>
<tr>
<td>0.040</td>
<td>0.296</td>
<td>0.543</td>
<td>2.563</td>
</tr>
<tr>
<td>0.050</td>
<td>0.350</td>
<td>0.591</td>
<td>2.916</td>
</tr>
<tr>
<td>0.100</td>
<td>0.545</td>
<td>0.736</td>
<td>3.826</td>
</tr>
<tr>
<td>0.150</td>
<td>0.643</td>
<td>0.799</td>
<td>3.987</td>
</tr>
</tbody>
</table>
Table 8: Number of false negatives in the pool testing strategy for different pool sizes

<table>
<thead>
<tr>
<th>Probability, p</th>
<th>$N=100$, $k=10$</th>
<th>$N=500$, $k=20$</th>
<th>$N=1000$, $k=20$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$</td>
<td>$\sigma$</td>
<td>$\mu$</td>
</tr>
<tr>
<td>0.010</td>
<td>0.601</td>
<td>0.773</td>
<td>4.858</td>
</tr>
<tr>
<td>0.020</td>
<td>0.909</td>
<td>0.959</td>
<td>7.850</td>
</tr>
<tr>
<td>0.030</td>
<td>1.227</td>
<td>1.100</td>
<td>10.283</td>
</tr>
<tr>
<td>0.040</td>
<td>1.493</td>
<td>1.212</td>
<td>12.237</td>
</tr>
<tr>
<td>0.050</td>
<td>1.732</td>
<td>1.304</td>
<td>13.809</td>
</tr>
<tr>
<td>0.100</td>
<td>2.582</td>
<td>1.583</td>
<td>17.804</td>
</tr>
<tr>
<td>0.150</td>
<td>3.032</td>
<td>1.709</td>
<td>18.510</td>
</tr>
</tbody>
</table>

The simulated results for the false-positives and false-negatives in the testing scheme are provided in (Tables 5, 6, 7, and 8) above. In the next section, we provide the discussion of our findings and the conclusion to the study.

Discussion and Conclusion

Computer intensive pool-testing strategy has been proposed in this study. The developed model is viable for statistics users but who are not interested in intensive computer programming or mathematical development of the procedures. From the simulated results, (Tables 1, 2, 3 and 4) it is clear that the number of diseased increase with increase in the incidence probability $p$ which affirms the idea that pool-testing is only ideal if the prevalence rate is small (Dorfman, 1943). It is also evident from the results that as $p$ increases the number of tests increase hence a decrease in savings. Therefore, for high prevalence rates the savings are minimized but may give rise to misclassifications. This assertion is evident in (Tables 5, 6, 7 and 8). We also note that false-positive increase when the pool-size are large hence pool size should not be relatively large with a view to maximizing the savings with minimal errors or high sensitivity/ specificity kits should be used to minimize the misclassification rates.

References


APENDIX
Matlab code (1)

This program generates moments for the Dorfmans' procedure we used se and sp for sensitivity and specificity respectively, p is the incidence probability, k is the pool size, N is population size, n is number of pools and pro is probability of classifying a group positive. Note that to obtain the desired results, the number of simulations r should be considerably large e.g. r=1000. Since MATLAB does not recognize some Greek notations, we change our notations for the sake of the program. pro give the probability of classifying a pool as diseased. Note that the program still works even for un-equal values of se and sp. For various assumed values of p, se,sp,k,N,n for example:

```
p=0.2;
se=0.99;
r=1000;
sp=0.89;
k=10;
N=100;
n=N/k;
pro=(1-p)^k*(1-sp)+(1-(1-p)^k)*(se);
to obtain the number of the diseased pools from r simulations
x = zeros (1,r);
```
U = rand (r,n);
for i = 1:N
    ind = find(U(i,:) <=pro);
    x (i) = length (ind);
end
m=mean(x);
s=std(x);
k1=kurtosis(x);
s1=skewness(x);
to obtain the number of diseased individuals then clearly notice that the pool size is 1 and so for r simulations we have
pro2= (1-p)*(1-sp) + (1-(1-p))*(se);
x1 = zeros (1,r);
U = rand (r,n);
for i = 1:N
    ind = find(U(i,:) <=pro2);
    x1 (i) = length (ind);
end
m1=mean(x1);
s2=std(x1);
k11=kurtosis(x1);
s12=skewness(x1);

Matlab Code (2)
to obtain the number of diseased individuals then clearly notice that the pool size is 1 and so for r simulations we have
pro2= (1-p)*(1-sp)+(1-(1-p))*(se);
x2 = zeros (1,r);
U = rand (r,n);
for i = 1:N
    ind = find(U(i,:) <=pro2);
    x2 (i) = length (ind);
end
m1=mean(x2);
s2=std(x2);
k11=kurtosis(x2);
s12=skewness(x2);
since the average number of diseased might be less than 1 we find the false negatives and false positives by using the computation formulae for
finding mean and variance for example. Let fse be probability of false positive
fse=1-se^2;
fp=m1*fse
sp1=(1-(1-p)^(k-1))*(se*sp+(1-sp))+(1-p)^(k-1)*((1-sp)*sp+sp);
fsp=1-spl;
to obtain the negatives its obviously and hence false negatives is obtained as; (Let fn be probability of false negative)
m2=N-m1;
fn=m2*fsp
Land and Soil Resources and their Management for Sustainable Agricultural Production in Kenya: Current Position and Future Challenges

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Abstract

Land and soil make up the cardinal resource-base in any agricultural production system and, hence, their proper management is vital for sustainable agricultural production. A study was conducted to evaluate the availability and accessibility as well as constraints to utilization of land and soil resources for sustainable agricultural production in Kenya. The study covered 33 districts drawn from 6 out of the 8 administrative provinces in the country. The selected districts were drawn from agriculturally active provinces. The methodology used in data gathering included collection of information from secondary sources, use of questionnaires, field visits and personal interviews. The country has an area of 583,000 km², out of which land and water occupy 571,770 and 11,230 km², respectively. About 33% and 67% of Kenya’s land mass are designated as available agricultural land and arid and semiarid land, respectively. Less than 40% of the available agricultural land is utilized. Only 5% of the country’s drainage potential is developed. Besides, most of the semiarid land is of high potential for agricultural production provided irrigation is applied. The country’s irrigation potential is estimated at 54,000 ha out of which 20% is developed. More land may be reclaimed for agricultural production from the semi-arid land by applying irrigation. Agricultural land availability is, therefore, not limiting to agricultural production. Skewed land ownership that leaves most of the land in the hands of few people is a factor that undermines access to, and development of, the land. Erosion risk and soil shallowness are important constraints. Suitable land/soil conservation measures targeting all forms of soil degradation processes in relation to agricultural systems in given agro-ecological zones need to be developed if the land/soil resources of the country are to be utilized for sustainable agricultural production.

Key words: Agricultural land, land availability, natural resources, soil degradation.
Introduction

There has been progressive decline in the rate of per capita food production in sub-Saharan Africa over the past several decades (FAO, 1986; FAO, 1996). In Kenya, a sub-Saharan country, the per capita food production has been declining over the years (GK, 1997; CBS, 2002, 2003, 2006), and both income and food accessibility have been declining (PAPPA, 2001). A number of possible causes to the declining agricultural production have been espoused, and they include rapid population growth, drought, conflicts (FAO, 1996; Dyson, 1996), lack of appropriate technology for use by farmers (World Bank, 1988/89), inappropriate land tenure and unavailability of credit for farmers (Bender and Smith, 1997), and misuse of land (Lal, 1987; 1988) as well as mismanagement of water resources (JICA, 1992a). Sanchez et al. (1997) reported that soil fertility is the fundamental biophysical root cause for declining per capita food production in the region. The situation is thus complex and calls for integrated approach to management based on resources available to farmers. The logical step in promoting agricultural production in any system is to address the most limiting constraint(s) in priori. The pertinent questions that inevitably follow are: (i) Does the country have adequate land resource for agricultural production? (ii) Which are the limiting constraint(s)? and (iii) What strategies should be pursued in addressing the identified constraint(s) so as to promote sustainable agricultural production in the country?

Land is the most cardinal natural resource on which agricultural production depends. In an effort to address agricultural production it is vital to ascertain if land is sufficiently available in good quality for agricultural purposes. It is also relevant to re-evaluate its management vis-à-vis the current and future uses and, finally, identify challenges if the land resource is to be used for sustainable agricultural production. There is unquestionable need, for an agricultural country like Kenya, to plan for a sustainable agricultural production system. By conceptualization, a sustainable agricultural system is one that conserves land and soil resources, plant and animal germplasm, and is environmentally non-degrading, technically appropriate, economically viable, and socially acceptable (Anon, 1991). Besides, sustainable agricultural system is a dynamically stable and continuous production system that achieves a level of productivity satisfying prevailing needs and is adapted continuously to meet future demands (Okigbo, 1991). The objectives of this study were to assess (i) adequacy and potential of available agricultural land, (ii) constraints to utilization of land/soil resources for sustainable agricultural production in Kenya, and (iii) identify challenges in addressing the constraints.

Materials and Methods

Survey Unit

The current administrative infrastructure in Kenya is such that planning and implementation of the Government sponsored programmes are organized within the administrative units, particularly district, with some co-ordination at the provincial level. For this reason the "survey unit" used in this study was district. The 33 survey units (districts) drawn from 6 out of the 8 provinces represented the country. The selected Districts were drawn from agriculturally active provinces. The remaining two Provinces – Nairobi and North Eastern- are characterized by low agricultural land and short length of growing period (lgp), respectively. Length of growing period is the number of days in any given rainfall season when there is sufficient water stored in the soil profile to support crop growth (Cooper et al., 2009).

Table 1: Survey units in the selected Provinces

<table>
<thead>
<tr>
<th>Province</th>
<th>Survey Unit (District)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western</td>
<td>Busia, Bungoma, Butere-Mumias, Kakamega, Vihiga</td>
</tr>
<tr>
<td>Nyanza</td>
<td>Kisii, Nyamira, Homa-bay, Rachuonyo, Kisumu, Siaya</td>
</tr>
<tr>
<td>Central</td>
<td>Kiambu, Muranga, Laikipia, Nyeri</td>
</tr>
<tr>
<td>Eastern</td>
<td>Embu, Meru Central, Machakos, Makueni, Kitiui</td>
</tr>
<tr>
<td>Coast</td>
<td>Tana River, Kwale, Kilifi, TaitaTaveta, Lamu.</td>
</tr>
<tr>
<td>Rift Valley</td>
<td>Narok, Kajiado, Trans Nzoia, West Pokot, Nandi, Nakuru, Uasin Gishu, Kericho</td>
</tr>
<tr>
<td>North Eastern</td>
<td>None</td>
</tr>
<tr>
<td>Nairobi</td>
<td>None</td>
</tr>
</tbody>
</table>

Data Collection and Analysis

The methodology deployed in data collection included collection of information from secondary sources, use of different questionnaires to different extension agents, field visits and personal interviews to verify secondary information and gather primary data. Secondary sources of information used included published materials and government records obtained from libraries, internet and/or documentation centres of the visited research and training institutions. A total of 16 research and training centres as well as regional development authorities from the six Provinces were visited. Questionnaires were sent to district environment and land development officers, district crops development officers and provincial irrigation and drainage officers because they are the officers directly involved in the management of land/soil resources. During the field visits it became necessary to interview key personalities in certain Institutions/Agencies. The aim was to interview as many officers as possible but...
only 35 were interviewed using a prepared interview schedule. The interviewees were drawn from visited Institutions and district extension and training officers relevant to land management in the country. Collected information on land availability, potential land use, constraints to land/soil productivity, and land/soil management was subjected to descriptive statistical analysis using Genstat (VSN International, 2007) to provide percentages and means.

**Results and Discussion**

**Availability and Accessibility of Agricultural Land**

Kenya has a total area of 583,000 km² out of which water occupies 11,230 km² (CBS, 2001). The country’s land area is variously reported as 571,770 km² (CBS, 2001), 569,250 km² (CIA, 2003), 576,700 km² (Macharia, 2004), and 569,000 km² (FAO, 2005). The CBS (2001) statistics are adopted for the purpose of this report. About 5 million of the 57 million ha land area is occupied by forests and civil structures. The remaining 52 million ha designated as agricultural land is categorized as high (12%), medium (6%), and low potential (73%) (CBS, 2001; FAO, 2005). The classification of land potential to agricultural production is based primarily on rainfall. High potential land falls within an area characterized by mean annual rainfall of at least 857.5 mm, while medium and low potential lands receive mean annual rainfall of 735 – 857.5 mm and 612 mm or more, respectively. About 33% of the 52 million ha (17 million ha) are endowed with some rainfall to support crop production (Jaetzold and Schimdt, 1982). Occurrence of land with impeded drainage is widespread in the country (Ministry of Agriculture, 1991).

**Table 2: Categories of agricultural land in Kenya**

<table>
<thead>
<tr>
<th>Category</th>
<th>Proportion (%)</th>
<th>Coverage ('000 ha)</th>
<th>Mean annual rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High potential</td>
<td>11.8</td>
<td>6,785</td>
<td>≥858</td>
</tr>
<tr>
<td>Medium potential</td>
<td>5.6</td>
<td>3,157</td>
<td>735 – 858</td>
</tr>
<tr>
<td>Low potential</td>
<td>74</td>
<td>42,105</td>
<td>≤612</td>
</tr>
<tr>
<td>All other</td>
<td>8.6</td>
<td>4,867</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>100</strong></td>
<td><strong>56,914</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Source:** CBS (2003)

Seventeen out of 33 surveyed districts (52%) reported impeded drainage problems. The drainage potential of Kenya is estimated at 600,000 ha, out of which only 30,000 ha (5%) have been developed (KARI, 1991; GK, 2002; FAO 2005). Although the locations of areas with impeded drainage have been known...

to exist in nearly all the districts, no precise assessment had been made.

Table 3: Land and soil problems common to many surveyed districts

<table>
<thead>
<tr>
<th>Problem</th>
<th>District</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil erosion</td>
<td>All the surveyed districts except Lamu</td>
</tr>
<tr>
<td>Insufficient water (Need for water conservation/irrigation)</td>
<td>Busia, Bungoma, Homa-bay, Rachuonyo, Kisumu, Siaya, Muranga, Laikipia, Embu, Machakos, Makueni, Kitui, Tana River, Kwale, Kilifi, TaitaTaveta, Lamu, Narok, Kajiado, West Pokot, Nakuru</td>
</tr>
</tbody>
</table>
| Soil acidification                           |Twenty districts (>60%) reported the need for water conservation and/or irrigation (Table 3). About two-thirds of Kenya's land area (38 million ha) require irrigation (FAO, 2005). The areas requiring irrigation comprise arid (IGP: 1-59 days), semi-arid (IGP: 60-119 days), and parts of sub-humid (IGP: 120-179 days) with poor/irregular rainfall distribution. This shows very high irrigation demand while the country's irrigation potential is only estimated at 540,000 ha (KARI, 1991; GK, 2002; FAO, 2005) out of which only 109,350 ha (i.e., 20%) are developed (GK 2002). The 540,000 ha irrigation potential of Kenya is distributed amongst the country's 6 drainage basins as follows: Tana River (38%), Lake Victoria (37%), Kerio Valley (12%), Athi River (7%), and Uwaso Ng’iro North and South (6%) (Table 4)

Table 4: Estimated irrigation potential distributed amongst the country’s drainage basins

<table>
<thead>
<tr>
<th>Drainage basin</th>
<th>Covered area (’000km²)</th>
<th>As a % of total land area</th>
<th>Irrigation potential (’000 ha)</th>
<th>As a % of total potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Victoria</td>
<td>49</td>
<td>8.5</td>
<td>200</td>
<td>37</td>
</tr>
<tr>
<td>Rift Valley</td>
<td>127</td>
<td>21.8</td>
<td>65</td>
<td>12</td>
</tr>
<tr>
<td>Athi River</td>
<td>70</td>
<td>12</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>Tana River</td>
<td>132</td>
<td>22.6</td>
<td>205</td>
<td>38</td>
</tr>
<tr>
<td>Ewaso Ng’iro North</td>
<td>120</td>
<td>20.5</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>Ewaso Ng’iro South</td>
<td>85</td>
<td>16.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>583</td>
<td>100</td>
<td>540</td>
<td>100</td>
</tr>
</tbody>
</table>

Sources: FAO (2005); Philips-Heineman (1991)

More irrigation projects, both major and small-scale schemes, covering a total of 118,240 ha are proposed [JICA 1992 b]. If all these proposed irrigation projects are implemented to completion then a total of 170,240 ha would be under irrigation. This will be a mere 34% of the country's irrigation potential.

Irrigation systems in use in Kenya are sprinkler (60%), surface (38%), and drip (2%). The current situation is that many of the major irrigation schemes in the country are not performing (FAO, 2005), possibly due to inadequate funding and/or mismanagement.

Less than 40% of the 17 million ha with rain to support crop production is utilized; leaving over 10 m ha unutilized (GK., 1981; FAO, 2005). Furthermore, the classification of land potential to agricultural production based on rainfall is not entirely correct. Rainfall is not an inherent soil characteristic that may be used to categorise land potential productivity. The use of rainfall as the sole criterion in designating land potential productivity is based on the premise that agricultural production in Kenya must depend for ever only on rainfall, with no consideration for irrigation. Civilized agriculture world-over manages water availability to crops by either draining off excess water or applying irrigation wherever and whenever precipitation is in excess or insufficient for the crops, respectively. Considering the soil's biological and physicochemical properties the land in the low rainfall areas - 67% of the country’s land mass - with its high Percent Base Saturation and near neutral reaction, is of higher agricultural potential than the hitherto so-called high and medium potential. Soils under high rainfall regimes are characterized by low concentrations of basic cations and are usually acidic. Rejuvenating such soils would require major inputs in fertilizer, lime, conservation and development of different farming systems (Ahmad, 1996).

Clearly more agricultural land may be made available by reclaiming land. The term "Land reclamation" as used in this report is defined as the bringing into agricultural use that parcel of land that, for various physicochemical reasons, could not be used. Flooded land (i.e., land with impeded drainage), arid land (i.e., land with insufficient moisture for crop growth) and degraded land (i.e., eroded, acidified, salinized, etc) may be rehabilitated or reclaimed. Soil moisture is the major limitation to the use of semiarid lands (GK., 2006). Thus, with irrigation semiarid lands would avail enormous agricultural land; much more than the 540,000 ha designated as the country’s irrigation potential. More land could also be made available for agricultural production by developing the drainage potential of the country. This is in addition to 60% of the 17 m ha of the land with sufficient rainfall for crop production that lies unutilized. For long there was no law to limit the size of land and individual can own in the country. Consequently, land accessibility/ownership is skewed with few people owning large tracks of land while the majority own small parcels (GK, 2006). Evidently, availability of agricultural land can be increased 3 or 4 folds and is, therefore, not a limitation. It is land accessibility that is a problem.
Constraints to Land Utilization

Land utilization may be constrained by land/soil inherent properties, management and/or climatic factors. Degradation of both cropland and grazing land was found to be widespread in Kenya. Accelerated soil erosion was reported a serious problem in all surveyed districts with exception of only Lamu District (>96%) (Table 3). Gullies along livestock tracks down the slopes were a common feature in the districts. Some of the gullies are very large and deep and are “growing” in depth and width season after season (Figure 1).

The walls of the gullies collapse with successive rainy seasons. Such gullies covered extensive areas in some districts (Figure 2) rendering land uncultivable and, in some cases, uninhabitable.
Soil erosion is the leading soil-inherent constraint to soil productivity in Kenya (Figure 3).

Figure 2: Gulleys covering a large expanse of land

Figure 3: Constraints to soil productivity in selected African countries
Cases of soil acidification (i.e., lowering of soil pH) suspected to be caused by continuous use of acid-forming fertilizers such as ammonium sulphate and diammonium phosphate (Sigunga, 1993) were reported in 5 districts (>16%) (Table 3). Copper deficiency was also reported in all these districts.

Land/soil degradation may be viewed as the wearing down of a land surface or the progressive modification of soil by leaching into more acid varieties (Chesworth, 2008). The implication is that the degraded soil becomes unproductive since acid soils are unproductive. A more inclusive view defines soil degradation as diminution of the soil’s current or potential capacity to produce crops as a result of one or more degradation processes (UNEP, 1981). The degradation processes include erosion, desertification, lateritization, hard-setting, fragipan formation, clay-pan formation, salt accumulation, excessive leaching, acidification, nutrient imbalance (Lal, 1988; Lal et al., 1989), build up of toxic elements such as aluminium and manganese as well as inundation leading to reduced soil conditions and poor aeration (Okoth, 2003). Some of these processes are purely natural (e.g., lateritization, hard-setting and fragipan formation), some are anthropogenic (e.g., soil compaction, salt accumulation, nutrient mining, and desertification), while others are effected by a combination of geological vicissitudes and anthropic phenomena (e.g., erosion, acidification, salt accumulation and nutrient imbalance). These processes act singly or interactively in influencing soil's capacity for self-regulation and its productivity (Lal et al., 1989). Soil erosion and shallowness are the most important constraints to soil productivity in Kenya (Figure 3).

The situation in Kenya with respect to land/soil degradation is not very gloomy if compared with the neighbouring Uganda and Tanzania cases (Figure 3). Erosion, the worst of the soil degradation processes in Kenya, is manageable. Soil erosion due to water and/or wind is merely a symptom that unsuitable methods of farming are being practiced for that area and ecosystem (Derpsch, 2008). Civil construction such as roads and buildings with improper runoff management also trigger off soil erosion. Farming methods suitable in specific AEZs and for particular farming systems need to be instituted to arrest soil erosion risks. Ground surface must not be left bare since lack of soil cover is a precursor to soil erosion by water (Brown, 2008). Low economic capacity exacerbates land/soil degradation by operating as a constraining factor on poor rural households’ ability to avoid land/soil degradation or to invest in mitigating strategies (Barber, 1999). Therefore, the Government should provide some fund and/or subsidy to contain soil erosion risks, particularly in subsistence household lands.
Copper deficiency in wheat in some of the wheat growing areas of Rift Valley Province has been with farmers for many years (Holmes and Sherwood, 1954; Butters, 1954). This problem is serious in Nakuru and Narok Districts especially in the areas known to be marginal in soil copper content (Nyandat and Ochieng’ 1976). It was reported by many respondents in Nakuru and Uasin Gishu districts (80%) that fertilizers used on wheat in the same cu-marginal regions are diammonium phosphate, monoammonium phosphate and urea which are NH$_4^+$-based. It is established that NH$_4^+$ ions reduce the absorption of Cu$^{2+}$ ions (Sigunga et al., 1986; Tills and Alloway, 1981), and enhance the uptake of negatively charged ions like H$_2$PO$_4^-$, HPO$_4^{2-}$ and SO$_4^{2-}$ (Blair et al., 1970; Smith and Jackson, 1987). Tills and Alloway (1981) had observed that high levels of NH$_4^+$-containing fertilizers used on soils with low copper content could cause copper deficiency in wheat. In Kenya no consideration has been given to the influence of nitrogen source, whether NH$_4^+$ or NO$_3^-$-based, on wheat crop performance in relation to the cu-deficiency problem in cu-marginal soils.

The constraints that are normally difficult to manage such as salinity, aluminium toxicity and high phosphorus fixation do not affect large proportions of the agricultural land in Kenya (Figure 3).

Many agricultural extension officers (80%) found it difficult to associate such soil degradation processes as soil acidification, nutrient imbalance, soil compaction, crust formation, fragipan formation and salt accumulation with soil degradation. Soil degradation control or conservation is the deployment of environmentally sound activities and techniques in the production of crops based on a sustainable use of land, species and ecosystems (RSCU, 1992). Conservation of land/soil is, thus, concerned with not only protecting the soil against erosion but also against physical, chemical and biological deterioration (Chesworth and Lavigne, 2008). To conserve soil is to enhance (and/or prevent diminution of) the soil's potential to produce crops. In this context, it is necessary to identify and understand soil degradation processes in various AEZs in the country. Organized soil conservation work started in Kenya in 1937, and the conservation measures applied were copied from the United States of America, while the traditional soil conservation measures were stiffly discouraged by the colonialists (Erikkson, 1992). In Kenya the concept of soil conservation is reduced to mean little more than soil erosion control, and Ministry of Agriculture books on soil conservation contain mainly information on types of soil erosion and their proposed remedies (Wenner, 1979; 1980; and 1981). Equating soil conservation with soil erosion control has lead to planning measures and projects in which erosion is thought of in terms of loss of soil...
material, and its control is treated in isolation from other aspects of agricultural improvement (Young, 1990). Besides, detrimental effects of soil erosion on the quality of surface and underground waters are largely ignored (Lomborg, 2001).

Soil and Water Conservation Branch of the Ministry of Agriculture and, indeed, many agencies participating in soil and water conservation work in Kenya were concerned only with accelerated soil erosion. Other processes of soil degradation and their effects on soil productivity were largely ignored. Soil degradation is effected by many processes other than soil erosion (Lal, 1988; Lal et al., 1989). In fact, there can be serious soil-degradation problems even in areas where erosion is not a problem (Young, 1990). Amir (1996) emphasized that prevention of soil degradation depends on a number of interacting factors, which include climatic, edaphic, and socio-economic, as well as cropping systems and agro-engineering. Many governments and donor-funded soil conservation projects in Africa continue to treat land/soil degradation as a "biophysical" problem without cognizance of economic aspect (Barber, 1999).

Although emphasis is placed on soil erosion control no "Soil Erosion Classification" had been done in 29 out of the 33 districts (88%) surveyed. Besides, the "recommended" soil erosion control measures in the country (Wenner, 1979; 1980 and 1981) have not been subjected to research to evaluate their feasibility in terms of technical effectiveness, economic benefits and societal suitability across different levels of the landscape hierarchy. It would be illogical to assume that any of the soil erosion control measures could be suitable under all cropping systems, topographical features, soil characteristics, climatic constituents, and land preparation techniques. This lack of empirical data inevitably leads to lack of understanding of the so-called "recommended" measures and, consequently, agricultural extension workers are not clear about what to do when. Erikkson (1992) summarized this sorry situation as follows:

"Kenyan extension workers have laid too much emphasis on cut off drains and fanya juu terraces instead of giving advice on the use of grass trips and conservation farming. There has been a dearth of flexibility and imagination all the way from the ministerial headquarters to the grassroots work...Too often the authorities introduce terracing where it is hardly needed, and where the farmers are consequently not interested".

Okoth (2003) report that in Kenya the erosion processes have been assumed to occur in a uniform manner at all levels of the landscape hierarchy and, hence, the results of one level observation are often factored to cover other levels for which data was not collected. De Haan (2000) maintains that the success of
particular soil conservation measures depends on socio-economic and climatic factors. Though soil erosion is a major soil degradation process in Kenya understanding of all soil degradation processes in terms of what they are, the comparative magnitudes of their impacts with respect to various soils under different agroecological zones (AEZs), cropping systems, and socio-economics are pre-requisites in generating appropriate technologies and formulating beneficial management policies necessary for sustained soil productivity.

Due to various factors, mainly agronomic and moisture, crop production per unit land area in the country is low necessitating putting of more land under crop production. Production of the 4 most important crops – maize, wheat, rice and sugar cane - for social and political stability in the country is always much lower than the potential yield (Table 5) and demand levels (Figures 4, 5, 6, and 7).

The short fall is met with imports. Raising crop production per unit land area in respect of these 4 crops could drastically reduce the demand for more land.

**Table 5:** Yield ranges of the most important food crops in Kenya compared with potential yields

<table>
<thead>
<tr>
<th>Crop</th>
<th>National yield range</th>
<th>Potential yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>1,294 – 1,392 kg ha(^{-1})</td>
<td>8,000 kg ha(^{-1})</td>
</tr>
<tr>
<td>Wheat</td>
<td>1,900 – 2,271 kg ha(^{-1})</td>
<td>6,000 kg ha(^{-1})</td>
</tr>
<tr>
<td>Rice</td>
<td>2,000 – 3,000 kg ha(^{-1})</td>
<td>7,000 kg ha(^{-1})</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>70 – 80 t ha(^{-1})</td>
<td>120 t ha(^{-1})</td>
</tr>
</tbody>
</table>

**Sources:** FAO (2010); Kenya Sugar Board (2008).
Figure 4: Trends of maize production, demand and imports over years

Figure 5: Trends of wheat production, demand and imports over years

Figure 6: Trends of rice production, demand and imports over years

Figure 7: Trends of sugar production, consumption, imports and exports over years

Challenges and Way Forward

The country faces daunting challenges in the management of land in order to realize sustainable agricultural production. Whereas availability of agricultural land is not a limitation as at now, accessibility is a problem. This is because of skewed land ownership whereby a few people own large expanses of land, most of which is not utilized while the majority of the people own very small parcels hardly enough for homestead and subsistence cultivation. In this regard, there are provisions in both the new constitution and the new Land Policy to look afresh at the issues of land ownership/accessibility and use (National Council for Law Reporting, 2010). The challenge is, therefore, to formulate enforceable laws to effectively address these issues.

Soil moisture control is an essential component of modern agriculture. Excess soil moisture must be drained off to allow plant roots access oxygen, and irrigation must be applied where soil moisture is tending to wilting point. There is need to not only conduct precise assessment of the drainage potential of the country but also to develop it. The estimate of the irrigation potential of the country given as 54,000 ha is too low given that the main limitation to agricultural use of the semi-arid lands (ca 34 m ha) is water. Irrigation potential of the country needs to be re-assessed. Substantial part of the semi-arid land should be brought under arable use with irrigation.

Crop yields per unit land area are too low with respect to most annual crops across the majority AEZs in the country. Next to soil-moisture stress, soil degradation is the major factor behind low crop productivity in Kenya. Degraded or infertile soil cannot support high crop yields. Soil degradation processes should, therefore, be studied with the view of understanding their impacts on land productivity in different soil types, landscape hierarchy, cropping systems, and climatic regimes. Developed soil degradation control measures must be evaluated not only in terms of technical efficacy but also in terms of economic viability, and social acceptability. Soil fertility is a crucial component of soil productivity, and deserves serious attention if crop production per unit land area is to be raised. Other agronomic practices such as good pest control and plant population density must be developed and applied.

Crop production technologies in the country are either meant for mechanized (tractor power) farming common in large-scale commercial farms or non-mechanized (human power) characteristic of subsistence household farmers. There is need to develop crop production technologies and policies that promote the use of draught power in order to raise subsistence farmer from relying on
human power. This is necessary to raise crop productivity per unit land area as farm operations would be timely and proper. With increased crop production per unit land area there will be less need to open more land.

**Conclusions and Recommendations**

There is plenty of good land for agricultural production; the problems are land distribution and management. Land/soil conservation is poor. Land/soil degradation processes, other than soil erosion, are largely ignored. Recommended soil erosion control measures have not been subjected to adequate testing with regard to technical efficacy, economic benefits, and social acceptability across AEZs. Crop management is poor with the consequence of low crop yields per unit land area necessitating imports of essential foods. Both drainage and irrigation potentials of the country are yet to be developed.

Both the drainage potential and irrigation potential of the country need not only to be reassessed but also to be developed. Evaluate land/soil degradation control measures in the context of land use types and AEZs. Strategies to shift away from using human power to using draught power and light machineries in farm operations should be put in place.

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**References**


Land and Soil Resources and their Management for Sustainable Agricultural

Accra, Ghana.


Comparative Ecological Analysis of Fish Parasitofauna in a Hub-Plot Aquaculture System: Implications for Aquaculture in Kenya

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Abstract

Aquaculture is an important source of food globally. The Kenya Government through the economic stimulus package (ESP) in a supplementary budget of 2009/2010, allocated Kshs.1.12 Billion for the establishment of 200 fish ponds in each of the 140 potential constituencies in an effort aimed at enhancing food security by increasing fish production in the country. Fish diseases and parasitic infections have been and still remain peripherally recognized as one of the detrimental and limiting factors in the development of the aquaculture sector. This research was set to study the diffusion and dispersion of parasites in Oreochromis niloticus fish cultured in a typical hub and plot system in an integrated cage fish culture in Kenya. The system was based on a hub (source of seed) from Sagana Aquaculture Centre and a plot (farm) in Machakos, Kwa-Ngeki reservoir. Specific objectives were to detect and identify parasites, determine their prevalence, mean intensity and abundance; and compare the distribution of major parasites in the hub and plot. Using routine necropsy and parasitological examination procedures a total of 370 Oreochromis niloticus fish (57 caged and 313 from open ponds) were examined for the presence of parasites. Ecological community diversities and similarity indices were used to compare the parasitofaunal assemblages between the hub and the plot. Data on prevalence, mean intensities, abundance, diversities and similarity indices on distribution of parasites as well as their implications in this rapidly growing sector of the economy are presented in this paper.

Keywords: Aquaculture, parasites, hub-plot system, ecological indices, Oreochromis niloticus, Kenya
Introduction

Aquaculture, which is broadly defined as the farming of finfish and shellfish in water systems is rapidly expanding in both size and culture systems and is therefore among the fastest growing industries, second to biotechnology (Drucker, 2002). Aquaculture is a fast-expanding mode of food production in the world. Currently, fish farming accounts for more than one-quarter of the total fish directly consumed by humans, using about 220 finfish and shellfish species. In comparison with the rest of the world’s aquaculture production, Africa’s contribution is insignificant (FAO, 2002). The continent as a whole contributes a mere 0.9% to the world aquaculture production (FAO, 2002; 2003). This production, however, increased from 37,000 tonnes in 1984 to 189,000 tonnes in 1998 (FAO, 2002). In 2002, the total aquaculture production of Africa amounted to 451,537 tonnes and the major fish species cultured include the freshwater Carp and Tilapia (FAO, 2003). Leading producers are Egypt (376, 296), Nigeria (30,663), Zambia (4,200), South Africa (4,177), Madagascar (7, 966), Ghana (6,000) and Uganda (4,915) all in tonnes. These figures reveal the low level intensity of aquaculture in sub-Saharan Africa as compared to 553,933 tonnes produced by Norway (FAO, 2002; 2003). Aquaculture activities can provide sources of supplementary high protein food and additional income to rural communities in developing countries (Fioravanti et al., 2007).

FAO (2002) reports that aquaculture is still essentially a rural, secondary and part-time activity taking place in small farms in small freshwater ponds. Extensive to semi-intensive cultural systems produce limited fish yields which are mostly consumed directly, bartered or sold locally as a cash crop. From the 20 different species known to be cultured, only three species (Nile Tilapia, African Catfish and common Carp) are mostly farmed throughout Africa (FAO, 2002).

Aquaculture activities are capable of creating an industry employing and supporting a substantial number of fish farmers, feed manufacturers, fish processors, traders and other actors. Hecht & Endemann (1998) reported that one of the major constraints observed to hold back productivity in fish farming include parasites and diseases. Therefore, to achieve a highly productive and profitable venture, all the constraints faced in aquaculture must be addressed. Information on occurrence, prevalence and pathogenicity of fish parasites and diseases is essential in aquaculture. Such information enables aquaculturists to apply correct control measures for fish diseases to reduce the cost of production, and period of growth thereby
increasing the profit margins (Akoll, 2005).

According to Jadwiga (1991) the presence of fish parasites is to a large extent detrimental to fisheries and the fishing industry. Even in well adjusted host-parasite systems, the host does not remain indifferent to the presence of parasites. There are various modes in which pathogenic effects of parasites on hosts manifest themselves. Epizootics and mass mortality brought about by parasitic infections are very frequent in fishes. More common is a prolonged gradual die-off which may, for some time, go unnoticed, particularly when the few sick and dead fish are prey to predators including piscivorous birds. Economic effects of parasites on fishes are mass mortality, rejection of infected fish by the market when parasites and/or lesions are visible and more importantly, retarded growth and weight losses of the infected fish (Jadwiga, 1991). Furthermore, some fish parasites are potentially pathogenic to man when the parasitized fish are consumed while not well cooked, cold smoked, marinated or just raw (Jadwiga, 1991). In this paper, a prototype model of a hub and plot system was analyzed for parasites and ecological indices employed for comparisons of the community parasitofauna to establish the link between the source of seed (hub) and the distribution of disease (farm).

Materials and Methods

Site 1: Sagana Fish Farm
Sagana Fish Farm is sited S 0°39' E 37°12', at an altitude of 1230 metres above sea level and comprises 20 hectares of ponds on a 50 hectares farm in Kenya's Central Province (Fig. 1). Ponds have been dug in black cotton soils formed from volcanic rocks on a gently sloping plateau, approximately 60 km south of Mt. Kenya. Sagana Fish Farm is located 2 km outside Sagana Township, Kirinyaga District, about 105 km Northeast of Nairobi (PD/CRSP, 1998).

Site 2: Ngeki’s dam in Machakos
Ngeki’s dam in Machakos is 3 km outside Machakos township, situated at an altitude of 1634 metres above sea level, S 01°30,882’, E 37°13,028’. It is located in Machakos district, Southern Kenya between Nairobi and Mombasa in Eastern Province.

Experimental Design
The fish in Machakos Ngeki’s dam were subjected to treatments namely; treatment 1 (T1): Stocked into cages (cage factor) and the conditions
included restricted movement and high densities per unit area of culture; Treatment 2 (T2): Stocked in open pond (open pond factor). The conditions here included free movement and low densities per unit area of culture. Treatment 3 (T3): The Sagana fish were handled under treatment 3, which acted included the fish in Sagana Aquaculture Centre as the controlled treatment in which fish were reared in open ponds with free movement and low densities per unit area of culture.

Data Collection

Abiotic Parameters

Dissolved Oxygen, pH, conductivity and temperature were measured in situ using probes (Universal meter, Model: Multi 350i, Wissenschaftlich Technische Werkstätten, Germany).

Biotic Data

For the fish samples a seine net was used for open pond fish sampling using line transects (where length of the seine net represented one transect from one edge of pond pulled along the length of the ponds) while cages were lifted and hand nets used to obtain the caged fish at bi-monthly intervals for one year. The sample size (n) was determined using the formula according to FSRRN, (2007); given as:

\[
n = \frac{Z^2 p (1 - p)}{e^2}
\]

where,

- \(Z\) is the Z value for the corresponding confidence level (1.96 for 95% confidence);
- \(e\) is the margin of error (± 3%) and
- \(p\) is the estimated value for the proportion of a sample that have the condition of interest (50 for 50%).

Fish morphometrics (measurement of total length) prior to dissection and observation of external fish condition was done. Dissection procedures in Aloo et al. (2004) were used in the laboratory to examine the parasite infections and diseases in fish.

Necropsy and parasitological analyses were performed on all sampled fish, following specific diagnostic protocols compiled on the basis of standard procedures (Fioravanti et al., 2007). For the identification of the parasite groups, the taxonomic keys from Dawes (1956), Ukoli (1966), Kabata (1970), Lom & Dyková (1992), Hoffman (1999), Scholz et al. (2004)
and Paperna (1996) were used. The identification of protozoan and myxozoan parasites was mainly based on the morphological characters of fresh specimens, while metazoans were isolated and fixed in 70% ethanol and then identified after clarification and/or staining. Intensity of infection, abundance and prevalence were calculated for all recovered parasites according to Bush et al., (1997). The identification of monogeneans was carried out with the aid of a phase-contrast microscope after partial digestion of the parasite and mounting in ammonium picrate and glycerine, following the method of Harris & Cable (2000). Data of the fish parasites observed, identified, counted and preserved were recorded in standard protocols. For protozoans and myxozoans a scale according to Fioravanti et al., (2007) was used to assign levels of intensity as follows: <10 individuals per field (low), between 10-30 (medium), between 30-50 individuals per field (high) and >50 individuals per field (very high).

**Data Analysis**

**Community Similarity**

The Morisita index ($I_M$) of community similarity where $\lambda$ is Simpson's index of dominance (calculated separately for each community, as $\lambda_1$ and $\lambda_2$ for community 1 and 2 respectively), $n_i$ is the number of individuals of species $i$, and $N$ is the total number of individuals sampled, was used (James & Jerrold, 1977).

$$I_M = \frac{2 \sum n_i n_{i2}}{(\lambda_1 + \lambda_2)(N_1 N_2)}$$

Simpson's index of dominance describing the probability that two randomly selected individuals from a community will be of the same species, where $s$ is the total number of species in the community was evaluated (James and Jerrold, 1977).

$$\lambda = \frac{\sum_{i=1}^{s} [n_i(n_i-1)]}{N(N-1)}$$

The Morisita index ranges from 0 to 1. With 0 indicating that no species are shared between the two communities and 1 indicating complete identity. Because the index takes species abundance into account, communities that contain the same species but have different species abundance will have an index value of less than 1.

The Sørensen index (Sørensen’s similarity coefficient), is a statistical calculation used for comparing the similarity of two samples (James &
where $A$ and $B$ are the species numbers in samples A and B, respectively, and $C$ is the number of species shared by the two samples. This expression is extended to abundance instead of incidence of species. This quantitative version of the Sørensen index is also known as Czekanowski index. The Jaccard index, (Jaccard similarity coefficient) is a statistical calculation used for comparing the similarity and diversity of sample sets (James & Jerrold, 1977).

The Jaccard coefficient measures similarity between sample sets, and is defined as the size of the intersection divided by the size of the union of the sample sets:

$$J(A, B) = \frac{|A \cap B|}{|A \cup B|}.$$  

The Jaccard distance, which measures dissimilarity between sample sets, is complementary to the Jaccard coefficient and is obtained by subtracting the Jaccard coefficient from 1, or, equivalently, by dividing the difference of the sizes of the union and the intersection of two sets by the size of the union.

$$J = \frac{M_{11}}{M_{01} + M_{10} + M_{11}}$$

Where $M_{11}$ is number of common species in the two communities, $M_{01}$ and $M_{10}$ are the numbers of species in communities one and two, respectively.

For species diversity: the Shannon-Weaner diversity index, $H'$ was calculated as follows (James & Jerrold, 1977):

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

Where $p_i$ is the relative abundance of the $i^{th}$ species to the total abundance, computed for all the species observed.

Results and Discussion

Environmental Factors

Abiotic Parameters

The mean monthly abiotic parameters measured during the study were within the normal range for successful culture of *O. niloticus*. For all the sites the pH range was 6.47 to 9.44 while dissolved Oxygen concentration range was between 2.84mg/L observed in Sagana fish ponds in fertilized, eutrophic ponds during the morning to 10.46 mg/L during the mid-day which was the highest. Temperatures ranged from 22.38°C to 31.8°C while conductivity ranged from 93.5μs/cm to 930μs/cm. These parameters were within the tolerable range for *O. niloticus* fish which was a significant finding because adverse abiotic factors may cause significant stress to the fish hence increasing its susceptibility to disease (Paperna, 1996).

Biotic Parameters

Quantitative Parasitological Data: Prevalence Rates, Intensities and Abundances

A total of 370 *O. niloticus* fish were examined comprising of 57 caged fish in Machakos Ngeki’s dam, 86 open pond fish from Machakos Ngeki’s dam and 227 open pond fish from Sagana aquacultural centre. The sample size is shown in Table 1.

Table 1: Sample size of *O. niloticus* fish distribution by date, system and site over the entire study period

<table>
<thead>
<tr>
<th>Sampling period</th>
<th>Year 2007</th>
<th>Year 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>March</td>
<td>May</td>
</tr>
<tr>
<td>Machakos caged</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Machakos open pond</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Sagana open pond</td>
<td>54</td>
<td>17</td>
</tr>
<tr>
<td>Total sample size (n)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In Ngeki’s dam caged *O. niloticus* fish, *Tylodelphys* spp. were dominant in prevalence, mean intensity and abundance (Table 2). Protozoan parasite
**Trichodina** spp., monogenean Dactylogyroid (*Cichlidogyrus tilapia*), *Gyrodactylus* spp.; and *Clinostomum* spp. had higher prevalence rates and mean intensity (Table 2).

**Table 2:** Quantitative parasitological data in Machakos caged system for *O. niloticus* fish (Mean±SE)

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Prevalence (%)</th>
<th>Mean intensity</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protozoan</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichodina</em> spp.</td>
<td>11.61±7.35</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>Sessiline peritrichs</td>
<td>3.03±3.03</td>
<td>2</td>
<td>0.06±0.06</td>
</tr>
<tr>
<td><strong>Myxozoa</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Myxobolus</em> spp.</td>
<td>1.52±1.52</td>
<td>very high</td>
<td>high</td>
</tr>
<tr>
<td><strong>Helminthes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monogenetic trematodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dactylogyrids</td>
<td>17.85±6.57</td>
<td>3.4</td>
<td>0.59±0.21</td>
</tr>
<tr>
<td>Gyrodactylids</td>
<td>6.06±4.49</td>
<td>3.3</td>
<td>0.17±0.13</td>
</tr>
<tr>
<td><strong>Digeneic trematodes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black spot metacercariae</td>
<td>9.66±4.73</td>
<td>2.5</td>
<td>0.26±0.15</td>
</tr>
<tr>
<td><em>Clinostomatid</em> metacercariae</td>
<td>10.23±5.24</td>
<td>2.5</td>
<td>0.27±0.17</td>
</tr>
<tr>
<td><em>Euclinostomum</em> metacercariae</td>
<td>1.52±1.52</td>
<td>1.3</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>Liver metacercariae</td>
<td>3.33±3.33</td>
<td>1</td>
<td>1.47±1.47</td>
</tr>
<tr>
<td><em>Tylodelphys</em> spp.</td>
<td>47.25±9.45</td>
<td>7</td>
<td>3.3±1.40</td>
</tr>
<tr>
<td><strong>Nematoda</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.06±4.49</td>
<td>2</td>
<td>0.04±0.03</td>
<td></td>
</tr>
<tr>
<td>11.61±7.36</td>
<td>high</td>
<td>low</td>
<td></td>
</tr>
<tr>
<td><strong>Acanthocephalan</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acanthosentis</em> spp.</td>
<td>3.03±3.04</td>
<td>3</td>
<td>0.06±0.07</td>
</tr>
</tbody>
</table>

In the open pond of Ngeki’s dam, *O.niloticus* fish *Tylodelphys* spp. and black spot metacercariae (*Neascus* spp.) were dominant and had high prevalences. However the liver metacercariae species were predominant with a mean intensity of 48.8 that was highest than in other systems (Table 3).
Table 3: Quantitative parasitological data in Machakos open pond for *O. niloticus* (Mean±SE)

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Prevalence (%</th>
<th>Mean intensity</th>
<th>Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protozoan</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichodina</em> spp.</td>
<td>5.55±5.55</td>
<td>low</td>
<td>0.12±0.12</td>
</tr>
<tr>
<td><em>Cryptobia</em> spp.</td>
<td>2.22±2.22</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td><strong>Helminthes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monogenetic trematodes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dactylogyrids</td>
<td>14.97±3.28</td>
<td>1.9</td>
<td>0.28±0.08</td>
</tr>
<tr>
<td>Gyrodactylids</td>
<td>3.33±3.33</td>
<td>2</td>
<td>0.067±0.067</td>
</tr>
<tr>
<td><strong>Digenetic trematodes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black spot metacercariae</td>
<td>2.28±1.46</td>
<td>19</td>
<td>0.44±0.36</td>
</tr>
<tr>
<td><em>Clinostomatid</em> metacercariae</td>
<td>11.04±8.9</td>
<td>2.6</td>
<td>0.27±0.24</td>
</tr>
<tr>
<td>Diplostomatid metacercariae</td>
<td>3.92±3.92</td>
<td>1.7</td>
<td>0.06±0.06</td>
</tr>
<tr>
<td>Intestinal cysts</td>
<td>1.28±1.28</td>
<td>1</td>
<td>0.012±0.012</td>
</tr>
<tr>
<td>Liver metacercariae</td>
<td>15.07±7.02</td>
<td>48.8</td>
<td>7.37±4.73</td>
</tr>
<tr>
<td><em>Tylodelphys</em> spp.</td>
<td>41.58±11.77</td>
<td>4</td>
<td>1.65±0.38</td>
</tr>
<tr>
<td><strong>Nematoda</strong></td>
<td>3.95±2.66</td>
<td>2</td>
<td>0.07±0.05</td>
</tr>
<tr>
<td><strong>Cestoda</strong></td>
<td>11.27±7.09</td>
<td>2.1</td>
<td>0.25±0.18</td>
</tr>
<tr>
<td><strong>Acanthocephalan</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In Sagana open pond *O. niloticus* fish, *Tylodelphys* spp. had a lower prevalence than in Machakos systems but had a high mean intensity (13.5). The monogenean Gyrodactylids were significant in Sagana with a mean intensity of 11.5. Parasites that were found in Sagana had higher intensities than those of Machakos (Table 4) such as the case with the monogenetic trematodes and Diplostomatids.
Table 4: Quantitative parasitological data in Sagana open pond for *O. niloticus* (Mean±SE) (P=Prevalence, %; MI =Mean Intensity, A = Abundance)

<table>
<thead>
<tr>
<th>Parasite</th>
<th>P</th>
<th>MI</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protozoan</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichodina</em> spp.</td>
<td>25.68±5.85</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td><em>Cryptobia</em> spp.</td>
<td>5.0±5.0</td>
<td></td>
<td>low</td>
</tr>
<tr>
<td><strong>Myxozoan</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Myxobolus</em> spp.</td>
<td>1.67±1.67</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td><strong>Helminthes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Monogenetic trematodes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Dactylogyrids</em></td>
<td>17.84±8.21</td>
<td>3.76</td>
<td>0.67±0.44</td>
</tr>
<tr>
<td><em>Gyrodactylids</em></td>
<td>4.17±2.71</td>
<td>11.5</td>
<td>0.48±0.44</td>
</tr>
<tr>
<td><strong>Digeneic trematodes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Black spot metacercariae</em></td>
<td>5.0±5.0</td>
<td>13.2</td>
<td>0.66±0.66</td>
</tr>
<tr>
<td><em>Clinostomatid</em> metacercariae</td>
<td>18.2±7.13</td>
<td>4.3</td>
<td>0.78±0.23</td>
</tr>
<tr>
<td><em>Diplostomatid</em> metacercariae</td>
<td>1.67±1.67</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td><em>Euclinostomum</em> metacercariae</td>
<td>2.5±2.5</td>
<td>1</td>
<td>0.025±0.00</td>
</tr>
<tr>
<td><em>Tylodelphys</em> spp.</td>
<td>25.68±5.85</td>
<td>13.5</td>
<td>3.47±1.03</td>
</tr>
<tr>
<td><strong>Nematoda</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Armithalingamia macracantha</em></td>
<td>25.68±5.86</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td><strong>Acanthocephalans</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Acanthosentis</em> spp.</td>
<td>5.0±5.1</td>
<td>low</td>
<td>low</td>
</tr>
<tr>
<td><strong>Crustacean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lamproglena monodi</em></td>
<td>1.67±1.68</td>
<td>low</td>
<td>low</td>
</tr>
</tbody>
</table>

Ecological quantification of parasite community similarities between Machakos and Sagana open pond fish system parasite communities were 0.71 and 0.83 Jaccards (CCJ) and Sorensen’s (CCS) coefficients, respectively. Since both values were close to 1. It shows that most of the species found in this investigation were common in both communities.

**Comparison of Parasites occurring in *Oreochromis niloticus* Fish in Hub and Plot Systems**

Protozoan species identified, were: The ciliates, sessilin peritrichs, (*Epistilis* spp.) and mobile peritrichs (*Trichodina* spp.) and the flagellate *Cryptobia* spp. Except for *Trichodina* spp., which sometimes occurred on the skin scrapings, all the other protozoan parasites found in this study were observed exclusively on the gills of *O. niloticus*. While *Trichodina* spp. and *Cryptobia* spp. were highly motile, sessilin peritrichs were found attached to the gill epithelium. Sessilin peritrichs were detected only in caged fish at very low level of infection, may be due to the net fouling and the less active swimming behaviour of caged fish.
Attached sessilians rarely produce mechanical damage or peripheral tissue response in the gills. Fish mortality due to attached sessilians are observed during high intensity which could disrupt gas exchange through the respiratory epithelium by irritation and mucus over production by the fish hence thickening the surface required for effective diffusion of gases (Paperna, 1996).

The occurrence of *Trichodina* spp., sessiline peritrichs and *Cryptobia* spp. was a common finding due to their ubiquitous presence in water environments (open ponds or cages) and their opportunistic action on stressed or traumatised fish (Paperna, 1996). Among Myxozoans, *Myxobolus* spp. spores were often found encysted in white cysts in gills and muscle or sometimes they were sparsed in intestines and kidney. The prevalence of *Myxobolus* spp. observed in this study is lower than that reported in other studies in Africa (Gbankoto *et al.*, 2001, El-Mansy, 2005). The pathogenic effects of several myxosporean parasites have been described by several scientists: including myoliquefaction of muscle tissues after the death of the host (Barja & Toranzo, 1993; Pampoulie *et al.*, 1999), reduction of the respiratory capacity (Molnar and Szekely, 1999) or of host fecundity (Swearer and Robertson, 1999) and parasitic castration (Sitja-Bobadilla & Alvarez- Pellitero, 1993). A rich helminthic community was observed consisting of 12 different types (family/genus). Among Monogeneans, the Dactylogyrids and Gyrodactylids were observed, respectively. On gills and skin. This could have been due to the strict confinement and overcrowding of caged fish, favouring the easy contact and fish-to-fish transmission of Gyrodactylids, and invasion by oncomiracidia of Dactylogyrids. Furthermore, caged fish may have certain types of physical injuries that are specific to this farming system, and, when overstocked, fish may suffer from fin and skin damage caused by net abrasion (Woo, 1995), hence more susceptibility to pathogenic organisms if handled without care. Studies by McGuigan & Sommerville (1985) on the effects of cage culture of fish on the parasite fauna also agree with these findings. Among digeneans, *Clinostomum* spp. was observed dominantly as yellow cysts on the skin below the scales or as large cysts behind the gills, while *Euclinostomum* spp. was observed as large round cysts in anterior and posterior regions of the kidney. *Clinostomum* spp. are of economic importance since heavily infected fish, particularly those with cutaneous infections, when marketed are often rejected by consumers (Kabunda & Sommerville, 1984). Furthermore, some human cases caused by Clinostomatids have been reported in Japan, pointing out the potential zoonotic role of these parasites.
Encysted *diplostomatid* metacercariae were infrequently found in the eyes, while *Tylodelphys* spp. was predominantly found swimming actively in the vitreous humour of the eyes. The differences in infection levels of *Tylodelphys* spp. and other *Diplostomatid* metacercariae observed in caged and open pond fish in each system could have resulted from a number of factors. Concerning *Tylodelphys* spp., very little variation occurred in the mean intensities from different systems under study. Slightly higher prevalence values in Machakos caged fish may be due to their increased susceptibility to cercarial invasion in cage confinement. These findings are similar to those reported by Ching (1985) for *Diplostomum beari bucculentum* and Field & Irwin (1994). *Tylodelphys* spp. findings in this study on influence of temperature are different from findings by Buchmann & Bresciani (1997) that found very low prevalence in Danish rainbow trout. This could have been due to the fact that in the tropics ambient and water temperatures are always high as compared to the temperate regions. In Sagana fish ponds, the water temperatures were always high (>23°C) in all the sampling months and the highest value was recorded in November (28.6°C). The eye fluke *Tylodelphys* spp. neither showed seasonality in prevalence nor mean intensities in Sagana because the regulated water levels in the ponds tend to mitigate abrupt fluctuations in water temperatures. In Machakos Ngeki’s reservoir, prevalence and intensity of *Tylodelphys* spp. fluctuated with changes in water temperature with a high intensity in the dry months, may be due to high water temperatures which trigger cercarial shedding by snail host. As for *Diplostomum* spp. infestations, the damage due to this parasite was evident as exophthalmia, blind eyes and blackening of fish. Blind fish do not see food and therefore feed poorly leading to stunted growth which is of economic importance. The parasite infection level raises some concern as the pathogenicity (Shariff *et al.*, 1980, Brassard *et al.*, 1982) and the growth reducing effect (Sato *et al.*, 1976; Buchmann & Uldal, 1994) of eye fluke infections are well known for fish feeding by trouts (Buchmann & Uldal, 1994), but concerning tilapias further studies need to be assessed.

One species of Acanthocephala; *Acanthosentis* spp. was observed in the intestinal lumen. These results show that this parasite has a tendency for aggregation. Aggregated parasite distributions are common among parasites (Rohde, 1993) and increase the chances of mating (Kennedy, 1976). In rare cases copepods, *Lamproglena* genera were found in gills.
Sagana fish had a rich and diverse helminthic community (Shannon-weaner diversity index, $H' = 1.314$) as compared to the Machakos open pond fish (Shannon-weaner diversity index, $H' = 1.174$) suggesting perhaps an abundant population of intermediate hosts. These findings are similar to findings by Hechinger & Lafferty (2005) and Marcogliese (2005) which indicate that a high density of host populations can increase the rate of transmission of parasites and propagation of disease. This is because communities with high species richness and high abundance of intermediate hosts fosters parasitism: as many parasites rely on a predator-prey relationship to reach the next hosts in their life cycles. The occurrence of a parasite in a host organism not only indicates the presence of other organisms that participate in the parasite’s life cycle, but also trophic pathways in which the hosts participate both up and down the food chain.

Morisita’s indices (IM) for community similarities between Machakos caged and Machakos open pond *O. niloticus* parasitofauna, was 0.15, Machakos open pond and Sagana Open ponds was 0.22 and between Machakos caged fish and Sagana open ponds fish was 0.46. The IM value for the case of Machakos caged fish and Sagana open ponds fish was the highest at 0.46 suggesting a strong relationship between source of seed fish veterinary quality and the veterinary quality of fish after establishment in a farm.

**Conclusions and Recommendations**

There were no significant variations in parasite types that infected caged and open pond *O. niloticus*. Three protozoans, fourteen helminths and one copepod genus were identified in *O. niloticus* in this study. No significant differences in mean prevalence, intensities and abundances of these parasites were found between caged and open pond fish. However, enclosure of fish in cages could influence the interaction of fish with intermediate hosts of digenetic trematodes, cestodes and nematodes. Certain parasites were found to have higher prevalence in caged than in open pond fish, such as parasites with a direct life cycle, i.e. protozoans and monogeneans.

The occurrence of parasites in this study leads to the conclusion that the source of fingerlings is an important determinant of the distribution and occurrence of parasites. This has a big implication on aquaculture in Kenya. The quality of seed produced in hatcheries should be regulated. Therefore, this paper recommends thorough screening and sanitary control of fingerlings from hatcheries before restocking activities in farms to avoid
diffusion and spread of parasites and diseases. Finally, this study also recommends further studies on the life cycle of major parasites observed such as diplostomatids, clinostomatids, nematodes, acanthocephalans, in order to improve the measures to prevent and control these parasites.

References


Modeling and Analysis of AC-DC Converter PID Controller Optimized with Pattern Search Algorithm

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Abstract

The paper presents a tuning methodology for the parameters of a PID controller in a three phase Pulse Width Modulation (PWM) Three-Level AC-DC converter system, often referred as Improved Power Quality Converters (IPQC). A PID Controller is a generic control loop feedback mechanism (controller) widely used in industrial control systems. A PID controller calculates an "error" value as the difference between a measured process variable and a desired point. Three-phase Three-level AC-DC converters have been developed to a matured level with improved power quality in terms of power-factor correction, reduced total harmonic distortion at input ac mains, and regulated dc output. However, for best performance, the PID parameters used in the calculation must be tuned according to the nature of the system – while the design is generic, the parameters depend on the specific system. The parameters of converter, which vary with the operating conditions of the system, are adapted in order to maintain desirable response for output voltage and power factor. A Pattern Search Optimization (PSO) algorithm is employed in order to obtain the controller parameters assuring improved response at selected load. The Three-level AC-DC converter PID-PSO controller is modeled in MATLAB environment. The response of the developed controllers is compared to that of the controller whose parameters are tuned using the well-known Ziegler-Nichols method. The developed method is more proficient in improving the controller loop settling time, the rising time and overshoot and hence the disturbances do not affect the performances of Three-Level AC-DC converter.

Keywords: PID controller, Pulse Width Modulation (PWM), Three-Level AC-DC converter, Pattern Search Optimization (PSO) algorithm, Improved Power Quality Converters (IPQC).
**Introduction**

Nowadays voltage source converters (VSC) are standard element of the drive application. The most commonly used topology is the two-level converter. However, their capabilities are limited when high power medium voltage loads are considered. For this reason multi-level inverters were introduced in early 1980s when first three-level diode clamped converters were developed. Shortly after that breakthrough, there appeared new topologies with the higher number of levels, three-level converter in Neutral Point Clamped (NPC) configuration (Figure.1 and figure.2) is the most often applied on the market of high power applications. Advance made in solid state power devices and progress in computational power of today’s processors means that three-level converters can be successfully be used in fast switching frequency applications.

![Figure.1 The three-level AC-DC converter](image1)

![Figure: 2 Equivalent circuit of phase-a](image2)

Standard two-level inverter is composed of only two complementary switching devices per phase. This causes that the each switch has to withstand a full DC-link voltage. In consequence, for power converters rated in MV, fast switching IGBTs has to be replaced with slower but able to handle higher voltage GTOs. On the other hand three-level converters employ 4 (or 6) switches per phase but each switch has to handle only half of the DC-link voltage. This allows using IGBT switches even for high power applications. Moreover, due to increased number of levels losses and harmonics are smaller in comparison to two-level converter (Bhim Singh et al., 2004).

In this paper, for fast and robust dc voltage and power factor controls, a feedback technique is applied to the control of PWM converter. By tuning the three constants in the PID controller algorithm, the controller can provide control action designed for constant output DC voltage and unity power factor in input ac network power. The controller can be described in terms of the responsiveness of the controller to an error, the degree to which the controller overshoots the set point and the degree of system oscillation.

**Theoretical Review**

Traditionally, AC-DC converters, which are also known as rectifiers, are developed using diodes and thyristors to provide controlled and uncontrolled unidirectional and bidirectional dc power. They have the problems of poor power quality in terms of injected current harmonics, resultant voltage distortion and poor power factor at input ac mains and slowly varying rippled dc output at load end, low efficiency, and large size of ac and dc filters. In view of their increased applications, a new breed of rectifiers has been developed using new solid-state self-commutating devices such as MOSFETs, insulated gate bipolar transistors (IGBTs), gate-turn-off thyristors and (GTOs). Such converters are generally classified as switch-mode rectifiers (SMRs), power-factor correctors (PFCs), pulse width-modulation (PWM) rectifiers, multilevel rectifiers or multi-pulse rectifiers. Because of the strict requirement of power quality at the input ac mains, several standards (Howard G and Murphy P.E, 1992) have been developed and enforced on the consumers. Because of the severity of power quality problems some other options such as passive filters, active filters (AFs), and hybrid filters (M. El-Habrouk et al., 2000) along with conventional rectifiers have been extensively developed, especially in large rating and already existing installations. However, these filters are quite costly, bulky, and have reasonable losses, which reduce overall efficiency of the complete system. Even in some cases the rating of converter used in active filters is almost
close to the rating of the load. Under such circumstances, it is considered a better option to use such converters as an inherent part of the system of AC-DC conversion, which provides reduced size, high efficiency, and well controlled and regulated DC to provide comfortable and flexible operation of the system (Bhim Singh et al., 2004).

Various control strategies have been proposed in recent work for this type of PWM converters. The main objectives in the control of PWM AC-DC converters are to achieve a high power factor and minimum harmonic distortion of input line currents. Proposed control strategies include the phase and amplitude control (PAC), (R. Wu et al., 1991; E. Wernekink and A. Kawamura, 1991), hysteresis current control (HCC), (Dorin O. Neacsu, 2004). The PAC provides a good switching pattern to reduce steady-state current harmonics and output voltage ripple. However, it has a dc current component (current offset) that appears on the ac side of the converter which deteriorates the dc load current and voltage waveforms during transients. The hysteresis current control has a fast dynamic response, good accuracy, no dc offset, and high robustness, but the major problem of HCC control is that its average switching frequency varies with the dc load current, which makes the switching pattern uneven and random, thus resulting in additional stresses on switching devices. The predicted current control with a fixed switching frequency shows a fast dynamic response and has a good switching pattern that reduces the switching device stresses. However, it is sensitive to parameter variations.

On the other hand, the control strategies proposed in (J. W. Dixon et al., 1987) require a dc capacitor large enough to ensure a good stability margin, making the system transient response slower. The load current control method proposed in (D. R. Veas et al., 1994) overcomes this problem by providing a very strong stability that neither depends on the input inductance nor on the size of the dc capacitor. It is shown in (Y. Guo et al., 1994), which the pole-placement control through state feedback provides not only stability, but also fast transient response when the dc capacitance is substantially reduced. The state feedback-based transient control method proposed in (Dong-Choon Lee et al., 2000) compensates the current offset that results from the PAC method and reduces the oscillations of dc current and voltage waveforms during transients. These control strategies yield various advantages and disadvantages related to control circuit complexity, switching frequencies, and transient responses. The common disadvantage is that they cannot guarantee system stability against large-signal disturbances. It is well known that the state space-averaged models of PWM AC-DC converters obtained with these control strategies are nonlinear systems.
In spite of the simple structure and robustness of these methods, optimally tuning gains of PID controllers have been quite difficult. The PSO methods have been employed successfully to solve complex optimization problems. Generally, PSO is characterized as a simple concept, easy to implement, and computationally efficient. Unlike the other heuristic techniques, PSO has a flexible and well-balanced mechanism to enhance the global and local exploration abilities (Matlab help manual, 2008).

**Modeling of PWM Converters**

Dynamic Modeling and Control Strategy for a whole control block diagram of the proposed scheme is shown in Figure.3 and figure.4. The control block consists of the conventional d-q current regulators is constructed in the synchronous reference frame (SRF). It should be noted that three-level sinusoidal PWM (SPWM) is utilized here.

![Controller configurations](image)

*Figure 3: Controller configurations*
A power circuit and a per-phase equivalent circuit of a PWM voltage-source converter are shown in Figure 1. It is assumed that a resistive load is connected to the output terminal. A voltage equation is derived from Figure 2 as

\[ e_s = R_i + L \frac{di}{dt} + V_r \]  

Where

- \( e_s \) source voltage;
- \( i_s \) current;
- \( V_r \) converter input voltage;
- \( R, L \) are resistance and inductance of the inductor, respectively.

Assembling equations of the three phases and transforming them into a synchronous d–q reference frame, then

\[ L \frac{di_d}{dt} + \omega Li_d + R_i = e_d - v_d \]  
\[ L \frac{di_q}{dt} + \omega Li_q + R_i = e_q - v_q \]

Where \( \omega \) is the angular frequency of the source voltage. For fast voltage control, the input power should supply instantaneously the sum of load power and charging rate of the capacitor energy. By the power balance between the ac input and the dc output

\[ e_s = R_i + L \frac{di}{dt} + V_r \]  

Where

- \( e_s \) source voltage;
- \( i_s \) current;
- \( V_r \) converter input voltage;
- \( R, L \) are resistance and inductance of the inductor, respectively.
\[
\frac{1}{2}(e_d i_d + e_q i_q) = V_{dc} i_{dc}
\]

Where \(V_{dc}\) and \(i_{dc}\) are the dc output voltage and current, respectively, and the resistance loss and the switching device loss are neglected. On the dc output side

\[
i_{dc} = C_d \frac{dV_{dc}}{dt} + i_L
\]

Where \(i_L\) is the load current. From (4) and (5)

\[
\frac{1}{2}(e_d i_d + e_q i_q) = CV_{dc} \frac{dV_{dc}}{dt} + V_{dc} i_L
\]

Equation (6) represents that the system is nonlinear with regard to \(V_{dc}\). From (2), (3), and (6), a state-space modeling of the system is given

\[
\begin{bmatrix}
i_d \\
i_q \\
V_{dc}
\end{bmatrix} =
\begin{bmatrix}
-\frac{R}{L i_d} + \omega i_q \\
-\frac{R}{L i_q} - \omega i_d \\
\frac{1}{2}C V_{dc} (e_d i_d + e_q i_q) - \frac{i_L}{C}
\end{bmatrix} +
\begin{bmatrix}
\frac{1}{L} \\
0 \\
0
\end{bmatrix} [e_d - V_d]
\]

\[
\begin{bmatrix}
i_d \\
i_q
\end{bmatrix} =
\begin{bmatrix}
i_d \\
i_q
\end{bmatrix} +
\begin{bmatrix}
\frac{1}{L} \\
0
\end{bmatrix} [e_q - V_q]
\]

The converter generates harmonic voltages, as is typical for switched power-electronic equipment. In order to reduce harmonic-related contamination of the controller’s input signals a set of signal conditioning analog filters are normally used prior to the controller inputs. In this paper second order low-pass filters are used for this purpose.

Unity power factor and constant DC link voltage are achieved with the current commands.

\[
i_d = (V_{dc} \text{ref} - V_{dc}) (i_{p1} + \frac{K_i}{s})
\]

\[
i_q = 0
\]

Where \(K_p\); \(K_i\) are PI gains. The d, q-axis voltages coming out of the current regulators are given by

\[
v_d = e_d + \omega L i_q - (i_d \text{ref} - i_d) (k_{p2} + \frac{K_i}{s})
\]

\[
v_q = -\omega L i_d - (i_q \text{ref} - i_q) (k_{p3} + \frac{K_i}{s})
\]

Where \((\omega L i_q\) and \(\omega L i_d\) are the feed forward compensation terms for the d, q-axis decoupling.
Voltage Synthesis for SPWM

To achieve the desired performance, the voltage vector \( (V_d; V_q; ) \) in (10) and (11) should be mapped into the stationary a-b-c reference frame.

\[
\begin{bmatrix}
V_a \\
V_b \\
V_c
\end{bmatrix} = K_1(\theta) \begin{bmatrix}
V_d \\
V_q
\end{bmatrix}
\tag{12}
\]

Where \( K_1 \) and \( \theta \) are frame transformation constants.

A three-level SPWM scheme for one phase is described in Figure 5. Unlike two-level SPWM, three-level SPWM uses two triangular carriers, i.e., \( Tu \) and \( TL \). The three level PWM wave for \( V_a \) is made with the following rules: When \( V_a \) is larger than the upper triangular carrier \( Tu \), the first switching device GTO-A in Figure 1 is turned on, otherwise it is turned off. The second switching device GTO-B is turned on and off by comparison with the lower triangular carrier \( TL \). A rule in three-level switching is that only two contiguous devices can be turned on at the same time. As a result, the wave form of GTO-C is opposite to that of GTO-A. Likewise, the wave form of GTO-D is opposite to that of GTO-B. The resultant a-phase PWM voltage \( V_a \) has the 3-level-step-shaped waveform as shown in Figure 5.

\[\text{Figure 5: Gating signals- phase-a of input waveform is considered}\]
Optimization of Control System

In this section the Pattern Search Optimization is used for tuning the control system of the converter. The Pattern Search Optimization (PSO) is an evolutionary optimization technique and a derivative-free optimization method that is suitable to solve a variety of optimization problems that lie outside the scope of the standard optimization methods. In practice PSO is robust, and rarely gets trapped in bad local minima (Mat lab help manual, 2008). PSO attempts to generate a set of search directions in such a way that one of them will be a descent direction (otherwise a stationary point has been reached). Generally, PSO has the advantage of being very simple in concept, easy to implement and computationally efficient. Unlike other heuristic algorithms, such as genetic algorithms, GPS possesses a flexible and well balanced operator to enhance and adapt the global and fine tune local search (V. Torczon, 2001; Kazem Haghdar and Heidar Ali Shayanfar, 2010; R. M. Lewis and V. Torczon, 2001).

The PSO algorithm proceeds by computing points that may or may not approach the optimal value. The algorithm starts by forming a set of points called a mesh, around the given point. This current point could be the initial starting point provided by the user or it could be computed from the previous step of the algorithm. The mesh is formed by adding the current point to a scalar multiple of a set of vectors called a pattern. A pattern is a set of vectors \{\mathbf{V}_i\} that the PSO algorithm uses to determine which points to search at each iteration. The set \{\mathbf{V}_i\} is defined by the number of independent variables in the objective function, N, and the positive basis set. Two commonly used positive basis sets in pattern search algorithms are the maximal basis, with 2N vectors, and the minimal basis, with N+1 vector. In this research the later method is used.

With PSO, the collections of the vectors that form the pattern are fixed-direction vectors. For example, if there are two independent variables in the optimization problem, the default for a 2N positive basis consists of the following pattern vectors: \mathbf{V}_1=[1 0], \mathbf{V}_2=[0 1], \mathbf{V}_3=[-1 0], \mathbf{V}_4=[0 -1] they may be called the direction vectors. An N+1 positive basis consists of the following default pattern vectors: \mathbf{V}_1=[1 0], \mathbf{V}_2=[0 1], \mathbf{V}_3=[-1 -1], If a point in the mesh is found to improve the objective function at the current point, the new point becomes the current point at the next iteration.

The PSO optimization algorithm will repeat the illustrated steps until it finds the optimal solution for the minimization of the objective function. The algorithm stops when any of the following conditions occurs:

- The mesh size is less than mesh tolerance.
- The number of iterations performed by the algorithm reaches a predefined value.
- The total number of objective function evaluations performed by the algorithm reaches a pre-set maximum number of function evaluations.
- The distance between the point found at one successful poll and the point found at the next successful poll is less than a set tolerance.
- The change in the objective function from one successful poll to the next successful poll is less than a function tolerance.

The purpose of the optimization is to improve the transient response of the converter to obtain the fastest transient response with minimum ripple in the ac system voltage as well as keep power factor as near to one as possible. This is done by selecting appropriate gains and time constants of the PID (parameter vector $x$ in (13)). For the purpose of the optimization, an objective function (OF), which depends on the controller parameters is used. It penalizes the deviations in the output voltage $V_{dc}$ and power factor of ac network from their respective set points. Note that the explicit relationship between the objective function $F(x)$ and the parameter vector $x$ is not available as an analytical formula, but is computed from the simulation.

$$F(x) = C_1 \int (V_{dc}^{ref} - V_{dc}) dt + C_2 \int (I_q^{ref} - I_q) dt$$  \hspace{1cm} (13)

Where $x = (K_{p1}, K_{p2}, K_{i1}, K_{i2}, K_{d1}, K_{d2}, T_d)$, $C_1$ and $C_2$ are weighting factors.

Note that this OF penalizes both poor transient response as well as steady state ripple due to harmonics or sustained oscillations; as in either case, there would be non-zero deviations. The 7-dimensional OF in (13) is minimized using the pattern search optimization.

### Applications

THREE-PHASE ac–dc conversion of electric power is widely employed in adjustable-speeds drive (ASDs), uninterruptible power supplies (UPSs), HVDC systems, and utility interfaces with nonconventional energy sources such as solar photovoltaic systems (PVs), battery energy storage systems (BESSs), in process technology such as electroplating, welding units, etc.,
battery charging for electric vehicles, and power supplies for telecommunication systems.

**Materials and Methods**

**Materials**
1. Converter rating: 500 Volts DC, 500 kW
2. AC Supply: three-phase, 600 V\text{rms} (phase-phase), 30 MVA, 60 Hz system.
4. DC-Link capacitors of 75000 uF (two)
5. Controllers: uses voltage and current controllers as shown in figure.5 to control the DC voltage while maintaining a unity input power factor for the AC supply.

**Methodology**

Initially a 200kw the converter is loaded with 200kw load. At t=0.2s, a 200-kW load is switched-on. We can see that the dynamic response of the DC regulator to this sudden load variation (200kW to 400 kW) is satisfactory. The DC voltage is back to 500 V within 0.03 sec and the unity power factor on the AC side is maintained. This is shown in figure 6, figure 7 and figure 8.

**Results**

![Figure 6: Voltage waveform of V_{ab}](image)

Figure 7: Expanded view of voltage waveform of Vab showing input pulses

Figure 8: Input $V_{ab}$ voltage and input current $I_a$
Table 1: Control system parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimization-PSA</th>
<th>Optimization-ZN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage controller</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>1.5e-2</td>
<td>2e-2</td>
</tr>
<tr>
<td>I</td>
<td>1.6</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>1e-6</td>
</tr>
<tr>
<td>Current Controller</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.3</td>
<td>0.35</td>
</tr>
<tr>
<td>I</td>
<td>20</td>
<td>23</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Time constant of derivative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Td</td>
<td>0.2e-3</td>
<td>0.5e-3</td>
</tr>
<tr>
<td>Rise time</td>
<td>0.01sec</td>
<td>0.01sec</td>
</tr>
<tr>
<td>overshoot</td>
<td>25%</td>
<td>27%</td>
</tr>
<tr>
<td>undershoot</td>
<td>3%</td>
<td>6%</td>
</tr>
<tr>
<td>Steady state error</td>
<td>0.2%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Settling time</td>
<td>0.04 sec</td>
<td>0.1 sec</td>
</tr>
</tbody>
</table>

Results Discussions

Within the first 0.05 sec power factor is low as the controller is under transient conditions, there after power factor is almost unity. At t=0.3s, a "Stop Pulsing" signal is activated (pulses normally sent to the converter are
blocked). We can see that the DC voltage drops to 315 V. A drastic change in the primary current waveform can also be observed. When the pulses are blocked, the Three-Level Bridge block operation becomes similar to a three-phase diode bridge. As it can also be noticed in table1, optimizing PID parameters improves the systems dynamic and static response as noted overshoot improves to 25%, while undershoot improves to 3% from 6%. Settling time is also shortened a factor of 2.5 i.e. from 0.1 seconds to 0.04 seconds.

**Conclusion**

The paper presented an optimization problem using Pattern Search Optimization (PSO) for tuning and analysis of the control system for a Three-Level AC-DC converter. The paper used a simulation-based optimization tool to adjust the gains and time constants of the control of a converter. It has shown in the paper that using the optimization tool makes it possible to easily improve the transient performance of the converter by automatically adjusting the controller gains and time constants. Table1, figure.8 and figure.9 shows improved performance of controller characteristics.

**Recommendation**

Standard three-level NPC topology (Figure.1) can have a problem of unbalanced input which may lead to flow of current in neutral line hence causes unbalanced voltages across the dc-link capacitors. In such situation control strategy need to be introduced to eliminate such imbalance of dc-link voltages across capacitors.

**References**


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