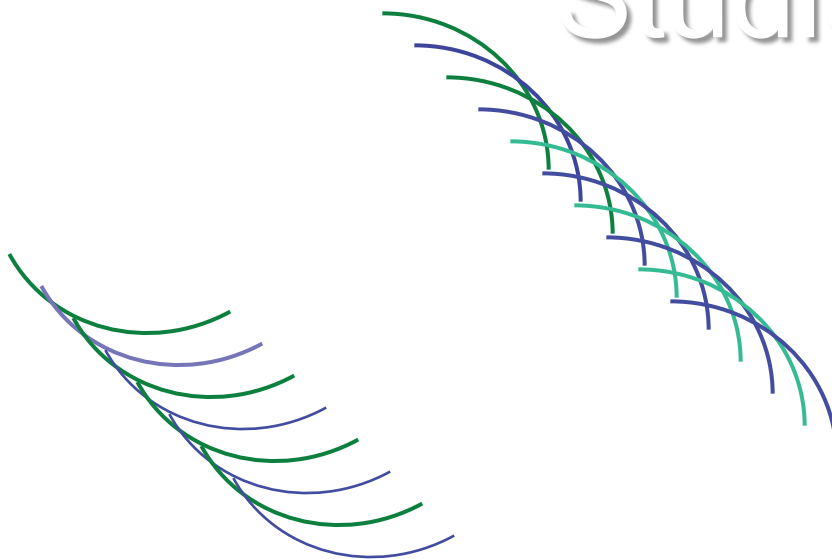


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Message from the Editor-in-Chief

It is with much joy and anticipation that we celebrate the launch of Malawi Journal of Agriculture, Natural Resources and Development Studies (MAJANDS) with this inaugural issue. On behalf of the MAJANDS Editorial Team, I would like to extend a very warm welcome to the readership of this journal. I take this opportunity to thank our authors, editors and anonymous reviewers, all of whom have volunteered to contribute to the success of the journal. I am also grateful to the Norwegian Government for funding this issue through the Capacity Building for Managing Climate Change (CABMACC) programme jointly coordinated by Lilongwe University of Agriculture and Natural Resources (LUANAR) and Norwegian University of Life Sciences (NMBU). Let me also thank LUANAR management for thinking strategically to revamp this journal which will ensure wider dissemination of research being done at LUANAR and outside.

MAJANDS is dedicated to the rapid dissemination of high quality research papers to help us meet the challenges of the 21st century. Manuscripts that articulate disparate orientations will be welcomed, given that this journal will be cross-disciplinary and cross-theoretical. Indeed, papers will emanate from numerous disciplines, agriculture, natural resources, climate change, and development studies that can demonstrate both short and long-term practical usefulness, particularly contributions that take a multidisciplinary approach because many real world problems are complex in nature.

We have come up with an initiative to publish articles related to development challenges and steps to solve them. So, in this regard I take an opportunity to invite authors to write articles (research papers, review, commentary, short communications, survey reports, or documentary) for publication in our journal.

MAJANDS provides an ideal forum for exchange of information on all of the above broader themes, and it is intended to be published twice a year. To ensure rapid dissemination of information, we aim at completing the review process of each paper within 3 months of initial submission. Interested authors can send their queries and/or manuscripts to the following email; majands@bunda.luanar.mw and more information can be found at our website <http://www.luanar.ac.mw>

Finally, we wish to encourage more contributions from the scientific community and industry practitioners to ensure a continued success of the journal. We also welcome comments and suggestions that could improve the quality of the journal.

It is our sincere hope that you will find MAJANDS informative.

Dr Daud Kassam (Associate Professor)

Editor-in-Chief

December 2015

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Sizes and Geometrical Characteristics of Watercans Used in Dimba Bucket Irrigation in Malawi

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Abstract

Bucket irrigation in wetland dambos and river valleys is wide-spread and growing throughout Malawi. A first-ever comprehensive national study was done to document the geometric characteristics and sources of watercans used in dimba bucket irrigation. Watercan sizes and geometric characteristics were measured in the dry season using four measuring tapes of the same design in twenty (20) of the 28 districts of Malawi over a four-year period (May-August between 2007 and 2010). A total of 753 watercans were randomly sampled in 70 towns, trading centres and market centres. Key parameters measured were source of the watercan, height and diameter of watercan; funnel distance from the top and bottom as well as the angle of inclination of the funnel. From the watercan diameter and height, the watercan volume was calculated for each watercan using a formula for a cylinder. Trigonometry was used to calculate the angle of inclination of the funnel and the diameter of the funnel. Descriptive statistics were used to analyze the data. The study found out that watercans were made locally by tinsmiths within the district. Vendors sourced the watercans from one trading centre to sell to another within the district. The geometric characteristics and volumes of the watercans did not vary significantly across the districts. Commonest volume of the watercans ranged from 12 to 15 litres while the most occurring heights and diameters ranged from 28 to 35 cm and 23 to 26 cm respectively. This would seem to imply that watercan tinsmiths must have been trained using a common watercan design.

Keywords: bucket irrigation; dambo; dimba; Malawi; shallow wells; watercan.

Introduction

Malawi has a unimodal rainfall pattern and highly dependent on rain-fed agriculture as the main way of crop production (Malunga, 2009 and MoG, 2010). Coupled with erratic rainfall due to climate change, Malawi finds herself persistently vulnerable to food insecurity. Dimba gardens in dambos (wetlands) used for winter cultivation significantly contribute to household food security and income (Chinsinga, 2007). Dimba cultivation offers farmers increased food security, by either supplementing meagre harvests from the rain-fed crop production or income from vegetable sales. Mulwafu (2003) argued in support of dimba cultivation and noted that dimbas present lasting opportunities for the improvement of living standards of peasant farmers in Malawi and consequently alleviating rural poverty.

Watercan or bucket irrigation is the simplest form of overhead informal irrigation. It is usually practiced along river valleys or low lying water holding areas (dambos) (Wiyo and Kasomekera, 1994). IWMI (2006) defined informal irrigation as those sections of the irrigation sector which have established themselves without public funding and official recognition. Generally, the informal irrigation sectors are managed by farmers without technical assistance from government or Non Governmental Organizations (NGOs) (Malunga, 2009). Smallholder informal irrigation is an important livelihood of the rural communities and it is widely spread not only in Malawi but also throughout Africa (IWMI, 2006, Mwalwafu, 2003).

Dimba Farming in Malawi

Dimba cultivation in Malawi is usually practiced in flood plains or wetlands (locally known as dambos) and in river valleys used for winter cultivation where water is abundant even during the dry season. Wetland dambos are flat open spaces existing along river courses or near lakes. They may be swamps or low lying areas of land which are subject to inundation, usually with hydromorphic and calcimorphic soils. Dambos and river valleys are suitable for agriculture because of their available water and high

soil fertility (Malunga, 2009). It is in these dambo areas that smallholder farmers establish dimbas (gardens) (Wiyo and Kasomekera, 1994). It is estimated that Malawi has a total wetland area or dambo area of about 480,000 to 600,000 ha (Malunga, 2009). Out of this, dimba cultivation covers as much as 123,000 hectares of land compared to only 47,000 hectares which are under formal irrigation (Chinsinga, 2007). Over the years, dimba farming has spread to many districts of Malawi and government policy is now encouraging the growing of a second crop in dimbas during the dry season and has given birth to the Greenbelt Irrigation Initiative (Wiyo and Mtethiwa, 2014).

A wide range of crops are grown in dimbas for both home consumption and for income. The main crops in dimba gardens are maize, rice and a variety of vegetables (tomatoes, cabbages, onions, mustard and potatoes). These crops primarily primarily on residual moisture but in the event of critical moisture stress, farmers dig shallow wells and use watercans, pails/buckets and even plates to irrigate (Kambewa, 2005). Dimba cultivation has become a very important activity in the wake of the recurrent spells of droughts and erratic rainfall since the turn of the 1990s, although very little is known about it (Chinsinga, 2007).

Dimba gardens contribute substantially to family food security and income by providing farmers with the opportunity to cultivate additional crops, either to supplement their meagre rain-fed harvests or for sale. A study by Wiyo and Kasomekera (1994) in Ntcheu and Dedza districts of Malawi found out that dimba farming contributes close to 70 percent of the household income and hence its continuing popularity in these two districts. A study by Peters (2004) in Lake Chilwa basin of Malawi found out that dimba gardens constituted the major source of cash income to the families, as well as contributing towards the family food supply. It is thus not surprising that dimba cultivation constituted the policy package in the implementation of the 2003 subsidized Targeted Input Programme (TIP) by the government of Malawi in a quest of recovering from the 2002 drought and subsequent hunger crisis (Chinsinga, 2007).

Use of Watercans in Dimba Farming

Maintaining year-round maize and vegetable production depends on access to water sources for irrigation purposes during the dry season (Drechselet al., 2006; Wiyo et al., 2014). Dambos are subject to flooding during rainy season and retain some residual moisture during dry season (Malunga, 2009). Farmers resort to digging shallow (open) wells in the dambos in a quest to sustain winter cultivation while utilizing watercan (bucket) irrigation (Wiyo and Kasomekera, 1994); Wiyo et al., 2014 and Kambewa, 2005). The dry season in Malawi, when irrigation is a necessity, stretches from April to late November when effective rains begin. The residual moisture from rainfall is often depleted by July. This calls for supplementary irrigation through informal bucket irrigation from dug shallow wells using watercans. In most cases, advantage is taken of seasonal flooding to plant one crop of rice followed by vegetables on residual moisture or irrigated by watercans from shallow wells (Wiyo and Kasomekera., 1994; Wiyo et al., 2014).

Watercans are made from sheet metal by local tinsmiths. Malawi is self-sufficient in watercans production because of the many tinsmiths located in trading and market centres throughout the country. Tinsmiths were first trained by missionaries in vocational schools during colonial times. Hundred years later, it is common to find a tinsmith in every trading or market centre. As such very few watercans are imported while some are exported to Mocambique (Reserve Bank of Malawi, 2008). Major towns and market centres often receive watercans made by tinsmiths in surrounding small trading centres brought in by vendors.

Watercan or bucket irrigation has several advantages. A watercan is one of the low-cost irrigation technologies employed by small-scale farmers in Africa and is readily available (Malunga, 2009). IWMI (2006) observed that watercans are most commonly used to fetch water manually in informal irrigation systems like dimbas. IWMI (2006) argued that the watercan or bucket irrigation ensures precise water application on fragile vegetables; has low investment costs because they are mostly locally made; has low risk of theft, easy maintenance, and high level of spatial and temporal flexibility. Further, watercans or buckets allow economical water use and thus, exhibit high water efficiency (GoM, 1998).

Despite this favourable situation and advantages of watercans, watercan or bucket irrigation has some drawbacks. Wiyo and Kasomekera (1994) and IWMI (2006) argued that a watercan or bucket irrigation is labour intensive but has high water application rates (640-1600 mm per year). Further, the weight of the water (10-15 litres for a watercan and about 20 litres for a bucket) limits its use to nearby streams or shallow wells/ponds resulting in only a small area being irrigated (IWMI, 2006).

The objective of the study was to assess and compare the sizes (height, diameter and volumes) and geometric characteristics (funnel size and inclination) of watercans used by dimba farmers in twenty districts of Malawi. Secondly, the study wanted to assess the spatial and temporal variability and stability of the watercan design over the years of fabrication since the colonial times. Thirdly, the study wanted to establish whether the watercans are locally made or brought in by vendors from outside the trading centre. This is the first-ever comprehensive national survey on watercans in Malawi. This study was necessitated by the

growing importance of dimba cultivation throughout the country and the Malawi government's desire to introduce other means of lifting water from the shallow wells using treadle pumps and motorized pumps in an attempt to reduce labour demands of bucket irrigation (Wiyo et al. 2014). This study fulfils a future research aim to investigate the water management practices of dimba farmers by estimating the variation in water volumes applied to the crop by a watercan and the labour demands associated with it.

Materials and Methods

Watercan sizes and geometric characteristics data were collected over four years in twenty (20) of the 28 districts of Malawi in the dry season (May-August) between 2007 and 2010 as shown in Figure 1. Due to logistical challenges and financial limitations, we were not able to reach the eight remaining districts (Likoma, Nkhonkhotakota, Ntchisi, Machinga, Mangochi, Mwanza, Chikwawa and Nsanje). This was part of research activities by the authors to understand dimba farming water management aspects throughout Malawi. Bucket irrigation using watercans is wide-spread throughout Malawi and is a key activity during the dry season. In order to carry out this research, trading/market centres where watercans are produced and sold by tinsmiths were visited on market days. A total 753 watercans were randomly sampled in 70 towns, market centres and trading centres of the 20 districts of Malawi (Table 1 and Figure 1). This was the first comprehensive national survey on watercans in Malawi. The 20 districts and the 70 towns and market centres in Table 1 were selected because of their close proximity to all dimba cultivation areas of Malawi.

Watercans were selected at random and their sizes and geometric characteristics measured using four measuring tapes of the same design and accurate to the nearest millimetre. Depending on availability, the watercan sample size for each of the trading or market centres ranged from 5 to 36. The sizes measured were height (H) and diameter (D) of watercan while geometric characteristics measured were the distance from the bottom of the watercan to the lower end of the funnel, the distance from the top end of the watercan to the top end of funnel as well as the horizontal distance from the edge of the watercan to the end of the funnel. From these dimensions, the angle of inclination of the funnel was calculated using trigonometry. Among the socio-economic data collected was the source of the watercans indicating whether the watercans were locally made by tinsmith at the market or trading centre or were brought in from outside the market or trading centre with the source centre name recorded. From the watercan diameter (D) and height (H), volume of the watercan was calculated using a mathematical formula for a cylinder ($\frac{22}{7} * D^2 / 4 * H$). The width of the funnel was found by subtraction of the top and bottom heights while trigonometry was used to calculate the angle of inclination of the funnel. Descriptive statistics (means, maximum, minimum and standard deviation) of the measured and calculated parameters were found by district using excel spreadsheet.

Results and Discussions

This section presents the findings of the study and discusses the implications of the study. Findings are divided into sources and suppliers of watercans, geometric characteristics of the watercans as well as sizes and volumes of the water cans.

Table 1: Watercan survey location by district, sample size and number of market centres

District (Sample N)	Market centres by district (Number)
Balaka (29)	Boma, Zalewa Turn-off (2centres)
Blantyre (40)	Limbe market, Ndirande market, Blantyre market, Chilomoni, Lirangwe, Lunzu, Mpemba, Chilobwe (8centres)
Chiradzulu (15)	Njuli, Ngulidi, Boma (3centres)
Chitipa (11)	Boma (1centre)
Dedza (57)	Bembeke, Dedza Town, Chimbiya, Lobi (4centres)
Dowa (44)	Mtengowanthena, Mponela, Madisi, Mvera (4centres)
Karonga (10)	Boma (1centre)
Kasungu (49)	Buwa, Boma, Nkhamenya (3centres)
Lilongwe (161)	Nathenje, Nanjiri, Mkwinda, Chiseka, Mitundu, Msundwe, Nkhoma, Kamphata, Lumbadzi, Namitete (10centres)
Mchinji (35)	Boma, Kamwendo (2centres)
Mulanje (17)	Chonde, Chitakale, Boma, Border (4centres)
Mzimba (27)	Ekwendeni, Boma (2centres)
Neno (5)	Zalewa (1centre)
Nkhatabay (22)	Boma, Chitheche (2centres)
Ntcheu (121)	Manjawira, Bawi, Nsipe, Kampepuza, Boma, Tsangano Turn off, Mlangeni, Lizulu, Masasa (9centres)
Phalombe (30)	Holy family, Boma, Mthuka, Nyezelera, Naminjiwa, Migowi (6centres)
Rumphi (9)	Boma (1centre)
Salima (36)	Kamuzu Road (1centre)
Thyolo (25)	Luchenza, Goliati, Bvumbwe, Mikolongwe (4centre)
Zomba (10)	Zomba market, Chinamwali (2centres)

Table 2: Sources of watercans used in dimba irrigation in Malawi by district

	Supplier Tinsmith	Supplier Vendor	Source Locally Made	Source Brought from Somewhere
Balaka	✓	✓	✓	
Blantyre	✓	Some	✓	✓ Manyowe
Chiradzulu	✓			✓ Yasini
Chitipa	Some		✓	
Dedza	✓		✓	
Dowa	✓		✓	
Karonga	✓		✓	
Kasungu	✓		✓	
Lilongwe	✓	Some	✓	✓ Ngwenya, Nkhoma
Mchinji	✓		✓	
Mulanje	✓	Some	✓	✓ Nkhonya, Mulanje
Mzimba	✓		✓	
Neno	✓		✓	
Nkhatabay	✓		✓	
Ntcheu	✓	Some	✓	Mlangeni, Tsangano
Phalombe	✓			
Rumphi	✓		✓	
Salima	✓		✓	
Thyolo	✓	Some	✓	✓ Bangwe, Brantyre
Zomba	✓		✓	

Table 3: Sources of watercans by vendors

Market Centre	Source Trading Centres by Vendors
Blantyre Market	Manyowe, Blantyre
Lirangwe, Blantyre	Matindi, Blantyre
Chilobwe, Blantyre	Chimwankhunda, Blantyre
Njuli, Chiradzulu	Mbulumbuzi, Chiradzulu
Chiradzulu, Boma	Yasini, Chiradzulu
Nanjiri, Lilongwe	Ngwenya, Nkhoma, Lilongwe
Mkwinda, Lilongwe	Nkhoma, Lilongwe
Chitakale, Mulanje	Nkhonya, Mulanje
Tsangano Turn-off, Ntcheu	Mlangeni, Ntcheu
Mlangeni, Ntcheu	Tsangano Turn-off
Bvumbwe, Thyolo	Bangwe, Blantyre

Table 4: Mean geometric characteristics of water cans by district

District	Height (cm)	Diameter (cm)	Funnel Angle of Inclination (°)
Balaka	30.0	24.03	48.40
Blantyre	29.4	23.7	35.87
Chiradzulu	29.0	24.3	32.44
Chitipa	30.0	23.9	52.56
Dedza	33.1	24.4	46.60
Dowa	30.2	23.7	49.32
Karonga	30.5	24.1	53.86
Kasungu	30.3	23.8	49.44
Lilongwe	30.0	23.9	42.32
Mchinji	30.1	23.7	50.59
Mulanje	29.5	24.9	32.01
Mzimba	30.2	24.1	53.54
Neno	30.6	24.4	38.61
Nkhatabay	30.1	24.1	54.59
Ntcheu	32.1	24.1	47.05
Phalombe	29.6	24.3	41.10
Rumphi	30.3	23.9	48.94
Salima	29.3	23.6	47.45
Thyolo	28.8	24.1	34.55
Zomba	28.5	24.1	34.60
Mean	30.4	24.0	44.90
SD	1.69	0.69	7.62
CV(%)	5.6	2.9	17.00
Min	23.5	21.4	15.95
Max	35.5	29.5	61.11
CI	23.80-37.03	21.30-26.71	

CI= confidence interval at 95 percent probability.

Table 5: Mean watercan volumes by district

District	N	Mean(??)	CV(%)	Min(l)	Max(l)
Balaka	29	13.60	3.89	12.8	14.62
Blantyre	40	13.00	8.61	10.22	15.50
Chiradzulu	15	13.50	7.70	11.49	14.97
Chitipa	11	13.50	14.09	12.12	19.10
Dedza	57	13.50	9.56	11.08	22.22
Dowa	44	13.40	4.56	11.08	17.19
Karonga	10	13.86	2.62	13.10	14.4
Kasungu	49	13.50	3.61	12.47	14.22
Lilongwe	161	13.48	4.96	11.01	14.98
Mchinji	35	13.29	5.04	12.05	14.22
Mulanje	17	14.38	10.01	12.11	17.68
Mzimba	27	13.81	11.09	11.90	19.10
Neno	5	14.27	1.65	13.87	14.48
Nkhatabay	22	13.77	9.37	12.12	19.10
Ntcheu	121	14.60	9.37	12.30	18.59
Phalombe	30	13.72	5.52	11.44	14.95
Rumphi	9	13.66	4.03	12.58	14.58
Salima	36	12.80	4.36	11.64	13.67
Thyolo	25	13.19	4.20	12.26	14.97
Zomba	10	12.99	8.80	11.64	14.85
Total/Mean	753	13.69	6.69	11.96	16.17

Figure 1: Map of Malawi showing location of districts surveyed. Likoma, Nkhonkhotakota, Nchisi, Machinga, Mangochi, Mwanza, Chikwawa and Nsanje were not surveyed due to logistical challenges



Figure 2: A scatter plot of diameter versus height of water cans indicating concentration of diameter (23-26 cm) and height sizes (28-35 cm).

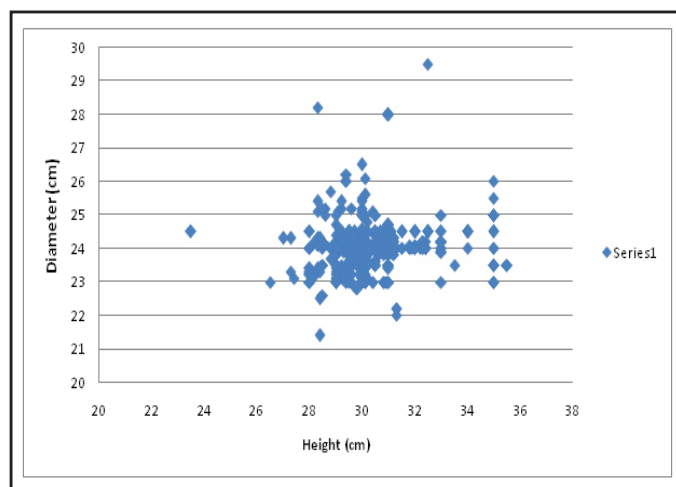
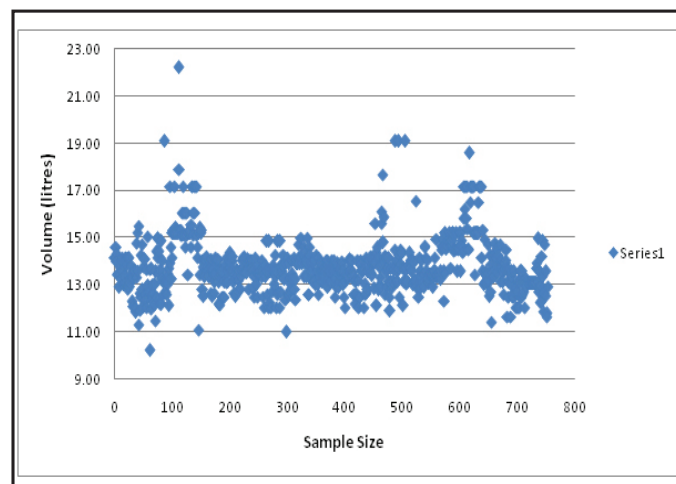


Figure 3: Scatter plot of volume of watercan versus sample size showing most sizes are concentrated between 11 to 15 litres



Sources, suppliers and movement of watercans

Table 2 shows the suppliers of watercans and where the watercans are produced. The major suppliers of watercans are the local tinsmiths resident at trading or market centres followed by vendors who bring watercans from somewhere. The results indicate that most of the watercans sold at trading or market centres are locally made by local tinsmith while a few of the watercans are brought by vendors from nearby trading/market centres within the same district. Movement of watercans across districts is very rare. Watercans are locally made within the district with some little movement from nearby trading centres within the district.

This is consistent with literature such as Malunga (2009) and GoM (1998) that most water cans are locally made within the vicinities where dimba irrigated cultivation is practiced. This implies that dimba farmers surrounding the market centre can easily access these watercans as they are affordable and there is no need to travel long distances to buy the water cans and hence their popularity. Fabricated watercans are a bulky product not easily transported over long distances. It therefore makes sense that watercans movement and trade is limited to nearby towns and market centres within the district.

Role of vendors in supplying watercans

Vendors play an important role in facilitating access to watercans by dimba farmers. As earlier pointed out, watercans are rarely brought in from areas outside the district. Table 2 indicate that the majority of watercans sold in the trading/market centres are locally made while a few are brought in by vendors from nearby towns (Table 2). Table 3 indicates that vendors get the watercans from somewhere in the district and sell the watercans in market centres within the same district. There is very little inter-district trade in watercans.

Geometric characteristics and volume of watercans

Table 4 and Figure 2 indicate that there were no significant differences in height as well as diameter of watercans across the 20 districts and 70 trading/market centres. The watercan height ranged from 28 to 35 cm while the watercan diameter ranged from 23 to 26 cm with small coefficient of variation ($CV < 5.7\%$). This is within the 95 percent confidence interval (CI) for both height (23.80-37.03cm) and diameter (21.30-26.71cm). However, there was great variation in the angle of inclination of the funnel. The mean inclination angle was 44.87° with a minimum of 15.95° and a maximum of 61.11° degrees. The coefficient of variation was higher (17.0 %) perhaps a reflection of the difficulties of fixing the watercan funnel at one standard angle by different tinsmiths. It is difficult for each tinsmith to maintain the same funnel angle as the soldering is being done during fabrication.

Table 5 shows that the watercan volumes ranged from 12-15 litres with the overall coefficient of variation (CV) of less than 7 percent. The maximum CV (10-11%) were observed in Chitipa, Mzimba and Mulanje. Other districts had a CV of less than 10 percent. The volume sizes are within the expected volume range for watercans of 10-15 litres (IWMI, 2006).

The fact that the height, diameter and volume of watercans have low spatial and temporal variability would seem to imply that Malawian tinsmiths were trained using a common standard design that has been maintained from tinsmiths to tinsmiths over the years through apprenticeships.

This is plausible given the fact that the first watercans were brought into the country during colonial times by the missionaries who trained a few local tinsmiths in vocation schools to make these. In turn, these trained others through apprenticeships until today the design has not changed much in over 100 years. This is remarkable stability in the replication of the design from one tinsmith to another.

Conclusion

Watercan or bucket irrigation is one of the most relevant irrigation methods to smallholder farmers in Malawi. Water cans are mainly used to irrigate vegetables in dimbas. The study found out that most of the water cans were made locally by local tinsmith and inter-district movement of the watercans was rare. In areas where the watercans were being brought from somewhere, vendors played a crucial role in moving and facilitating access to water cans by small scale farmers. Specifically, vendors sourced the water cans from within the districts where the market centres are located. Of major importance, the geometric characteristics and volumes of the water cans and did not vary significantly across the districts. The most common volumes of the water cans ranged from 12 to 15 litres while the most occurring heights and diameters ranged from 28 to 35 cm and 23 to 26 cm respectively. This implies that the makers of the water cans must have been trained from a common source of design and over the years have passed the design knowledge from generation to generation through apprenticeships.

Acknowledgements

The authors acknowledge the financial support of Bunda College, University of Malawi and Concern Universal to the study. The assistance of Dola Biswick, Gift Kamwamba and Madalo Mtsinje in collecting data in the twenty districts of Malawi is acknowledged.

Author Contributions

Kenneth Wiyo the principal investigator and Jean Mtethiwa the co-investigator originated the study concept, designed the study and the study tools. They analyzed the data, found and discussed the results and drew conclusions. Joseph Kanyamula helped with some of the graphical analyses and populated the first draft using a template provided by the two investigators. Lastly, Kenneth Wiyo and Jean Mtethiwa did the editing and finalization of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript and in the decision to publish the results.

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The potential for using anaerobic digester effluents in recirculating hydroponics system for lettuce production

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Abstract

Fertiliser costs along with public demand for sustainable production of organically grown produce and the need for removing nutrients from anaerobic digester effluents prior to discharge into receiving waters have necessitated research on using anaerobic digester effluent as an alternative fertilizer for hydroponics systems. This study evaluated the potential for replacing inorganic chemical fertilizers in hydroponics systems with anaerobic digester (AD) effluent by comparing primary-treated anaerobic digester (PTAD) effluent and aerobically-treated anaerobic digester (ATAD) effluent as nutrient solutions for hydroponically grown lettuce. A commercial nutrient solution was used as control. The response of the lettuce crop and the level of nutrient removal were assessed in three recirculating hydroponics systems employing a combination of the nutrient-film technique (NFT) and the ebb-and-flow method. The results from the study have shown that the PTAD effluent could not support the growth of lettuce while the ATAD effluent was able to produce a vigorous crop of lettuce whose yield was calculated to be 70 – 75 percent of the control crop. The results have reinforced the findings from previous studies that raw anaerobic digester effluents were not suitable as nutrients solutions for hydroponics systems due to their high ammonia concentration which have a toxic effect on most crops. The success of the ATAD effluent supports the recommendation for nitrification to improve the quality of anaerobic digester effluents. The hydroponics system was capable of removing large amounts of ammonia, nitrate and phosphorus from the nutrient solution. This signals that the hydroponics system can effectively be used as a technology for removing nutrients from treated anaerobic digester effluents while producing a valuable crop.

Keywords: anaerobic digester effluent; hydroponics system; nutrient removal; nitrification; nitrates; phosphates.

Introduction

Most crops are grown in field soils but the soil itself is not essentially necessary for plant growth. The soil simply acts as a reservoir for water and nutrients and also provides support for the plants. Plants can therefore be produced in soilless cultures which artificially provide the plants with support and a reservoir for water and nutrients. A soilless culture in which plants are either grown in a static pool of water or in a channel with a recirculating flow of water is known as a hydroponics system. In a hydroponics system, the water in the system is the sole source of the dissolved minerals and other ingredients that are fed to the plant through the roots. The advantages of hydroponics include close control of crop nutrition, a reduction in labour requirement, ease of irrigation, economy of water usage, lack of weeds, improved yields, and avoidance of soil-borne diseases and pests (FAO, 1990; Jones, 1997; Resh, 1998). In addition, hydroponics systems avoid the problem of nitrate contamination of groundwater (Ayaz and Saygin, 1996).

The two most common hydroponics systems in use today are the nutrient film technique (NFT) and the ebb-and-flow or flood-and-drain technique. The NFT growing system consists of a series of channels through which nutrient solution is recirculated from a supply tank. The plants are placed in the channels in which a small film of liquid is maintained. The ebb-and-flow technique utilizes a channel filled with thoroughly cleaned and sterilized pea gravel or some other inert material of similar size (Johnson, 2000). The plants are grown in the pea gravel in the channel. The channel is plumbed to a nutrient solution reservoir and is flooded with the nutrient solution and drained periodically rather than continuously. The pea gravel in the channel is maintained wet and is not allowed to dry.

In a typical hydroponics system, inorganic salts or fertilizers are dissolved in water to produce a nutrient solution that supplies all the nutrients for the plants. Commercial nutrient solutions are also available for use in hydroponics systems. Essential nutrients for plant growth include the

macronutrients (N, P, K, Ca, Mg, and S) and the micronutrients (Fe, B, Mn, Zn, Cu, Mo, and Cl). These nutrients must be in sufficient amounts and in the right concentrations (FAO, 1990; Jones, 1997; Resh, 1998; Decoteau, 2000). The required concentrations for the nutrients in the nutrient solution vary with the type of crop but there are some standard formulations that have been adopted for general hydroponics use (Johnson, 2000; Cooper, 1976). There is need for a routine analysis of the nutrient solution, growing media, and crop. The analysis of the nutrient solution should include pH, EC, and concentrations of the major elements N, P, K, Ca and Mg (Jones, 1997; FAO, 1990).

Fertiliser costs along with public demand for sustainable production of organically grown produce and the need for removing nutrients from anaerobic digester effluents prior to discharge into receiving waters have necessitated research on using anaerobic digester effluent as an alternative fertilizer for hydroponics systems (Ayaz and Saygin, 1996; Boyden and Rababah, 1996; Mackowiak et al., 1996; Strayer et al., 1997; Garland et al., 2000; Rababah and Ashbolt, 2000; Liedl et al., 2006; Alhattab and Ghaly, 2012; Krishnasamy et al., 2012; Neal and Wilkie, 2014). Anaerobic digester effluent contains adequate amounts of plant nutrients including nitrogen and can be used to grow crops hydroponically. However, the nitrogen in the effluent is in ammonium form (Moller and Muller, 2012). A number of studies have shown that the nitrogen form affects growth and yield of many vegetables (Gamiely et al., 1991). In plant nutrition, the main difference between nitrate (NO₃) and ammonium (NH₄) is that high rates of ammonium are highly toxic to plants since free ammonium irreversibly disrupts the structure of the thylakoid membrane (Wakiuchi et al., 1971; Simonne et al., 2001).

The ammonium nitrogen can be transformed to nitrate through nitrification in an aerobic process. Nitrification is a sequential conversion of ammonium ions to nitrites and then nitrates by aerobic bacteria. For plants that have a preference for nitrate over ammonium, nitrification is needed for better fertilizer assimilation. The nitrification

of anaerobic digester effluent has been widely studied (Xu et al., 2014). Nitrosomonas and Nitrobacter are the two examples of bacteria genera responsible for nitrification of ammonia within the environment. As the bacteria are always present in most well aerated soils, nitrification of anaerobic digester effluent is not an issue when the effluent is applied to the soil (Tanabe and Sato, 2014). However, in hydroponic production, the nitrification rate may be limited by a lack of nitrifying bacteria, which are not present in the effluent of a digester (Neal and Wilkie, 2014).

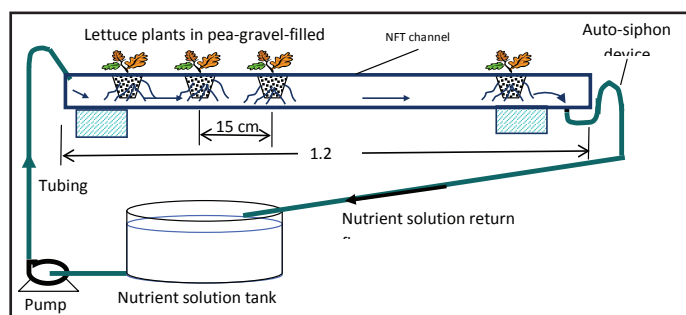
This study evaluated the potential for replacing inorganic chemical fertilizers in hydroponics systems with anaerobic digester (AD) effluent by comparing primary-treated anaerobic digester (PTAD) effluent and aerobically-treated anaerobic digester (ATAD) effluent as nutrient solutions for hydroponically grown lettuce. The study specifically evaluated lettuce response to the two effluents and determined the level of nutrient removal from the solution. A commercial nutrient solution was used as control.

2. Materials and methods

2.1 Recirculating hydroponics system

The study was conducted at Bunda College of Agriculture in the Agricultural Engineering Department. Three recirculating hydroponics systems were specifically designed for use in this study. Each system was a combination of the NFT and the ebb-and-flow technique. The NFT channel was constructed from a 75-mm HDPE pipe that was 1.2 m long. Each channel had nine 6-cm diameter holes cut at a centre-to-centre distance of 15 cm for fitting planting pots. Each system had nine 750-ml plastic cups that served as the planting pots. The planting pots were perforated and were filled with pea gravel. The pea gravel provided support to the plants while also retaining moisture. The flow of nutrient solution was achieved using a plumbing system consisting of a 10-mm tubing, a Masterflex® Easy-Load® II Laboratory/Standard high precision peristaltic pump and a 5-L nutrient solution tank. An auto-siphon device ensured automatic drainage of the nutrient solution once it reached the top level of the channel. Figure 1 is a schematic diagram of one of the hydroponics systems.

Figure 1 : Schematic diagram of a recirculating hydroponics unit



2.2 Greenhouse

The three hydroponics systems were located in a 1.5 m long, 1.5 m wide and 2.0 m high greenhouse which was completely covered with a single layer of transparent polyethylene sheet. Ventilation and air circulation inside the greenhouse was accomplished through the use of openings and electric fans. A lighting power of 110 W/m² was provided inside the greenhouse through the use of six 40-W General

Electric Plant/Aquarium® wide spectrum fluorescent tubes that were placed parallel to the NFT channels and 300 mm above the plants. The humidity and carbon dioxide levels in the greenhouse were not controlled or monitored but the temperature was maintained below 20°C to satisfy the requirement for lettuce.

2.3 Nutrient solutions

The nutrient solutions for the study were obtained from an anaerobic digester for dairy manure that was operated with a retention time of 20 days under ambient conditions (average ambient temperature of 20°C). The settleable solids in the effluent were allowed to settle and the clarified supernatant was collected and stored in a refrigerator at 4°C for use as primary-treated anaerobic digester (PTAD) effluent. One half of the PTAD effluent was aerobically-treated in a 20-L complete-mix reactor (CMR). Compressed air was supplied to the CMR at a rate of 2.5 litres/min through an air stone located at the bottom of the CMR. The primary objective of the aeration treatment was to convert the ammonia and any organic nitrogen in the PTAD effluent into nitrate. The CMR was operated at a retention time of 10 days to ensure complete nitrification of all the ammonia. The effluent from the CMR was clarified in a settling tank to produce clarified aerobic-treated anaerobic digester (ATAD) effluent. The ATAD effluent was also stored in a refrigerator at 4°C for use in the hydroponics system. A control nutrient solution was prepared using a commercial plant nutrient mix, Grow More Tropical Plant Food® containing 20% nitrogen, 6% P₂O₅, 16% K₂O, 1.0% calcium, 0.5% magnesium, 1.0% sulphur, 0.05% copper, 0.1% iron, 0.05% manganese, 0.0005% molybdenum and 0.05% zinc.

2.4 Lettuce seedlings

Leaf lettuce, of the cultivar Black Seeded Simpson, was selected for this study because of its short maturity period and ability to grow successfully in hydroponics systems. Lettuce seedlings were produced in plug trays that were filled with potting soil. A single seed was placed into each plug. Watering of the seeds was done manually at a frequency that ensured that the trays were moist all the time. Once the seeds had germinated the amount of water was increased to ensure that the trays were always wet. It took two weeks for the seedlings to reach transplanting stage.

2.5 Tests

Two sets of tests were conducted to evaluate the potential for the PTAD effluent and the ATAD effluent as nutrient solutions for hydroponically grown lettuce. Both tests were designed as a single factor experiment with the two nutrients solution as treatments and the commercial nutrient mix as control. The two treatments and control were assigned at random to the three hydroponics systems. Samples of the nutrient solutions were analysed for total ammonia-nitrogen (TAN), nitrate-nitrogen (nitrate-N), and ortho-phosphates (ortho-P) as PO₄³⁻ using the procedure described in APHA (2005). The pH and electrical conductivity (EC) of the nutrient solutions were measured using an Accumet® AR 50 dual channel pH/ion/conductivity meter.

2.6 Transplanting of seedlings

Lettuce seedlings having an average mass of 1.3 ± 0.5 g were transplanted into clearly marked planting pots in the three NFT channels of the hydroponics system. Prior to

transplanting the seedlings, all the soil was removed from the roots by thoroughly cleaning them in a bucket of tap water. The seedlings were assigned at random to the two treatments and control. Nine seedlings were allocated to each treatment and control. After transplanting all the twenty-seven seedlings into the planting pots, the hydroponics systems were turned on.

2.7 Operation of the hydroponics system

The operation of the pumps was controlled by a ChronTrol® controller and timer which were programmed to turn on the pumps for 5 minutes every 2 hours during the first week following transplanting and thereafter every hour. The flow rate was maintained at 1.0 L/min so that the entire volume of nutrient solution was circulated during each pumping cycle. The nutrient solution was pumped from the nutrient solution tank to the head of the NFT channel from where it flowed through the channel by gravity and in the process wetting the gravel-filled planting pots and then flowed back into the nutrient solution tank in a closed loop configuration. To ensure that the gravel in the planting pots was completely flooded with nutrient solution, the auto-siphon device installed at the lower end of the NFT channels ensured that the nutrient solution filled the entire depth of channel before discharging the solution out of the channels. The gravel was able to retain enough moisture for the entire period when the pumps were off.

2.8 Management of the nutrient solution

In the two tests, the nutrient solutions were maintained in the hydroponics systems for 18 days before replacement with a fresh set of nutrient solutions. In those 18 days, fresh water was added to make up for any loss of water but no nutrients were added. Before replacing the nutrient solutions, samples were taken and analysed for TAN, nitrate-N, ortho-P, pH, and EC using the procedure described in APHA (2005). The percentage reduction in the nutrients (TAN, nitrate-N, and ortho-P) was calculated from the initial and final nutrient concentrations.

2.9 Harvesting of the lettuce

The lettuce was harvested 36 days after transplanting. Harvesting involved the careful removal of the entire lettuce plant from the planting pot. Each lettuce plant was cut into two parts: the top part which consisted of leaves and the bottom part consisting of roots. The two parts were immediately weighed to obtain their wet masses. The mass of the plant tops was used as the measure of lettuce yield. A one-way analysis of variance (ANOVA) test was conducted to test for any statistical differences in the lettuce yield. A means test was conducted to compare the mean lettuce yields from the two treatments and control. The statistical analysis was done using the statistical package Statistix® for Windows.

3. Results and Discussion

3.1 Characteristics of nutrient solutions

The characteristics of the nutrient solutions for the two tests are given in Table 1. As expected, the PTAD effluent had a high ammonia concentration and zero nitrate while the ATAD effluent had a high nitrate concentration and almost zero ammonia concentration. The nitrification process had managed to convert all the ammonia into nitrate. The ortho-phosphates were, however, reduced to almost zero in the ATAD effluent. The removal of the ortho-phosphates

was largely due to uptake by bacteria during the nitrification process as observed by Sedlak (1991). The bacterial cells that had taken up the ortho-phosphates were removed as sediments through gravity settling. The PTAD effluent used in the second test was derived from that used in the first test by using a dilution factor of three.

Table 1 Characteristics of nutrient solutions in the two tests

Test	Treatment	Nutrient Solution	TAN (mg/L)	Nitrate-N (mg/L)	Ortho-P (mg PO ₄ ³⁻ /L)	pH	EC (mS/cm)
1	PTAD	PTAD effluent	223	0	182	7.40	2.75
	ATAD	ATAD effluent	2	355	2	8.15	2.24
	Control	Commercial solution	144	244	132	7.05	2.90
2	PTAD	PTAD effluent	70	0	61	7.92	2.54
	ATAD	ATAD effluent	2	355	2	8.15	2.24
	Control	Commercial solution	144	244	132	7.05	2.90

3.2 Lettuce growth

In both tests, all the lettuce in the two treatments and control survived after transplanting. It was however noted that the lettuce plants in the ATAD effluent treatment and the control solution grew vigorously and appeared healthier than those in the PTAD effluent treatment. The plants in the ATAD effluent treatment and control treatments had big green leaves and a well developed and extensive root system. The lettuce plants in the PTAD effluent treatment were all stunted with yellow leaves. It was observed that the root tips for the plants in the PTAD effluent treatment were continuously dying. Although the second test had a lower TAN concentration in the PTAD effluent treatment than in the first test, there was no significant improvement in the lettuce growth. After the plants were harvested the roots for all the lettuce were weighed and the results are given in Table 2. The results clearly showed that the lettuce in the PTAD effluent treatment had poor root growth and therefore could not produce a vigorous crop of lettuce.

Table 2 Average mass of lettuce roots (wet basis)

Test	Treatment	Nutrient solution	Mean (g)	Std. Dev (g)
1	PTAD	PTAD effluent	1.7	0.7
	ATAD	ATAD effluent	14.2	1.2
	Control	Commercial solution	9.9	0.9
2	PTAD	PTAD effluent	1.9	0.6
	ATAD	ATAD effluent	16.4	1.5
	Control	Commercial solution	11.1	1.1

3.3 Lettuce yield

The average lettuce yield for the two treatments and the control in the two tests is presented in Table 3. An ANOVA test performed on the yield indicated that there were significant differences ($P < 0.05$) in the lettuce yield of the two treatments and control. In both tests, the yield of the control lettuce was the highest while that of the PTAD effluent treatment was the lowest. Figure 2 shows the lettuce plants at harvest stage. The results reinforced the findings from previous studies that raw anaerobic digester effluents are not suitable as nutrient solutions for hydroponics

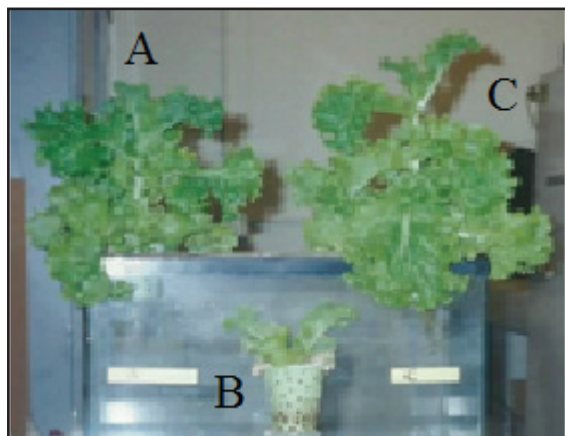
systems due to their high ammonia concentration (Neal and Wilkie, 2014; Krishnasamy et al., 2012; Liedl et al., 2004). The lettuce in the ATAD effluent treatment did not suffer the same fate as those in the PTAD effluent treatment signifying that the nitrification process improved the nutrient quality by transforming the ammonia into nitrate. This supports the recommendation made by Neal and Wilkie (2014) that nitrification was required to make anaerobic digester effluents suitable as nutrient solutions for hydroponics systems. The yield from the ATAD effluent treatment was calculated to be between 70 – 75 per cent of the control lettuce. This could be attributed to the difference in the nutrients available in the solutions. The control solution had all the essential nutrients while the ATAD effluent did not have any phosphorus, but might have contained the other trace nutrients. However, no complete analysis of all the nutrients except for nitrogen and phosphates was done.

Table 3: Average lettuce yield

Treatment	Nutrient Solution	Yield (g) for Test 1		Yield (g) for Test 2	
		Mean	Std. Dev	Mean	Mean
PTAD	PSAD effluent	4.7 ^a	8.0	12.0 ^a	1.1
ATAD	ATAD effluent	52.6 ^b	5.8	68.1 ^b	7.2
Control	Commercial solution	75.3 ^c	6.5	91.0 ^c	8.7

In each column, means followed by the same letter are not statistically different (Tukey's test $P > 0.05$).

Figure 2: Lettuce plants at harvest stage: A is ATAD treatment, B is PTAD treatment and C is control



3.4 Nutrient removal

The nitrogen and phosphorus removed by the system was calculated from the TAN, nitrate and ortho-phosphates available in the system at the start and at the end of the tests and the results are presented in Tables 5 and 6. The results indicate that the hydroponics system was able to remove significant amounts of ammonia-N, nitrate-N and ortho-P from the nutrient solutions. The nutrient removal mechanisms and levels of removal were different in the two treatments and the control. The removal of the nutrients in the ATAD effluent treatment and the control could be attributed to plant uptake since there was good plant growth

capable of utilizing the nutrients. Plant uptake has been reported to be the major nutrient removal mechanism in planted wetlands (Zhang et al., 2007). These wetlands are similar to the hydroponics system that was used in this study. Other nutrient removal mechanisms such as denitrification could also have contributed to the reduction in the nitrate concentration.

In the PTAD effluent treatment, plant uptake could not have contributed much to the removal of the nutrients because of the poor plant growth. The removal of the ammonia could be attributed to volatilization and transformation into nitrates due to nitrification. The presence of nitrate at the end of the tests signified that nitrification was taking place in the PTAD effluent treatment. The removal of the phosphorus in the PTAD effluent treatment could not be attributed to plant uptake since the plants failed to grow. Studies in wetlands have shown that the mechanisms responsible for phosphorus removal have largely been those more related to gravel surface processes such as physical adsorption and chemical precipitation by Ca or Fe (Richardson, 1985; Faulkner and Richardson, 1989) than to biological processes such as plant and microorganisms uptake. Since the hydroponics system used in this study was similar to a wetland, physical adsorption onto the pea gravels in the growing pots and chemical precipitation can also be considered to be the mechanisms responsible for the removal of the phosphorus in the hydroponics system. In the control system, in addition to plant uptake, the potting pea gravel adsorption, chemical precipitation and denitrification could also have removed the nutrients in the system. In both tests, the decrease in EC and the increase in pH could be attributed to ionic nutrient uptake by the plants or the natural aging of the solutions.

4. Conclusions

The results from the study have shown that ATAD effluent is a suitable nutrient solution for hydroponically grown lettuce while PTAD effluent is not. The PTAD effluent could not support the growth of lettuce while the ATAD effluent was able to produce a vigorous crop of lettuce whose yield was 70 – 75 percent of the control crop. The lettuce plants that were grown using the PTAD effluent failed to grow because their roots could not develop. This reinforces the findings from previous studies that raw anaerobic digester effluents were not suitable as nutrients solutions for hydroponics systems due to their high ammonia concentration which have a toxic effect on most crops. The ability of the ATAD effluent supports the recommendation for nitrification to improve the quality of the anaerobic digester effluent prior to use as nutrient solution for hydroponics systems. The study has also shown that the hydroponics system can effectively be used as a technology for removing nutrients from treated anaerobic digester effluents prior to discharge to receiving waters. The results indicated that the hydroponics system was capable of removing significant amounts of ammonia, nitrate and phosphorus from the nutrient solutions.

5. Acknowledgements

The author is acknowledging the Agricultural Engineering Department at the Lilongwe University of Agriculture and Natural Resources (LUANAR) for providing laboratory space and equipment for the study.

Table 5 Initial and final nutrient solution characteristics and nutrient removal for Test 1

Parameter	PTAD effluent Treatment			ATAD effluent Treatment			Commercial solution Control		
	Initial	Final	Removal (%)	Initial	Final	Removal (%)	Initial	Final	Removal (%)
TAN (mg/L)	223	1	99.6	2	0	100.0	144	0	100.0
Nitrate-N (mg/L)	0	30	-	355	35	90.1	244	60	75.5
Ortho-P (mg/L)	182	9	95.1	2	0	100.0	132	23	82.6
pH	7.4	9.05	-	8.15	8.88	-	7.05	8.16	-
EC (mS/cm)	2.75	1.39	-	2.24	1.16	-	2.90	0.85	-

Table 6 Initial and final nutrient solution characteristics and nutrient removal (%) for Test 2

Parameter	PTAD effluent Treatment			ATAD effluent Treatment			Commercial solution Control		
	Initial	Final	Removal (%)	Initial	Final	Removal (%)	Initial	Final	Removal (%)
TAN (mg/L)	70	4	94.3	2	0	100.0	144	0	100.0
Nitrate-N (mg/L)	0	40	-	355	60	83.1	244	45	81.6
Ortho-P (mg/L)	61	11	82.0	2	0	100.0	132	14	89.4
pH	7.92	8.06	-	8.15	8.70	-	7.05	8.54	-
EC (mS/cm)	2.54	2.21	-	2.24	1.85	-	2.90	0.94	-

6. Conflict of interest

The author declares no conflict of interest in this work.

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Characterisation of breeding systems for Malawi Zebu cattle in Mzimba District, Northern Malawi

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Abstract

A study was carried out to characterise the cattle breeding systems and structures in Mzimba District, Northern Malawi as a preparatory step towards the definition of the breeding goal for Malawi Zebu cattle in the district. In 2010, a household survey was conducted in five randomly selected cattle breeding areas in five Extension Planning Areas (EPAs). A total of 87 farmers were randomly selected and interviewed using a semi-structured questionnaire. A census of livestock ownership was also carried out in the selected breeding areas. Rates of inbreeding in cattle populations were calculated based on the number of breedable bulls and cows using equations proposed by Mackay and Falconer. The rates of inbreeding ranged from 0.612% to 1.335%. Cattle that were transferred into the herds mostly came from surrounding villages (47%) and cattle markets (29%). Preferred outlet methods were cattle markets (40%) and middlemen (40%). Selection was not done for cows, and selection for breeding bulls was indirect, through selection of bull calves and young bulls to be castrated for draught power. The decision about which bull calf should be left in the herd for breeding was done by an individual farmer although such a decision affected the whole breeding area because the cattle mixed freely. About 64.6% of the farmers did not have any bulls in their herds. Farmers reported that they could predict the milk yield potential of a cow by its udder morphological characteristics (57.2%) and coat colour (14.3%); beef production potential by body size and structure (85.1%); power by body size and structure (40.9%) and strength (22.7%). There were no organised cattle breeding systems in the study areas, but there was potential to effect a community breeding programme.

Keywords: Breeding structures; breeding systems; inbreeding; crossbreeding; outcrossing

Introduction

The Malawi Zebu cattle dominate the cattle population in Malawi numerically and in terms of utilisation. In rural areas, beef and milk almost entirely come from Malawi Zebu cattle. In addition, Malawi Zebu cattle males are often castrated to provide draught power, and cows form the dam line for production of crossbred dairy cattle, as they possess adaptive characteristics that make the crosses perform better than pure breeds under low-input production systems (Gondwe 2011). The majority of beef produced in Malawi is also from the Malawi Zebu. Unfortunately, the Malawi Zebu is a low producer of milk and meat products compared to breeds introduced from developed economies (DAGRIS 2007). This deficiency in the Malawi Zebu was at first considered to be correctable by crossbreeding, but others thought that it is more sensible to improve the Zebu through within breed selection. Kasowanjete (1979) evaluated three breeding systems: within-Malawi Zebu breeding, upgrading Malawi Zebu to Charolais and Malawi Zebu/Charolais rotational cross breeding. Using total productivity as yearling weight, he found that the breeding systems involving cross breeding were superior to the within Malawi Zebu breeding. However, when the total productivity was evaluated economically, the within-Malawi Zebu breeding system was superior to the other two. He therefore concluded that under similar economic conditions, it was worthwhile to use the within-Malawi Zebu breeding. Zimba (1991) reported that when selection was done at the Government Livestock centres between 1953 to 1965, average weights increased from 18 kg to 21 kg at birth, from 127 to 154 kg at one year old, from 155 to 224 at two years, and from 228 to 300 kg at three years old. Strydom (2008) reported that beef cattle indigenous to Southern Africa can potentially produce quality meat economically comparable to exotic European beef breeds, based on results from many trials aimed at comparing the two groups of beef breeds. These research results suggest that there is need for functional breeding programmes aimed at within breed selection in the Malawi

Zebu. Simpson (2002) argued that given the extremely difficult conditions for the livestock sector in Malawi, we should be attempting to develop the local genetic resources (Malawi Zebu) instead on depending on exotic breeds as far as cattle are concerned. Unfortunately, no organised within-Malawi Zebu breeding system was ever implemented beyond the experimental stage, and emphasis in Zebu breeding has for a long time been put on cross breeding the Zebu with dairy cattle. One of the organisations that have taken up the idea of within breed selection is Better life for All (BELIFA), a local non-governmental organisation working in Mzimba District. The objective of BELIFA was to improve the livelihoods of the people in the district through increased beef off-take as well as through introduction of small holder dairy production. It was understood that it was possible for beef and dairy interventions to take different genetic improvement approaches but there was need understand the existing breeding practices and systems. BELIFA intended to make sure that this aspect of cattle management is well-addressed, considering that previously, new breeds were introduced without putting in place rules for sustaining the breeds through well-defined breeding programmes.

This study was therefore conducted to document existing breeding practices, systems and programmes for the Malawi Zebu. This was an entry point towards the definition of the farmers' breeding goal (Duguma et al 2010).

Materials and methods

The study was carried out in Mzimba District, where BELIFA operates. The district is located in the Northern Region of Malawi, bordered by Zambia to the west, Rumphi to the North, Nkhatabay and Nkhotakota to the East and Kasungu to the South. The district is the largest in Malawi by land size, located between the coordinates 11°30'S; 33°30'E. It covers an area of 10,430 km² and has a human population of 727,931 (NSO 2008). The district is culturally dominated by Ngoni tribe, who are traditionally cattle keepers.

Sampling procedure and data collection

The study first defined Malawi Zebu breeding areas through focus group discussions with farmers and assistant veterinary workers in five EPAs in which BELIFA operates: Champhira, Kazomba, Manyamula, Luwerezi and Njuyu (Figure 1). The corresponding breeding areas in each EPA were Kamalambo, Daniel Gausi, Manyamula, Popopo and Gowoka. A breeding area was defined as a cluster of villages in which the cattle generally graze as unit and breed, so that the animals in such an area can be recognised as constituting a distinct, closed breeding population (Table 1). Subsequently, a breeding area was randomly selected from five Extension Planning Areas (EPAs) where BELIFA is working in Mzimba District. Breeding system characterisation took place within these breeding areas.

The mean number of cattle farmers per breeding area was 23.8. The minimum number, n , of farmers required to be interviewed in each breeding area was determined by estimating the number of observations potentially needed to distinguish between breeding areas by 30% in some of the important farm variables (Mburu, et al 2007) using the formula $n = (zc/d)^2$, where $z = 1.96$ for 95% confidence interval, $c =$ coefficient of variation and $d =$ level of difference. Coefficient of variation used was 51% as estimated by Khonje (1989) in a study in a similar area (Mbawa) in Mzimba. Using the formula, the minimum number of farmers per breeding area was 14. A semi-structured questionnaire was used to collect characterisation data from the selected farmers. A total of 87 out of the 119 cattle farmers in the breeding areas were interviewed. In addition to use of questionnaire, a census of all livestock owned by all the cattle keepers in each breeding area was also conducted after it was noticed that there were no reliable livestock population figures in the study areas at sub-EPA level.

Data analysis

Cattle population data were analysed using descriptive statistics. Herd composition and herd dynamics data were used to estimate the flow of breeding stock within and between breeding areas. Effective population sizes and rates of inbreeding were estimated using the numbers of breedable males and females in the populations as proposed by Mackay and Falconer (1996). The rate of inbreeding was estimated using the equation:

$$\Delta F = \frac{N_m + N_f}{(4N_m N_f)}$$

where N_m and N_f are the numbers of breeding males and females, respectively, in the population. The multiple correspondence procedure of SPSS (IBM Corporation, 2011) was used to analyse the determinants of movement (transfers-in and out) of cattle within and between breeding areas.

Results

Demographic characteristics of the households

Ninety-six per cent of the households were male-headed. The majority of the respondents (75%) owned cattle, 9% cared for cattle but were not owners, while 16.1%

were caretakers of cattle for extended families. Over 93% of the farmers were literate, and majority of these (91%) had some primary education. About 63% of the farmers had some leadership position in the society and these included village heads (28%), religious leaders (21%) and leaders of organisations (7%).

Effective population sizes and estimated rates of inbreeding

There were 1397 cattle comprising 31.4% cows and 6.7% bulls, with an average herd size of 11.7 head of cattle per farmer (Table 2). These percentages correspond to a bull to cow ratio of about 1:5 for the communal breeding areas, which is far above the recommended level of between 1:10 and 1:60 for range conditions (Rupp et al, 1977; Healy et al, 1993). Ndebele et al (2007) reported a bull/cow ratio of 1: 20 in similar communal production system in the Gwayi region of South-Western Zimbabwe. The distribution of different classes of cattle in the different EPAs was the same (Table 3), with the exception of heifers, whose numbers were highest in Gowoka ($p=0.015$). About 71.4% of the farmers had at least a pair of oxen.

Effective population size is defined as the number of breeding individuals in an idealised population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration. The effective population sizes for Daniel Gausi and Manyamula breeding areas were below the acceptable minimum of 50 (Meuwissen 2009). The other breeding areas had effective population sizes between 50 and 100 (Table 4), within which inbreeding can be difficult to control unless the mating among the cattle is non-assortative.

Table 1: Breeding population structure and size of the breeding areas. The number of farmers per village and the number of villages per breeding area was taken as a measure of the size of the breeding area.

Breeding area	Number of villages per breeding population	Number of farmers	Mean number of farmers per village with cattle	Standard deviation
Daniel Gausi	6	22	3.67	1.25
Gowoka	11	21	1.91	1.08
Kamalambo	13	25	1.92	1.27
Manyamula	4	22	5.50	3.28
Popopo	18	29	1.61	0.76
Overall	52	119	2.29	1.76

Extent of exchange of genetic material within and between breeding areas

About 77% of the farmers reported inward or outward transfer of a total of 185 (13%) cattle of different age classes during the previous year. The major means of transfer off or heifer- and bull-calves into and out of the herds were through birth and deaths (Table 5). The transfer of cattle, especially heifers, bulls and oxen, through gifts and dowry was highly associated with Kazomba EPA. Luwerezi, Manyamula and Champhira EPAs showed a significant activity of middlemen. Gowoka Cattle Market (in Gowoka breeding area, Njuyu EPA) was mostly utilised by farmers from distant areas other than those from Gowoka Breeding Area itself. On the other hand, sale of steers was highly

associated with Gowoka Cattle Market (in Njuyu EPA). About 47% of the animals that moved into the herds were from surrounding villages, 29% from the market (within the breeding area) and the rest from the other sources (Figure 2). On the other hand, when selling out, the main channels were through cattle market (40%) and middlemen (40%). The relationships between the factors of the extent of exchange of genetic material within and between breeding areas are given in Figure 3.

Table 2: Overall herd structures for households in all the breeding areas (n = 119)

Class of cattle	Mean± Standard deviation	Minimum	Maximum	Median	Sum	Percentage
Bulls	0.790±1.10	0	7	1	94	6.73
Cows	3.68±3.04	0	22	3	438	31.4
Heifers	2.31±2.17	0	14	2	275	19.7
Heifer Calves	1.23±1.42	0	7	1	146	10.5
Bull Calves	1.23±1.34	0	8	1	146	10.5
Oxen	2.13±1.59	0	7	2	253	18.1
Steers	0.38±0.77	0	4	0	45	3.22
Overall	11.7±8.2	-	-	10	1397	100

Table 3: Cattle herd composition per household by breeding area

Class	Breeding area (Mean ± standard deviation)				
	Kamalambo	Daniel Gausi	Manyamula	Popopo	Gowoka
Bulls	1.00±1.19	0.545±0.671	0.500±0.673	0.655±0.769	1.29±1.79
Cows	3.64±2.36	4.41±4.16	2.86±1.67	3.55±2.84	4.00±3.73
Heifers	2.68±1.99 ^a	2.00±2.20 ^b	1.32±0.995 ^b	2.14±1.71 ^b	3.48±3.16 ^a
Heifer Calves	1.08±1.15	1.32±1.64	1.46±1.34	1.03±1.50	1.33±1.53
Bull Calves	1.04±0.935	1.41±1.71	1.18±0.958	1.35±1.37	1.14±1.68
Oxen	2.56±1.47	2.46±1.68	1.96±1.56	1.93±1.67	1.71±1.52
Steers	0.520±0.770	0.182±0.395	0.227±0.685	0.448±0.827	0.500±1.05

ab Means in the same row without a common letter are different at P<0.05

Table 4: Estimated population sizes and rates of inbreeding among Malawi Zebu cattle

Breeding area	Number of cows	Number of bulls	Estimated effective population size	Estimated rate of inbreeding (%)
Gowoka	84	27	82	0.612
Kamalambo	91	25	79	0.637
Daniel Gausi	97	12	43	1.17
Manyamula	63	11	37	1.34
Popopo	103	19	65	0.779
Overall	438	94	310	0.162

Criteria farmers use to select and cull their breeding animals within a breeding group

Parameters related to cattle selection decisions are given in Table 6. Majority (92%) of the farmers used all the available cows for breeding. Selection was done only in male animals starting at about 6 months when noticeable differences between bull calves could be used to choose superior bull calves. This selection was indirect. The default

practice was to castrate bull calves for sale and for power. The best bull calves were castrated so that they could be trained as oxen. When a farmer decided to have a bull, he left an average of about one bull calf uncastrated, after castrating the best ones in terms of size and conformation for power. About 74.6% of the farmers castrated all bull calves and young bulls by the time they were about 36 months old. Very few farmers (1.6%) maintained more than one bulls in their herds, but the bulls that were still in the herd but were no longer needed for breeding were castrated for draught power by the time they were about 48 months. The rest of the farmers (23.8%) sold all intact bull calves not intended for power, mainly to middlemen or at cattle markets. Consequently, a big proportion of the farmers (64.6%) did not have bulls in their herds. About 68% of the respondents had no idea what characteristics can be used to predict milk yield (Table 7). Of the ones that had an idea, about 57% reported that they could predict the milk yield potential of a cow by udder morphological characteristics and 14% reported using coat colour. The farmers that used coat colour to predict milk yield reported that black and white cows give more milk than cows with other colours. The farmers that could predict milk yield from teat and udder characteristics indicated that the bigger the udder the higher the milk yield.

Discussion

Demographic characteristics of the households

The farmers in the study site were generally literate. Level of education has been shown to determine the farmer's ability or readiness to adopt new technologies (Ampaire and Rothschild, 2011; Ndebele et al, 2007; Rahman, 2007). It is also noteworthy that a significant proportion of the farmers had some leadership position in the society. Farmers with leadership positions in non-farming or non-livestock activities can have tremendous influence in decision making concerning communally managed livestock resources such as grazing areas.

Effective population sizes and estimated rates of inbreeding

Manyamula breeding area had the highest rate of inbreeding followed by Daniel Gausi. This was so because in Manyamula and Daniel Gausi, the number of bulls was small compared to other breeding areas. Effective population sizes and subsequently inbreeding levels are largely dependent on the sex that has smaller numbers than the other. If the cattle in all the 5 breeding areas were treated as one population, the rate of inbreeding would be markedly low. This implies that if out-crossing is practised between the areas, the rate of inbreeding is going to be acceptably low (0.162%). Out-crossing can be achieved through the exchange of breeding bulls by farmers in different breeding areas. This may occur through sales, deliberate exchange of cattle within and between communities and through dowry and gifts. Encouraging farmers to engage in this kind of exchange may be a very effective tool for controlling inbreeding, especially in the areas where inbreeding might be a big problem such as Manyamula and Daniel Gausi.

Extent of exchange of genetic material within and between breeding areas

The association between some EPAs (Luwerezi, Manyamula and Champhira) and involvement of middlemen implied that the exchange of genetic materials in this areas is likely to be higher than in the other areas. These results

agreed with what was hypothesised by Changadeya et al (2012) that functional markets lead to a better exchange of genetic material. The results also suggested that EPAs with or in proximity to functional cattle markets were associated. Farmers preferred to buy from other farmers whom they know, or from a cattle market. This purchasing behaviour also confirms that the breeding areas can indeed be considered as closed populations.

farmer, and this was indirectly through the selection of animals for draught power. An individual farmer's selection decisions led to breeding consequences in the whole breeding area because a bull left in the herd for breeding would also breed with cows from other herds.

Table 5: Number of cattle that moved into and out of herds

Direction transfer	Mode of transfer	The class of the moving animal							Total
		Cows	Heifers	Heifer Calves	Bull Calves	Steers	Bulls	Oxen	
	Birth	-	-	7	11	-	-	-	18
	Received dowry	7	5	0	0	0	1	9	22
In	Bought	8	5	0	3	1	5	8	30
	Received as a gift	1	1	0	0	0	2	0	4
	Subtotal	16	11	7	14	1	8	17	74
	Dead	8	6	5	8	0	3	2	32
	Sold alive	11	1	0	6	2	0	5	25
Out	Lost	1	0	0	0	0	1	0	2
	Given out as a gift	0	1	0	0	0	0	0	1
	Paid dowry	1	5	0	0	0	0	0	6
	Subtotal	21	13	5	14	2	4	7	66
Overall		37	24	12	28	3	12	24	140

Table 6: Cattle section decisions parameters

Parameter	Percentage
Reason for not selecting cows (n=71)	
Use all available cows to increase the herd	75
Do not know about the need for selecting only the best cows for breeding	25
Fate of unselected bull calves (n=63)	
Sold	23.8
Castrate	74.6
Leave them intact	1.59
Reason for not having a bull (n=53)	
Easier to hire bull	2.24
Rely on other farmers' bulls during grazing	22.2
Always castrate bulls	57.8
Can't afford to keep a bull	11.1
Bulls are difficult to manage	6.7
Selection decision level (n=53)	
No selection done	29.3
Jointly by the Community	1.3
Individual owner of cattle	69.9

Criteria farmers use to select and cull their breeding animals within a breeding group

The results suggested that the objective of the farmers was primarily to increase herds, as indicated by lack of selection of cows for breeding, which makes sense because the mean number of cows per farmer is small (3.68). The

Although there was no organised system for selecting breeding animals at community, it was noted that selection decisions that individual farmer made were influenced by actions of other farmers. For example, when there was no bull in a village, a farmer was more likely to not to castrate all bull

calves and spare at least one for breeding. When there was a bull in a village, the farmer banked on the bulls of their colleagues, and sold or castrated their bull calves or young bulls. This meant that it was possible to effect a community based breeding programme in the breeding areas, since it is not difficult to have all unselected animals disposed or prevented from breeding. The farmers did report ability to select animals based on indicator traits such as coat colour and teat and udder characteristics. There are reports that colour is indeed related to milk yield, especially in temperate breeds raised in tropical climates (Becerril et al 1994; Ouma et al 2004). These results are also similar to what was reported by Rege et al (2001) from a study in Kenya where farmers put much emphasis on milk production, body size, coat colour and horn shape for selecting breeding animals. Ngowi, Chenyambuga and Gwakisa (2008) reported that farmers keeping Tarime cattle in Tanzania placed much emphasis on tolerance to diseases (73.4%), draught power (65%), meat taste (30.8%) and milk quality (14.2%) when selecting cattle for breeding.

It is therefore important to check if these teat and udder characteristics in Malawi Zebu cattle can be used as predictors of the milk yield and mothering ability, not only for dairy production, but also for beef production realised through faster growth rates of calves. Relationship between milk yield and teat and udder traits has been demonstrated in water buffaloes (Prasad et al 2010). The possibility of using teat and udder traits as indicator traits for selecting for economic traits is supported by classical studies by Lojda, Stavikova, and Polacek (1982), who indicated that the heritability for teat shape and teat end shape were moderately high and Tilki et al (2005). Lin et al (1987) reported udder height can be used as a good predictor of milk yield in cattle. The characteristics for power and beef were similar. The farmers expect more beef and power from animals which are big, stocky and have straight legs. This agrees with the observation that the farmers' quest for bigger animals is a reflection of their need for as much power output as possible, while at the same time obtaining maximum benefit from sales of beef (DFID 2006). Unfortunately, priority was given to castration of the best looking animals bull calves for power, and this could lead to perpetuation of inferior genes in the populations.

Table 7: Desirable characteristics of cattle for different uses

Desired product	Characteristics looked for	Per cent
Beef (n = 47)	Body size and structure	87.0
	Body size and Coat Color	2.2
	Coat Colour	2.2
	Straight legs	6.5
Milk (n = 36)	Strength	22.2
	Body Size	19.4
	Coat Colour	5.6
	Teat Length	2.8
	Teat Presence	2.8
Power (n = 22)	Teat Size	47.2
	Udder Size	40.9
	Body Size and Structure	40.9
	Colour	4.6
	Hard Skin, Tail near the knee	4.6
	Height and Length	4.6
	Length of Horns	4.6
	Length of legs	4.6
	Strength	22.7
	Strong legs	9.1
Temperament	4.6	
All products (n = 50)	Its body structure	16.0
	Its size	60.0
	Its color	4.0
	Performance of its relatives	14.0
	Its aggressiveness	6.0

Table 8: The desirable levels of traits for milk production (n=36)

Trait	Level	Percent
Body Size	Large body	19.4
	Medium body	2.8
Coat Colour	Black	2.8
	Black and brown	2.8
	Black and White	8.3
Teat Presence	All teats present	2.8
Teat Size	Large teats	2.8
Teat length	Long teats	5.6
Udder size	Big Udder	47.2

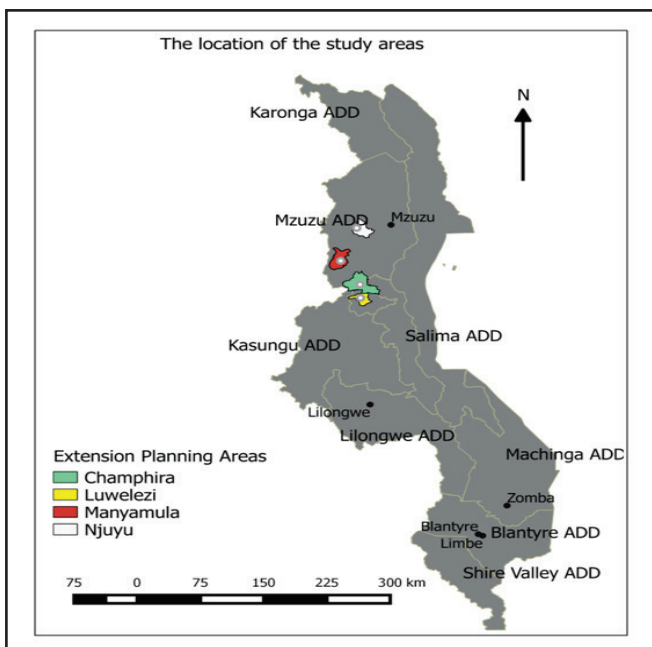


Figure 1: The location of the breeding areas in which data were collected within the EPAs

Conclusion

There were no organised cattle breeding systems in the study areas. Considering that the cattle were grazed together, one would expect communal agreements and structures for selection of breeding animals, especially males, but these were not available. The existing cattle breeding systems were primarily aimed at maintaining or increasing the cattle herds, with all cows being maintained in the herd for breeding without any selection. Selection of bulls for breeding purposes was indirect, through the selection of the best bull calves and young bulls for castration for power. This implies that the bull calves that were left in the herd for breeding may not make the best bulls for breeding for improvement of beef and milk. Farmers did have some knowledge about the relationship between certain traits and desired outputs. However, there was need to carry out research to validate some of the farmers' selection criteria (such as coat colour and udder morphology) as predictors of such traits as milk yield, beef and power. The verification would help in indirect selection in such systems where recording of important traits is not taking place.

Acknowledgment

The authors would like to thank NORAD that funded Better Life for ALL (BELIFA) through Agriculture Research and Development Program (ARDEP) to implement livestock improvement project in Mzimba District. The BELIFA team is commended for incorporating research into the development project. Also, appreciation goes to all farmers that participated in the study.

Author Contributions

All the three authors were involved in the design of the study. W Nandolo was responsible for field data collection, collation and management as well as the initial data analysis. Both T Gondwe and L J Banda were responsible for supervision of the data collection exercise, and validation of the data. T Gondwe analyzed part of the data. L J Banda assisted with re-organization and discussion of the paper including proof-reading.

Conflicts of Interest

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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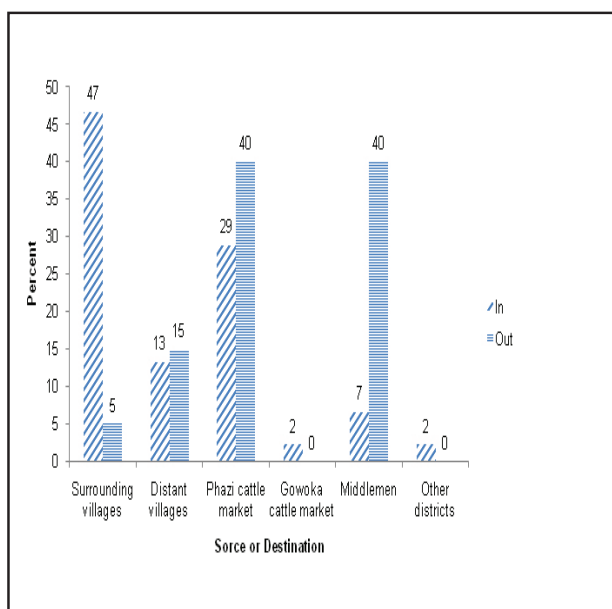


Figure 2: Sources and destinations of cattle that moved in and out of the herds

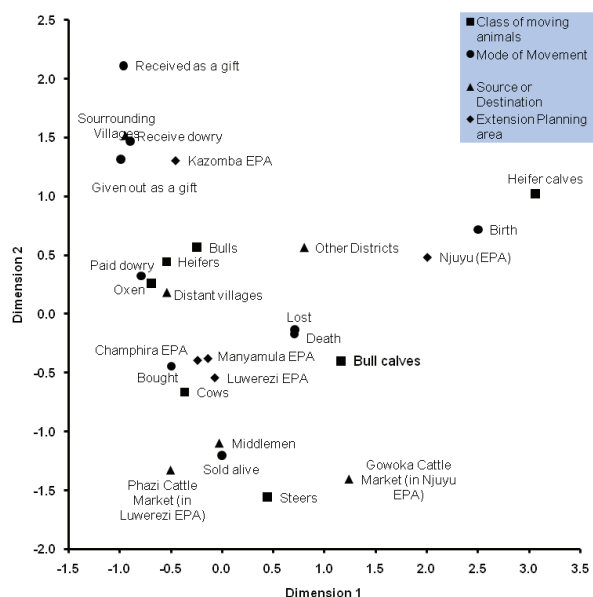


Figure 3: The relationship between class of moving animals, mode of transfer, source or destination of the moving animal and the EPA

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The breeding potential of local maize varieties as source of resistance to the maize weevil and larger grain borer in Malawi

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Abstract

Maize weevil (MW) (*Sitophilus zeamais* Motschulsky) and larger grain borer (LGB) (*Prostephanus truncatus* Horn) are the most important storage pests of maize in Malawi. Malawian farmers continue to cultivate local maize varieties partly due their perceived tolerance to storage pests. A study was conducted at Chitedze Agricultural Research Station in 2012 and the objectives were to determine levels of LGB and MW resistance among local maize varieties and to identify local varieties that can be exploited for LGB and MW resistance breeding. Sixty eight local varieties were assessed for MW and LGB resistance using fecundity, grain damage (%), grain weight loss (%) and flour weight. Significant differences were observed among the varieties for adult mortality, median development period, grain damage (%) and number of F1 progenies where MW was used as a test pest. About 14.5% of the varieties were resistant, 21.7% were moderately resistant, 24.6% moderately susceptible, 23.2% susceptible and 16% highly susceptible. Maize varieties, such as, 1772, 1983, 1992 and 3243 were resistant to MW. For LGB, significant differences were observed for insect mortality, total number of insects, grain damage (%), flour weight (g) and grain weight loss (%) and all varieties were susceptible. However, varieties 1992, 2012, and 1983 were less susceptible to LGB. Variation for resistance against MW and LGB exists among local varieties which can be exploited to improve resistance in productive maize populations. However, for LGB resistance, recurrent selection should be used to increase frequency of resistant genes in the identified varieties.

Keywords: larger grain borer; insect resistance; resistance variation; maize breeding; maize weevil

Introduction

Maize (*Zea mays* L) is an important staple food crop in Malawi. However, postharvest losses due to storage insect pests are becoming a serious challenge to food security at household level in the country (Denning et al., 2009). Maize weevil (MW) (*Sitophilus zeamais* Motschulsky) and larger grain borer (LGB) (*Prostephanus truncatus* Horn) are the most important postharvest pests in Malawi (Makoka, 2008; Singano et al., 2009; Kamanula et al., 2011). Yield losses ranging from 5% to 80% caused by maize weevil have been reported (Tigar et al., 1994; Pingali and Pandey, 2001; Dhliwayo et al., 2005). Larger grain borer is prevalent in Africa and is negatively affecting maize production (Tefera et al., 2011). For instance, in 2012, about 47000 tonnes of household grain losses of maize in Malawi were reportedly due to LGB (APHLIS, 2015) and from 1995 to 2001, weight loss of stored maize due to the pest increased from 5 to 16% (Denning et al., 2009; Singano et al., 2009).

The management of the two major storage insect pests of maize has relied heavily on the use of chemical compounds, such as Actellic Super dust, a cocktail of organophosphate and pyrethroid (Dhliwayo and Pixley, 2003; Ching'oma, 2009). Unfortunately, the use of storage insecticides to control insect pests such as maize weevil and larger grain borer is being threatened by development of insect resistance (Golob, 2002; Fragoso et al., 2005; Pereira et al., 2009). In addition, these storage insecticides are generally costly to smallholder farmers (Dhliwayo and Pixley, 2003). However, host resistance can be integrated into the pest management system and could provide a durable means of resistance to pest damage (Smith, 1994). Unfortunately, host resistance has largely been overlooked in Malawi, mainly due to the promotion of storage pesticide use against storage pests.

Understanding the variation for resistance that may exist among genotypes is an important step in breeding for durable pest resistance (Mwololo et al., 2010). Differential reaction of genotypes to insect pests can be exploited for

breeding purposes (Kitaw et al., 2001). For example, resistant varieties can be combined with other control measures like use of storage facilities e.g. metal silos to protect grains from *P. truncatus* and *S. zeamais* (Tefera et al., 2011). The combination of a biological agent (histerid beetle, *Teretrius nigrescens* Lewis) with both resistant and susceptible maize grains increases maize resistance to storage pests through reduced progeny numbers, grain weight loss and frass production (Bergvinson and García-Lara, 2011).

Genetic variation for resistance against the storage pests has been observed. Variable and useful maize weevil resistance has been reported by Kim and Kossou (2003) in both open pollinated and hybrid cultivars of maize in Africa. Derera et al. (2000) reported variation for resistance against maize weevil among maize genotypes sampled from Southern, Eastern and Western Africa. Arnason et al. (1992) reported the existence of weevil resistance variation among Mexican landraces. Abebe et al. (2009) reported variability in resistance against maize weevil in improved maize varieties in Ethiopia with the results showing a decrease in number of F1 progenies, low grain damage and low grain weight loss among resistant genotypes. Variation for resistance to LGB among maize varieties has been reported in Kenya (Ndiso et al., 2007) while in Malawi, variation in susceptibility among maize varieties against LGB was reported by (Kasambala, 2009). Kumar (2002) reported some 19 landraces from the Caribbean which showed resistance to LGB. The observed variations for resistance among the varieties were as a result of mechanical and biochemical factors, such as phenolic compounds that provide both mechanical resistance and antibiosis in maize grain (Arnason et al., 1992; Derera et al., 2000; Kumar, 2002; García-Lara et al., 2004).

Considering huge grain losses emanating from storage insect pests in Malawi, exploration for variation in maize resistance against maize weevil and larger grain borer among different local maize varieties would be an important step in identifying resistant varieties. The identified resistant varieties could be used for the development of insect resistant maize

populations and for improvement of resistance in productive maize populations in Malawi. The objectives of the study were therefore; to determine levels of larger grain borer and maize weevil resistance among local maize varieties in Malawi and to identify maize varieties that can be exploited for larger grain borer and maize weevil resistance breeding. The following hypothesis was tested; Genetic variation exists among local varieties in Malawi for resistance against larger grain borer and maize weevil. This variation can be exploited in a breeding programme to develop new maize populations and improve the resistance in productive varieties.

2. Materials and methods

2.1. Plant materials

The study was conducted at Chitedze Agricultural Research Station (CARS) where sixty eight (68) local maize varieties were collected from the National Gene Bank and smallholder farmers in Malawi. The list included one commercial hybrid (DK8053) and one local landrace locally known as Kanjerenjere with known resistance against maize weevil and larger grain borer as standard checks for susceptibility and resistance, respectively (Table 1).

Table 1: List and origin of local maize varieties that were used in the study

Variety	District	Longitude	Latitude	Variety	District	Longitude	Latitude
172	Nkhatabay	34° 03'	11° 38'	2027	Lilongwe	34° 04'	14° 02'
243	Mzimba	33° 32'	12° 05'	289	Karonga	33° 44'	9° 45'
322	Rumphi	33° 54'	11° 12'	1786	Dedza		
250	Mzimba	33° 20'	12° 14'	699	Zomba	36° 26'	15° 40'
1772	Ntcheu	34° 45'	15° 01'	2872	Likoma	34° 44'	12° 02'
740	Balaka	34° 54'	15° 15'	Local 1	Dowa		
787	Machinga	35° 32'	14° 52'	164	Nkhatabay	34° 14'	11° 35'
3414	Zomba	35° 04'	15° 31'	1992	Dedza	34° 25'	14° 18'
3411	Zomba	35° 11'	15° 23'	725	Balaka	35° 00'	14° 55'
629	Thyolo	35° 12'	15° 09'	148	Mzimba	35° 44'	11° 18'
163	Nkhatabay	33° 57'	11° 43'	206	Mzimba	33° 27'	11° 57'
1795	Dowa	34° 16'	13° 42'	315	Mzimba	33° 26'	11° 15'
218	Mzimba	33° 20'	11° 53'	1845	Ntchisi	33° 52'	13° 22'
696	Zomba	35° 21'	15° 34'	260	Chitipa	33° 41'	10° 20'
199	Mzimba	33° 37'	11° 56'	2012	Lilongwe	33° 58'	14° 09'
410	Chikwawa	34° 41'	16° 22'	445	Chikwawa		
752	Balaka	34° 55'	15° 03'	249	Mzimba	33° 29'	12° 13'
332	Mzimba	33° 54'	11° 12'	741	Balaka	34° 54'	15° 11'
145	Mzimba	33° 45'	11° 26'	193	Mzimba	33° 36'	11° 54'
2017	Lilongwe	33° 58'	14° 09'	811	Mangochi	35° 33'	14° 40'
310	Mzimba	33° 36'	11° 17'	1983	Dedza	34° 24'	14° 21'
139	Mzimba			226	Mzimba	33° 27'	11° 41'
569	Chiradzulu	35° 18'	15° 57'	1915	Kasungu	33° 23'	12° 47'
736	Balaka	35° 03'	14° 58'	Local 2	Lilongwe		
303	Mzimba	33° 38'	10° 52'	1850	Dowa	33° 46'	13° 28'
292	Karonga	33° 50'	9° 58'	403	Nsanje	35° 15'	16° 27'
240	Mzimba	33° 26'	11° 23'	Knjinj	Blantyre		
386	Nsanje	35° 10'	17° 05'	3243	Mzimba		
750	Balaka	34° 53'		2862	Karonga	34° 02'	10° 09'
3244	Mzimba			783	Machinga	35° 32'	14° 55'
203	Mzimba	33° 32'	11° 53'	539	Phalombe	35° 44'	15° 40'
1857	Dowa	33° 25'	13° 25'	637	Thyolo	35° 15'	16° 23'
584	Chiradzulu	35° 08'	15° 33'	1892	Mchinji	33° 50'	13° 57'
297	Karonga	33° 58'	10° 03'	154	Nkhatabay	33° 58'	11° 43'

2.2. Planting and experimental design

The maize varieties were planted at Chitedze Agricultural Research Station field during the 2011/2012 growing season using Alpha lattice design (10 incomplete blocks, each with 6 or 7 entries) and three replicates. Each replicate was 10 m wide and 124 m long, giving a total area of approximately 3720 m². One seed was planted per station using 25 cm spacing between plants and 75 cm between rows. The hybrid maize variety "DK 8053" was used in guard rows. Full-sib mating was done for each variety. Basal application of fertilizer was done using NPK (23:21:0 +4S) while top dressing was done using Urea (46% N) fertilizers at the rate of 100kg/ha which is the standard practice in Malawi. Field weeding was done twice and Karate (lambda-cyhalothrin) was applied to control termites. At maturity, cobs were harvested and dried until the moisture content was

between 12-13% for resistance evaluations in the laboratory.

2.3. Rearing of larger grain borer and maize weevil

The rearing of LGB and MW was done at the Chitedze Crop storage facilities according to the procedures outlined by CIMMYT (Tefera et al., 2010). Unsexed pests were reared in a controlled environment at 28± 1oC, 65±5% RH, with a 12h: 12h light: dark regime to minimize fluctuations in temperature and relative humidity and promote insect survival (Haines, 1991). The LGB and MW were cultured on susceptible mixed maize grain in glass jars covered with wire mesh lids which prevented insect migration in or out of the jars. The emerging adult insects were carefully monitored to ensure that insects were of the same generation.

2.4. Evaluations of maize varieties for maize weevil and LGB resistance

Maize varieties were evaluated for maize weevil and larger grain borer resistance under controlled laboratory conditions where temperature was at 28± 1oC while relative humidity was 65±5%. A Complete Randomised Block Design (CRBD) was used with four replications. Blocking was done to take care of variation that may result from placing containers on different shelves.

2.5. Data collected and analysis

2.5.1. Measurements for grain resistance to maize weevil

The following parameters were used for measuring weevil resistance among the varieties: **Adult mortality** was determined 10 days after infestation; both live and dead insects were counted and discarded. Insects were separated from maize grain using sieves. The **F1 progenies** were recorded 21 days after the 10 day oviposition, the recording was done every 3 days, until no more insects were expected. The **F1 progeny mortality** was assessed by separating dead progenies from the total number of **F1** progenies. **Damaged and undamaged grains** were counted based on 100 grains randomly selected from each jar. A scale based on percent grain damage was conveniently used to assess resistance among varieties and the varieties were categorised as follows, highly resistant (0%), resistant ($\leq 2\%$), moderately resistant (2.1-2.9%), moderately susceptible (3-3.9%), susceptible (4-4.9%) and highly susceptible ($\geq 5\%$). A **susceptibility index** developed by Dobie (1974) was used to assess the response of varieties as follows: $DSI = [\text{Loge } Y/t] \times 100$; where $DSI = \text{Dobie susceptibility Index}$, $Y = \text{total number of progenies emerging from the treatment}$, $t = \text{median development period (number of days from the middle of the oviposition (day 5) period to the emergence of 50\% of the F1 progeny (Derera et al., 2000))}$. However, where zero or 1 maize weevil emerged, the maximum median development period was calculated based on the last day of insect counting. The values calculated were assigned resistance/susceptibility categories as follows, highly resistant (0), resistant (≤ 2), moderately resistant (2.1-2.9), moderately susceptible (3-3.9), susceptible (4-4.9) and highly susceptible (≥ 5). A high susceptibility index signified that the maize varieties were susceptible and a low susceptibility index meant maize varieties were resistant. For comparison purposes, **grain weight loss** was also calculated using the damaged and undamaged grains (CIMMYT protocol, Boxall 2002) as follows: $\text{Weight loss (\%)} = \{(W_u \times N_d) - (W_d \times N_u) / W_u \times (N_D + N_u)\} \times 100$; where $W_u = \text{weight of undamaged grain}$, $N_u = \text{number of undamaged grain}$, $W_d = \text{Weight of damaged grain}$, $N_d = \text{number of damaged grain}$. The following categories were used to determine resistance based on grain weight loss: Resistant (grain weight loss $\leq 2\%$), moderately resistant (grain weight loss between 2.1% and 4%), moderately susceptible (grain weight loss between 4.1 and 6%), susceptible (grain weight loss of between 6.1% and 8%), highly susceptible (grain weight loss $\geq 8.1\%$).

2.5.2. Measurements for grain resistance to the larger grain borer

Due to the peak in laboratory activities at Chitedze crop storage laboratory, a different resistance screening methodology (CIMMYT Protocol) was adopted for LGB that does not require collection of data every 3 days as outlined in the section for measuring weevil resistance. Collection of data on resistant parameters was done 90 days after infestation. The following resistance parameters were collected: total number of insects, insect mortality, grain damage, weight loss and flour weight. **Total number of insects** was determined by a total count of both live and dead insects, **insect mortality** was assessed by separating dead insects from the total number of insects. **Percent grain damage** and Grain weight loss were determined as indicated in the above section on maize weevil. **Maize** flour produced

in the jars due to insect damage was separated from insects and maize using sieves of different hole sizes. **Weight of maize** flour was determined using an electronic weighing balance.

2.5.3. Data analysis

Data collected on flour weight (g), grain damage (%), grain weight loss (%) were angular transformed, while data collected on number of insects was transformed using log (base e) to normalize variance before subjecting it to the analysis of variance (ANOVA) and correlation analysis in GenStat (Payne et al., 2011). Table of means for grain resistance parameters are presented using original (untransformed) data. Broad-sense heritability for yield was calculated based on ANOVA as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma^2\epsilon/r}$$

$H^2 = \text{Broad -sense heritability}$

$\sigma_g^2 = \text{Mean sum of squares for varieties}$

$\sigma^2\epsilon = \text{Mean sum of squares for error}$

$r = \text{Replication}$

3. Results

3.1. Response of maize varieties to maize weevil infestation

Maize varieties showed significant differences for adult mortality, median development period, grain damage (%) and total F1 progenies but there were no significant differences for F1 progeny mortality and grain weight loss. Broad-sense heritability (H^2) among the parameters ranged from 0.84 for grain weight loss (%) to 0.92 for adult mortality (Table 2).

Results on adult mortality, total number of F1 progenies and median Development Period (MDP)

Results showed highly significant differences ($p < 0.001$) for adult mortality of the maize weevil among the varieties and the highest adult mortalities were observed from the following varieties 148, 3244, 2862, 445, 249 and Kenjerenjere (resistant check). Three varieties, namely 148, 3244, and 249 had mean insect mortality numbers of 39.25, 42.5 and 40.75, respectively. These varieties performed better than the resistant check which had a mean of 38.50. Highly significant differences ($p < 0.001$) were observed for total number of F1 progenies among maize varieties. Maize varieties 1992, 1772, 3243, 3244, and 403 had the lowest mean number of F1 progenies (< 1) and these varieties outperformed the resistant check "Kanjerenjere," which had a mean value of 2.0. Significant differences ($p < 0.05$) were observed among the varieties for the median development period, and the varieties 148, 315, 3243, 1992, and 3244 had the longest median development period compared to the resistant check "Kanjerenjere" (Table 3).

Results on grain damage (%) and grain weight loss (%)

Highly significant differences ($p < 0.001$) for percent grain damage were observed among maize varieties, where varieties 3244, 2012, 445, 250 and 218 had values of $\leq 1\%$ and these values were better than the resistant check (2.5%). Varieties 148, 322, 1772, 445, 386 and 218 experienced less

Table 2: Analysis of Variance (ANOVA) for grain resistance related parameters for maize weevil

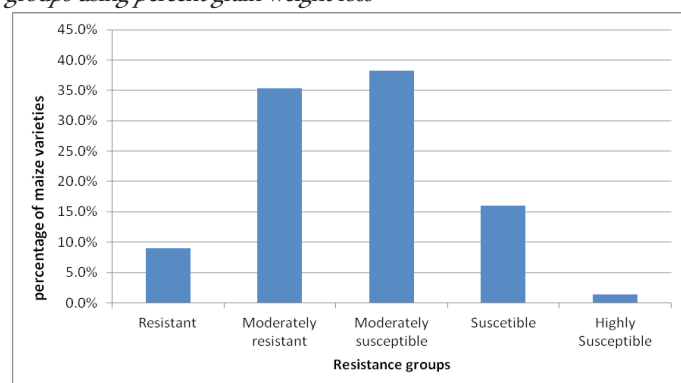
SOV	Df		Resistance parameters					F ₁ progeny mortality
			Adult mortality	F ₁ progenies	MDP	Grain damage (%)	Weight loss (%)	
Variety	68	MS	0.4756**	0.275**	0.033*	13.313**	5.116	0.2462
Block	3	MS	0.2461	0.1754	0.0332	4.233	5.218	0.165
Residual	204	MS	0.1684	0.1418	0.0238	7.623	4.446	0.1803
Total	275	MS	0.2452	0.1751	0.02614	8.99	4.581	0.1964
		CV (%)	12.8	22.4	4.3	26.3	29	37.1
		SED	0.2902	0.525	0.109	1.952	1.491	0.592
		H ²	0.92	0.89	0.85	0.88	0.84	0.85

** = significant at P<0.001, * = significant at P<0.05

Table 3: Table of means for grain resistance parameters against maize weevil for top 25 resistant maize varieties

Variety	Adult mortality	Total F1 progenies	MDP	Grain damage (%)	Grain weight loss (%)	DSI	Yield (tons/ha)
1772	32	0.25	43	1.3	1.4	0.4	3.76
1992	39	0.5	43	2	3.5	0.81	2.88
3244	42.5	0.75	39.8	0.8	3.4	1.16	3.91
3243	23.3	0.75	43	2.3	4.6	1.21	2.85
403	23.5	0.75	40.5	2.3	3.7	1.24	2.49
249	40.8	0.75	40.5	1.8	2.3	1.24	2.2
699	37.8	0.75	33.3	1.5	3.5	1.24	2.29
811	34	0.75	39.8	1.3	3	1.3	1.71
240	27.3	0.75	39.8	3	2.5	1.32	1.4
1995	31.5	1.25	39.8	5.8	7.6	1.49	
750	29	1.5	39.8	4	6.9	2.2	3.52
315	26.8	1.75	39.8	3.3	8.2	2.3	2.35
289	26.8	2.25	39.8	2.8	4.2	2.54	2.43
148	39.2	1.75	39.8	2	1.8	2.55	1.51
322	32	2	36.5	1.8	1.9	2.68	1.74
741	40	2	36.5	1.8	4.2	2.7	1.81
787	36.2	1.75	36.5	1.5	2.5	2.7	1.46
172	26	1.75	36	1.8	6	2.71	1.8
297	20.3	2	36.5	2.3	4.1	2.81	1.54
2862	36	1.75	36.5	2.8	7	2.88	
199	31.5	1.75	36.5	1.3	2.4	2.88	2.22
206	34.8	1.75	34	3.5	4	2.88	2.39
303	32.8	1.75	34	2.8	3.1	2.9	0.26
Kanjerenjere	38.5	2	33.3	2.5	8	2.9	2.31

Figure 1: Grouping of local maize varieties into maize weevil resistance groups using percent grain weight loss



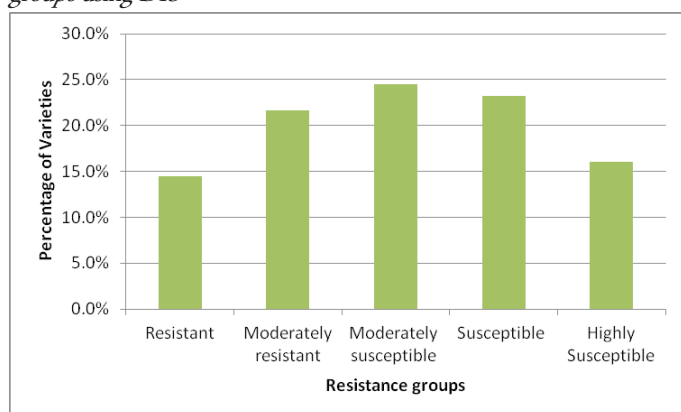
Dobie index of susceptibility (DSI)

Using Dobie index of susceptibility, 14.5% of the varieties were resistant, 21.7% were moderately resistant, 24.6% moderately susceptible, 23.2% susceptible and 16% highly susceptible to maize weevil (Figure 2). The most promising varieties were 1772, 1992, 811, 699, 249, 403, 1995, 240, 3243, 1983, 750, 752 and 3244. These varieties had index values <2 which were lower than the resistant check “Kanjerenjere” with an index of 2.9 (Table 3).

Table 3: Table of means for grain resistance parameters against maize weevil for top 25 resistant maize varieties

Variety	Adult mortality	Total F1 progenies	MDP	Grain damage (%)	Grain weight loss (%)	DSI	Yield (tons/ha)
1772	32	0.25	43	1.3	1.4	0.4	3.76
1992	39	0.5	43	2	3.5	0.81	2.88
3244	42.5	0.75	39.8	0.8	3.4	1.16	3.91
3243	23.3	0.75	43	2.3	4.6	1.21	2.85
403	23.5	0.75	40.5	2.3	3.7	1.24	2.49
249	40.8	0.75	40.5	1.8	2.3	1.24	2.2
699	37.8	0.75	33.3	1.5	3.5	1.24	2.29
811	34	0.75	39.8	1.3	3	1.3	1.71
240	27.3	0.75	39.8	3	2.5	1.32	1.4
1995	31.5	1.25	39.8	5.8	7.6	1.49	
750	29	1.5	39.8	4	6.9	2.2	3.52
315	26.8	1.75	39.8	3.3	8.2	2.3	2.35
289	26.8	2.25	39.8	2.8	4.2	2.54	2.43
148	39.2	1.75	39.8	2	1.8	2.55	1.51
322	32	2	36.5	1.8	1.9	2.68	1.74
741	40	2	36.5	1.8	4.2	2.7	1.81
787	36.2	1.75	36.5	1.5	2.5	2.7	1.46
172	26	1.75	36	1.8	6	2.71	1.8
297	20.3	2	36.5	2.3	4.1	2.81	1.54
2862	36	1.75	36.5	2.8	7	2.88	
199	31.5	1.75	36.5	1.3	2.4	2.88	2.22
206	34.8	1.75	34	3.5	4	2.88	2.39
303	32.8	1.75	34	2.8	3.1	2.9	0.26
Kanjerenjere	38.5	2	33.3	2.5	8	2.9	2.31
2012	29	1.75	33.3	0.5	2.5	2.99	4.57

Figure 2: Grouping of local maize varieties into maize weevil resistance groups using DSI



Correlation analysis among maize weevil resistance parameters and yield

Correlation analysis showed significant relationships among different resistance parameters. Importantly, highly significant ($p < 0.001$) and positive correlations were observed between percent grain damage and percent grain weight loss (0.637), and between percent grain damage and total number of F1 progenies (0.4299). Negative but highly significant correlations ($p < 0.001$) were observed between median development period and DSI (-0.8312), and between median development period and total number of F1 progenies (-0.6572). Correlation between yield and weevil resistance parameters was not significant (Table 4).

3.2. Response of maize varieties to larger grain borer infestation

Significant differences in response of maize varieties to larger grain borer were observed for insect mortality, total number of insects, grain damage (%), flour weight (g) and grain weight loss (%). Broad-sense heritability (H^2) ranged from 0.65 for grain weight loss to 0.97 for flour weight (Table 5).

Total number of insects, insect mortality and flour weight (g)

Maize varieties showed significant differences ($p < 0.01$) for total number of insects. Varieties 172, 164, 699, 410, and 322 experienced the lowest number of insects than the resistant check (42.75). Significant differences ($p < 0.05$) for insect mortality were observed among varieties. Varieties 1992 (33.5), 445 (36.5), 1983 (41.5), 292 (38.08) and 154 (41.25) had the highest number of dead insects and outperformed the resistant check “Kanjerenjere” (23). Highly significant differences ($p < 0.001$) for flour weight were observed among the varieties. The best performers with the least amount of flour produced were varieties 1983 (0.95g), and 1992 (1.8g). Resistant and susceptible checks had 3.225g and 4.225g of flour produced, respectively. The worst performers with high amounts of flour were varieties 304 (8.5g), 154 (8.1g), 1957 (7.95g), 260 (7.82g) and 310 (7.6g) (Table 6).

Table 4: Correlation among parameters for measuring maize weevil resistance and yield

Adult mortality	1	-	-	-	-	-	-	-	-
DSI	2	-0.5923***	-	-	-	-	-	-	-
Grain damage_%	3	-0.4056***	0.4258***	-	-	-	-	-	-
MDP	4	0.4108***	-0.8312***	-0.2678*	-	-	-	-	-
Total F1 progenies	5	-0.5755***	0.9369***	0.4299***	-0.6572**	-	-	-	-
Weight loss %	6	-0.2937*	0.3395**	0.637***	-0.2478*	0.3418**	-	-	-
Yield tons ha	7	-0.1266	0.0368	-0.0654	-0.0582	0.0337	0.206	-	-
		1	2	3	4	5	6	7	

Note: Correlation coefficients with * were significantly correlated $p < 0.05$, ** significantly correlated $p < 0.01$ and *** significantly correlated at $p < 0.001$

Table 5: Analysis of Variance (ANOVA) for grain resistance related parameters against larger grain borer

SOV	Df		Insect mortality	Number of insects	Flour weight (g)	Grain damage (%)	Grain weight loss (%)
Variety	66	MS	0.131**	0.078*	23.394**	94.75**	57.90**
Block	3	MS	0.627	0.175	17.897	15.53	6.42
Residual	198	MS	0.09	0.047	2.963	23.6	17.81
Total	267	MS	0.106	0.056	8.181	40.697	27.605
		CV (%)	9.2	5.5	14.7	21.5	23.3
		lsd (0.05)	0.4195	0.3021	2.400	6.696	5.885
		SED	0.2127	0.1532	1.217	3.396	2.984
		H ²	0.85	0.87	0.97	0.94	0.65

** = significant at $P < 0.001$, * = significant at $P < 0.01$

Table 6: Table of means for grain resistance parameters for larger grain borer among top 25 maize varieties

Variety	Total insect number	Insect mortality	Grain damage (%)	Grain weight loss (%)	Flour weight (g)	Yield (tons/ha)
1983	47.0	41.5	5.0	10.6	1.0	3.16
386	41.0	26.5	8.0	11.9	3.0	1.60
2012	38.0	29.5	6.8	12.0	2.1	4.57
3244	46.5	30.3	7.3	12.1	3.1	3.91
1992	42.8	33.5	6.8	12.9	1.8	2.88
1850	40.5	30.0	7.8	13.3	2.3	1.70
2017	45.0	28.3	7.8	13.4	3.9	1.01
699	26.0	26.0	8.5	13.4	2.7	2.29
3411	52.1	29.0	8.8	13.7	3.8	2.60
403	42.8	22.8	8.0	13.7	2.7	3.81
445	45.0	36.5	8.3	14.3	2.0	3.81
2027	50.5	30.0	10.0	14.4	2.7	3.17
289	46.8	33.0	8.3	14.7	2.4	2.43
315	43.3	28.3	10.8	14.7	3.0	2.35
741	41.3	28.3	10.3	15.0	2.7	1.81
3243	46.0	32.8	10.8	15.2	2.2	2.85
249	38.5	25.3	9.9	15.3	2.4	2.20
752	41.3	26.5	11.3	15.5	3.4	4.18
Kanjerenjere	42.8	23.0	10.8	15.6	3.2	2.31
637	41.8	27.5	11.0	15.9	4.0	2.58
569	43.8	31.8	12.3	16.1	3.1	4.09
410	40.8	29.0	13.5	16.1	4.0	1.65
332	49.3	29.5	13.3	16.6	4.0	2.44
539	49.8	30.0	12.5	16.6	3.2	1.35
2872	50.0	32.8	11.3	16.8	3.0	2.49

Grain damage and grain weight loss (%)

Highly significant differences ($p < 0.001$) for percent grain damage were observed among the varieties. Varieties 1983, 1992, and 2012 experienced the least grain damage ranging from 5-6.75%, while the resistant and susceptible checks had 10.75% and 13.50% grain damage (%), respectively. Percent grain weight loss showed highly significant differences ($p < 0.001$) among the varieties.

Despite showing significant differences, percent grain weight loss as a measure of resistance showed that all the varieties were highly susceptible to LGB. However, a good number of varieties such as 1983 (10.64%), 1850 (13.33%), 1992 (12.93%), 2012 (12.01%), 386 (11.89%) and 2017 (13.37%) performed better than the resistant control (Kanjerenjere) (15.62%). Varieties, such as 310, 260, 292, 303, and 154 performed worse than the susceptible commercial hybrid

(DK8053) (Table 6).

Correlation between LGB resistance parameters and yield

Highly significant correlations ($p < 0.001$) were observed between grain weight loss (%) and grain damage (%) (0.8828), between flour weight and grain damage (%) (0.9789), and between flour weight and grain weight loss (%) (0.8722). Correlations between yield and resistance parameters were not significant except for flour weight (0.3599) (Table 7).

Table 7: Correlation among resistance parameters for LGB and yield

Flour weight (g)	1	-					
Grain damage%	2	0.8828**	-				
Insect mortality	3	-0.1868	-0.195	-			
Total number of insects	4	0.7099**	0.6128**	0.3454*	-		
Weight loss %	5	0.8722**	0.9789**	-0.216	0.6137**	-	
Yield tons ha	6	-0.3599*	-0.2284	0.1226	-0.1858	-0.2345	-
		1	2	3	4	5	6

Discussion

Maize weevil resistance among local maize varieties

Substantial variation for resistance against maize weevil exists among local maize varieties in Malawi. This variation for resistance was confirmed by significant differences for adult mortality, total F1 progenies, median development period, percent grain damage, and Dobie index of susceptibility among local maize varieties. The results obtained in this study were in line with what Giga and Mazarura (1991) reported about the existence of significant variation for weevil resistance among the exotic, local open pollinated varieties and maize hybrids obtained from Malawi, Zimbabwe and Mexico.

Percent grain weight loss as an indicator of susceptibility appeared to be more conservative in identifying resistant varieties than Dobie Index of susceptibility. Results further showed a weak but significant correlation between percent grain weight loss and Dobie Index of Susceptibility (DIS) (0.3395) at $p \leq 0.01$. This weak but significant relationship probably could be an indication that the two indicators of susceptibility have a small chance of identifying similar resistant varieties. Hence, the two indicators only identified one common resistant variety (1772) among the top most resistant varieties. In addition, DSI significantly correlated with the other resistant parameters at $p \geq 0.001$. Combining DIS, percent grain weight loss, percent grain damage, total number of F1 progenies and adult mortality, maize varieties 1772, 1992, 3243, 3244, 148, 322, 445, 386, 218, 2012, 741, 699, 811, 1983, 249, 403 and 250, were identified as the most resistant varieties. It is also worth noting that only the distribution of variation for DIS was normal. Taking all above factors into consideration, DIS was a better parameter for discriminating maize varieties for weevil resistance than percent weight loss in this study.

The use of percent grain weight loss, percent grain damage, fecundity and DIS as indicators of susceptibility or resistance has been documented (Derera et al., 2000; Kitaw et al., 2001; Abbe et al., 2009; Mwololo et al., 2012). Mwololo et al. (2012) used grain weight loss, grain damage (%) and number of insects to differentiate levels of weevil resistance among maize varieties in Kenya. Resistant varieties showed

low grain weight loss, low grain damage (%) and low number of insects as also established by Abbe et al. (2009) among varieties in Ethiopia. Derera et al. (2000) and Kitaw et al. (2001) demonstrated that resistant varieties can be identified using adult weevil mortality, grain damage (%), progeny numbers, median development period and Dobie index of susceptibility.

From a breeding perspective, the grain resistance parameters showed good levels of broad-sense heritability.

For example, F1 progenies showed a broad-sense heritability of 0.89, adult mortality (0.92), weight loss (%) (0.85), MDP (0.85) and grain damage (%) (0.88). This indicated that these parameters are heritable as reported by Dhliwayo and Pixley (2003).

The resistance observed in maize varieties against maize weevil could be due to biophysical grain factors or antibiosis (Derera et al., 2000; García-Lara et al., 2004). For example, Mwololo et al. (2013) reported differences in grain hardness between resistant and susceptible varieties due to protein composition within the grain structure. Taking into consideration that many traits are involved in maize weevil resistance (Mwololo et al., 2013), a multi-trait breeding approach to maize weevil resistance breeding is crucial. For example, the use of molecular markers, exploitation of husk cover, physical grain characteristics and chemical composition (Meikle et al., 1998; Derera et al. 2000; García-Lara et al., 2004; Reif et al., 2004; Mwololo et al., 2013) can lead to a successful maize weevil resistance breeding programme. However, central to this approach is the identification of the nature of gene action controlling maize weevil resistance in maize materials (Derera et al., 2000; Dhliwayo and Pixley, 2003; Kim and Kossou, 2003; Dari et al., 2010). The nature of gene action would help in devising a strategy for enhancing resistance in the maize varieties.

Larger grain borer resistance among local maize varieties

Maize varieties showed significant variation in response to LGB infestation. The variation in resistance among maize varieties was shown by highly significant differences for flour weight, insect mortality, percent grain damage and percent grain weight loss. Variation for resistance to LGB was also reported among landraces along the coastal region of Kenya (Ndiso et al., 2007). Varietal differences in response to LGB are critical in the control of the pest (Rugumamu, 2006).

Exploitation of variation for flour weight, insect numbers, development periods, percent grain weight loss, percent grain damage to measure varietal resistance against LGB have been reported (Kasambala, 2009; Mwololo et al., 2010). For instance, Kasambala (2009) used insect numbers,

percent grain weight loss and percent grain damage to determine the existing variation for resistance against LGB among maize varieties in Malawi. The results revealed variation for grain weight loss ranging between 7.7 and 30.3%, percent grain damage (33-66.7%) and insect numbers (41 to 99). Kasambala (2009) identified Kanjerenjere (local variety) to be a resistant variety. Hence, Kanjerenjere was used as a resistant check in the current study.

Results of the current study showed that the total number of insects ranged from 38 to 79, percent grain damage ranged from 5 to 32% and percent weight loss ranged from 10.64% to 28.61%. These ranges did not differ significantly from the results obtained by Kasambala (2009). However, some varieties outperformed the resistant check (Kanjerenjere). These varieties could become new sources of resistant materials. Mwololo et al. (2010) reported significant differences in grain damage, flour weights, number of dead and live insects among varieties in Kenya. Importantly, these parameters are heritable. For example, in the current study, percent grain weight loss showed a broad-sense heritability of 0.65 and percent grain damage (0.94), flour weights (0.97), adult mortality (0.85) and insect numbers (0.85). Therefore, according to the present results, these parameters can reliably discriminate varieties against larger grain borer and could be used in insect resistance screening among maize genotypes.

Correlation analysis showed highly significant relationships between flour weights with grain damage (%), and between grain damage (%) and number of insects. These relationships are consistent with the manner in which LGB excavates the grain with its deflexed head and well-built mandibles (LI, 1988). Consequently, an increase in number of insects resulted in increased grain damage and high amount of flour produced. Using percent weight loss to measure susceptibility of varieties, all varieties were susceptible. However, varieties, such as 1992, 2012, 1850, 2017, 386 and 1983 had lesser percent weight loss and performed better than the resistant check (Kanjerenjere). The relatively low percent grain weight loss among the varieties was consistent with their respective percent grain damages, which were also relatively lower than the resistant check. This provides a new opportunity for new sources of resistance for use in breeding for insect resistance. It is also worth noting that varieties 1992 and 1983 also showed high levels of resistance to maize weevil. This provides an opportunity to select for both maize weevil and larger grain borer resistance, since both insect pests are generally found in the same environment within the storage facilities. The resistance observed in maize varieties against larger grain borer could be due to antibiosis (Kumar, 2002; Nhamucho et al., 2014). Of late, Mwololo et al. (2012) reported the effect of protein composition and lipids on maize resistance to LGB in tropical maize. Resistant varieties exhibited high levels of lipids and protein content. Arnason et al. (1992) also reported the role of grain moisture, grain hardness, vitreous endosperm and nutritional factors in LGB development and behaviour.

Yield and grain resistance

The correlations between yield and resistance parameters for both maize weevil and larger grain borer were not significant. This means that selection for resistance can be done without significantly affecting yield. In this regard, potential varieties that have been identified as having better resistance against maize weevil and larger grain borer can be

improved upon to enhance both resistance and yield.

Conclusion

Variation for resistance against maize weevil and larger grain borer exists among local maize varieties grown in Malawi. Varieties were largely resistant to maize weevil and susceptible to larger grain borer. The identified resistant varieties could become new sources of LGB and MW resistance and recommended for use in programmes that emphasize post-harvest insect resistance. Varieties 1772, 1983, 1992, 3243, 3244, 750 and 752 are good candidates for developing populations that are resistant to maize weevil while 1992, 2012, and 1983 could be used in developing LGB resistant populations. However, these varieties would require recurrent selection to increase the frequency of resistant genes. Varieties that showed useful levels of resistance to maize weevil and less susceptible to larger grain borer may be used as candidates for stacking MW and LGB resistance in new hybrids. Further tests on the potential varieties should be done to confirm their consistency in resistance levels, largely against larger grain borer to dispel pseudo-resistance among the varieties. The assessment of the top varieties should be done inclusive of other equally important agronomic attributes preferred by farmers.

Acknowledgement

The authors would like to thank the following; African Center for Crop Improvement (ACCI) for their financial support, Crop Storage Laboratory and the Malawi Gene Bank based at Chitedze Agricultural Research Station for their technical assistance and for providing maize materials used in this study, respectively.

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The interactive effect of water temperature and salinity on yolk absorption rate, growth and larval survival of African catfish *Clarias gariepinus* (Burchell 1822)

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Abstract

The study was undertaken to determine the interactive effect of water temperature and salinity on yolk absorption rate, growth and survival of *C. gariepinus* larvae. Fertilized eggs, from broodstock reared for commercial seed production were incubated in 1000ml glass flasks and received one of the nine temperature (25, 28, and 31°C) and salinity (0, 3 and 6ppt) combinations in triplicates. The experiment was kept viable until all the yolk was fully disorbed. Data collected included yolk absorption rate and period, average rate of growth in length and larval survival of *C. gariepinus*, while water temperature was monitored periodically. The results showed that: Both temperature and salinity and their interaction had a significant effect on yolk absorption rate and period, average rate of growth in length and larval survival of *C. gariepinus*. A temperature and salinity combination of 28-31°C and 0ppt was found to be suitable for nursing *C. gariepinus* larvae. This is evidenced by having the least yolk absorption rate and period, highest rate of growth in length and highest survival of *C. gariepinus* larvae. Hence this temperature-salinity combination is recommended for the improvement of catfish seed production which is a major problem in catfish industry.

Key words; *Clarias gariepinus*, Yolk absorption period, rate of growth, survival, temperature, salinity

Introduction

In Africa, full aquaculture potential of African catfish, *Clarias gariepinus*, has not yet been realized despite its possession of many qualities that make it suitable for commercial production. To a large extent, the main constraint facing its commercial culture in the continent has been the lack of adequate and reliable supply of quality fingerlings for stocking purposes (Rasowo et al., 2007). This has been attributed to its characteristic gonadal seasonal cycle i.e. gravid females may be found in freshwater from October (spring) until water temperatures drop in March/April (autumn) (Britz, 1991). It does not show any parental care, its inability to spawn naturally in captivity (Rasowo et al., 2007) and the low survival of its hatchlings in earthen ponds (De Graaf and Janssen, 1996). Considering that this species cannot naturally spawn in captivity, the current production is mainly based on induced breeding techniques and for this reason induced breeding techniques have been perfected, adequately described and are routinely practiced in many hatcheries (Hogendoorn, 1979; Hogendoorn and Vismans, 1980). However the shortage of seed for stocking ponds continues to persist in Africa (Rasowo et al., 2007). It is apparent that management protocols covering egg production, egg hatching, and particularly production techniques that enhance fry and fingerling survival need to be further studied and refined to ensure a sufficient supply of quality catfish seed.

Temperature and salinity are the two most important environmental factors affecting fish hatchery production (Aktas, 2004). Such abiotic factors influence fish eggs and larval physiology and they have a direct effect on growth and survival of fish (Holliday, 1969). However, information on effect of water temperature, salinity, and their combination is available only from fingerling to adult stages but not available on the yolk-sac larvae stage. Therefore this study aimed at unveiling how such factors affect growth, yolk absorption and survival of yolk-sac, a precursor of the subsequent

stages necessary to address the current shortage of catfish fingerlings.

Methods and Materials

Study site

The study was carried out at the National Aquaculture Center at Domasi, Zomba District, in Malawi. The timing was between September and December 2008, which is the natural breeding season of *C. gariepinus*.

Brood-stock management and hormonal treatment

The mature females and males of *C. gariepinus* broodfish were selected from a stock maintained for commercial breeding program at the National Aquaculture Center at Domasi (Malawi), with individual weights ranging between 500 and 700g. All broodfish were selected using external morphological characteristics; female broodfish which were selected had soft and distended abdomen from which matured eggs, based on their greenish coloration and singular occurrence, were stripped by gentle application of pressure in accordance to Janssen (1987). Male brood fish were selected if they possessed elongated and reddish pointed urino-genital papillae. Fifteen females and 10 males were selected. Males and females were kept separately in concrete tanks measuring 10 x 8 x 1.2 m. The broodstock were acclimatized in their new environment (concrete tanks) for 7 days at mean temperature of $28 \pm 2^\circ\text{C}$ and normal photoperiodic regimes with water pH around 7.1 ± 0.1 . The broodfish were fed on formulated pellets (35% crude protein) twice per day (7 am and 5 pm) at 5% of total fish biomass.

Prior to hormone injection, a total of four female and four male broodfish were randomly seined out from the tanks and kept singly in aerated 50L aquaria, with 25 litres of aerated water for 12 h. The randomly-selected females were measured in terms of weights and total lengths (TL) of 580, 660, 650 and 500g, and 47.5, 50.0, 49.5 and 58.0 cm, respectively, and males had weights and total lengths of 600, 650, 680 and 700g and 46.5, 40.0, 48.5 and 65.0 cm,

respectively.

Broodfish were injected with ovaprim®, a synthetic analogue of gonadotropin releasing agent (Syndel Laboratories, Canada), between 6 and 7 pm. Ovaprim was administered in liquid form at 0.5 ml/kg body weight of female fish. Each male was injected with half of the dose of the female in accordance to Legendre, 1986; Haniffa and Sridhar, 2002. The injected fish were returned and kept separately into their respective 50L aquaria for 12h, and water temperature was maintained at 28°C using thermostatically controlled water heaters.

Fertilization and preparation of test solutions

Ovulated females were stripped the following morning after injection at room temperature (25°C) to collect eggs. The ovulated eggs oozed out by slight thumb pressure onto the plastic bowl. The fish were stripped until traces of blood were observed which signified that the ovaries were empty. For male gametes, mature males were sacrificed (killed) and sperm sacs were collected, then incisions were made on the sperm sacs following Viveiros (2002). Milt was squeezed and spread over the eggs then mixed thoroughly with a soft clean feather. To this, 0.6% saline solution was added and further agitated for few seconds. Spermatozoa from one mature male were used to fertilize eggs stripped from three females while keeping the eggs from different females separately. The process from stripping to fertilization took about three minutes to accomplish. Three concentrations of the common salt, NaCl, (0, 3, and 6 g/l) were prepared by dissolving these three amounts of salt in a liter of natural freshwater used by National Aquaculture Center for breeding catfish, in order to obtain the required salinities, for all the tests on salinity tolerance of fertilized eggs and Yolk-sac larva. The mixture was tested with a salinometer, to confirm the salinities of the test solutions.

Egg incubation, Yolk absorption rate and period, rate of growth and survival

At incubation, a 3x3 factorial design was used where three temperatures levels (25, 28, and 31°C) and three salinity levels (0, 3 and 6ppt) levels each with three replicates were set. Immediately after fertilization, eggs were transferred into 1000ml-capacity beakers (100 eggs per each beaker), beakers were filled to 800ml mark with the test media. Aerators were placed in each water bath and in each beaker to ensure temperature homogeneity and oxygen supply respectively. At the end of hatching, the hatched larvae were left in the salinities and temperatures of incubation. Yolk absorption rate was determined by measuring the size (length and height) of the yolk sac of 27 larvae, 9 from each replicate using an ocular micrometer mounted on a light microscope daily until the yolks were fully absorbed in accordance to (Molokwu and Okpokwasili, 2002). The average rate of daily yolk absorption were then determined using the formula by Borode and Akin-James (2005):

$$\sum (nI - nF)/t$$

Where:

nI = initial yolk size per day,

nF = final yolk size per day,

t = rearing period

The yolk-sac period began from the end of the hatching period until when 50% of the larvae fully absorbed

their Yolk-sac. This was determined by visual observation and the time taken was recorded. Mortality was determined by counting and by recording on a daily basis the number of dead larvae until the Yolks were fully absorbed. Percentage survival was determined by the following formula according to Radonic et al. (2007)

$$(\%) \text{ Survival} = \frac{\text{Number of live larvae at the end of Yolk absorption} \times 100}{\text{Number of live larvae after hatching}}$$

The rate of growth (GR) was determined by measuring the length of 27 larvae, 9 from each replicate using an ocular micrometer mounted on a light microscope. The average rate of growth in length in relation to the various salt and temperature was calculated using the formula by Borode and Akin-James (2005):

$$\sum (Lf - Li)/t$$

Where:

Lf = final daily length of larvae,

Li = initial daily length of larvae

t = rearing period.

Other water quality parameters

The following Water parameters were monitored on a daily basis. Temperature was measured using a mercury in glass thermometer, pH were read using a pH meter model 191 CP-20 digital, Dissolved Oxygen (DO) level were maintained with RESUN LP- 100 low noise air pump while values were measured using the oxygen meter YS1 model 51B. Salinity values were monitored using portable refractometer salinometer.

Data analysis

Two-way analysis of variance after normality tests for ANOVA was used to analyse the data. When treatment means were found to be significant (P<0.05), multiple comparisons among means were done using Scheffe's test. Both ANOVA and Scheffe's tests were done using SAS.

Results

Effect of temperature on Yolk-sac larvae

The average rate of Yolk absorption per day increased with increasing temperature at all the three incubation salinities. However there was no significant differences (P<0.05) at 0 and 6ppt but there were significant differences (P<0.05), at 3ppt. The Yolk absorption period decreased with increasing temperature at 0ppt but it increased with increasing temperature at 6ppt and significant differences (P<0.05) were observed. On the other hand, the lowest Yolk absorption period was observed at 3ppt when temperature was at 28°C. However, there was no significant differences (P<0.05) at all the three incubation temperatures. The average rate of growth in length per day increased with increasing temperature but there were no significant differences (P<0.05) at 0ppt and 3ppt. Highest average rate of growth in length per day was at 6ppt and 31°C. The values were not significantly different (P<0.05) from that which were obtained at 28°C but significantly different (P<0.05) from that which was obtained at 25°C. In freshwater the survival percentage increased with increasing temperature and there were no significant differences (P<0.05) while in salty water,

it decreased with increasing temperature and significant differences were observed (Table 1).

Table 1: The effect of temperature and salinity on average rate of Yolk absorption, Yolk absorption period, rate of growth and survival rate (mean \pm SE., n=9) of *Clarias gariepinus* larvae

Salinity (ppt)	Temperature (°C)	Average rate of yolk absorption per Day (mm ³)	Yolk absorption period (h)	Average rate of growth in length per day (mm)	Survival (%)
0.00	25	0.997 \pm 0.110 ^a	74.00 \pm 1.46 ^a	0.894 \pm 0.0994 ^a	90.67 \pm 1.15 ^a
	28	1.013 \pm 0.112 ^a	62.00 \pm 1.76 ^b	0.675 \pm 0.0750 ^b	79.67 \pm 1.52 ^b
	31	1.050 \pm 0.116 ^a	48.00 \pm 1.56 ^c	0.668 \pm 0.0742 ^b	54.67 \pm 4.16 ^c
3.00	25	0.523 \pm 0.068 ^a	80.00 \pm 3.46 ^a	0.959 \pm 0.1065 ^a	92.00 \pm 2.00 ^a
	28	0.600 \pm 0.066 ^b	74.00 \pm 2.46 ^a	0.779 \pm 0.0866 ^b	74.00 \pm 8.71 ^b
	31	0.757 \pm 0.041 ^c	76.00 \pm 3.06 ^a	0.729 \pm 0.0809 ^b	34.00 \pm 4.00 ^b
6.00	25	0.305 \pm 0.033 ^a	88.00 \pm 2.06 ^b	1.011 \pm 0.1123 ^a	93.00 \pm 1.00 ^a
	28	0.360 \pm 0.040 ^a	92.00 \pm 3.46 ^{ab}	0.850 \pm 0.0945 ^b	32.00 \pm 6.93 ^b
	31	0.373 \pm 0.084 ^a	96.00 \pm 0.56 ^a	0.796 \pm 0.0884 ^{ab}	08.67 \pm 1.15 ^c

Means with the same superscript are not significantly different within columns (P>0.05)

Effect of salinity on Yolk sac-larvae

The average rate of Yolk absorption per day was significantly (P<0.05) highest in freshwater and it decreased progressively with increasing salinity at all the three incubation temperatures. Yolk absorption period was significantly (P<0.05) least in freshwater and it increased progressively with increasing salinity. The average rate of growth in length per day was significantly (P<0.05) highest in freshwater and increased with increasing salinity. Survival percentage was also significantly (P<0.05) highest in freshwater at all the three incubation temperatures and decreased with increasing salinity (Table 2).

Table 2: The effect of salinity on average rate of yolk absorption, yolk absorption period, rate of growth and survival rate (mean \pm SE n=9) of *Clarias gariepinus* larvae at a various temperatures.

Temperatures (°C)	Salinity (ppt)	Average rate of yolk absorption per Day (mm ³)	Yolk absorption period (h)	Average rate of growth in length (mm)	Survival (%)
25	0.00	0.997 \pm 0.110 ^a	74.00 \pm 1.46 ^a	0.894 \pm 0.0994 ^a	90.67 \pm 1.15 ^a
	3.00	0.523 \pm 0.068 ^b	62.00 \pm 1.76 ^b	0.675 \pm 0.0750 ^b	79.67 \pm 1.52 ^b
	6.00	0.305 \pm 0.033 ^c	48.00 \pm 1.56 ^c	0.668 \pm 0.0742 ^b	54.67 \pm 4.16 ^c
28	0.00	1.013 \pm 0.112 ^a	80.00 \pm 3.46 ^a	0.959 \pm 0.1065 ^a	92.00 \pm 2.00 ^a
	3.00	0.600 \pm 0.066 ^b	74.00 \pm 2.46 ^a	0.779 \pm 0.0866 ^a	74.00 \pm 8.71 ^b
	6.00	0.373 \pm 0.041 ^c	76.00 \pm 3.06 ^a	0.729 \pm 0.0809 ^b	34.00 \pm 4.00 ^b
31	0.00	1.050 \pm 0.116 ^a	88.00 \pm 2.06 ^b	1.011 \pm 0.1123 ^a	93.00 \pm 1.00 ^a
	3.00	0.757 \pm 0.084 ^b	92.00 \pm 3.46 ^{ab}	0.850 \pm 0.0945 ^b	32.00 \pm 6.93 ^b
	6.00	0.360 \pm 0.040 ^c	96.00 \pm 0.56 ^a	0.796 \pm 0.0884 ^{ab}	08.67 \pm 1.15 ^c

Means with the same superscript are not significantly different within columns (P>0.05)

Interactive effect of temperature and salinity on yolk-sac larvae

The highest average rate of yolk absorption per day was obtained at temperature-salinity combinations of 31°C and 0ppt, this was not significantly different (P<0.05) from those obtained at 28°C and 0ppt and 25°C and 0ppt, while the least mean average rate of yolk absorption per day was recorded at temperature-salinity combination of 25°C and 6ppt. The least yolk absorption period was obtained at temperature-salinity combination of 31°C and 0ppt, and the

highest yolk absorption period was recorded at temperature-salinity combination of 31°C and 6ppt. The highest average rate of growth in length per day was obtained at temperature-salinity combinations of 31°C and 0ppt, however, this was not significantly different (P>0.05) from those which were recorded at 28°C and 0ppt and 25°C and 0ppt. The highest survival rate was recorded at temperature-salinity combination of 31°C and 0ppt, however this was not significantly different (P>0.05) from those which was recorded at 28°C and 0ppt, and 25°C and 0ppt, while the least survival rate of 8.67 was recorded at temperature-salinity combination of 31°C and 6ppt.

Table 3: The interactive effect of temperature and salinity on average rate of yolk absorption, yolk absorption period, rate of growth and survival rate (mean \pm SE., n=9) of *Clarias gariepinus* larvae.

Temperatures (°C)	Salinity (ppt)	Average rate of yolk absorption per Day (mm ³)	Yolk absorption period (h)	Average rate of growth in length (mm)	Survival (%)
25	0.00	0.997 \pm 0.110 ^a	74.00 \pm 1.46 ^a	0.894 \pm 0.0994 ^a	90.67 \pm 1.15 ^a
	3.00	0.523 \pm 0.068 ^b	80.00 \pm 3.46 ^a	0.675 \pm 0.0750 ^b	79.67 \pm 1.52 ^b
	6.00	0.305 \pm 0.033 ^c	88.00 \pm 2.06 ^b	0.668 \pm 0.0742 ^b	54.67 \pm 4.16 ^c
3.00	0.00	1.013 \pm 0.112 ^a	62.00 \pm 1.76 ^b	0.959 \pm 0.1065 ^a	92.00 \pm 2.00 ^a
	3.00	0.600 \pm 0.066 ^b	74.00 \pm 2.46 ^a	0.779 \pm 0.0866 ^b	74.00 \pm 8.71 ^b
	6.00	0.360 \pm 0.040 ^c	92.00 \pm 3.46 ^{ab}	0.729 \pm 0.0809 ^b	34.00 \pm 4.00 ^b
6.00	0.00	1.050 \pm 0.116 ^a	48.00 \pm 1.56 ^c	1.011 \pm 0.1123 ^a	93.00 \pm 1.00 ^a
	3.00	0.757 \pm 0.084 ^b	76.00 \pm 3.06 ^a	0.796 \pm 0.0884 ^b	32.00 \pm 6.93 ^b
	6.00	0.373 \pm 0.041 ^c	96.00 \pm 0.56 ^a	0.850 \pm 0.0945 ^b	08.67 \pm 1.15 ^c

Means with the same superscript are not significantly different within columns (P>0.05)

Discussion

Water Temperature affected the average rate of yolk absorption per day and yolk absorption period the average rate of yolk absorption per day increased with increasing temperature while the yolk absorption period decreases with increasing temperature. A similar trend was reported by Haylor and Mollor (1995) who incubated *C. gariepinus* eggs in freshwater and reported the yolk absorption period of 74.40-90.24h at temperature range of 24-26°C, 63.06h at 28°C and 48.96-55.20h at 30-32°C. Increased metabolism at higher temperature could have been responsible for the direct relationship between the average rate of yolk absorption per day and temperature and the inverse relationship between temperature and yolk absorption period (Nwosu and Hertzlohner, 2000).

At 3 and 6ppt, the yolk absorption period decreased with increasing temperature while the least average rates of yolk absorption per day was obtained at 28°C. However, this was not significantly different from those obtained at 25°C and 31°C (Table 2). The inverse relationship between yolk absorption period and temperature could be due to the high metabolic rates at high temperature as reported by Nwosu and Hertzlohner, (2000). While the results obtained for the average rate of yolk absorption per day suggests that salinity modifies the effect of temperature on yolk absorption per day.

Water temperature affected the average rate of growth in length per day and survival of yolk sac larvae at a given salinity in that, at all the three incubation salinities (0-6ppt), the average rate of growth in length per day increased with increasing temperature. These results are

in line with those reported by Britz and Hecht (1986) who observed better growth of *C.gariepinus* larvae with increasing temperature. Similar results were also reported by Haylor and Mollor (1995) that number of day-degrees decrease with increasing temperature until hatching, first feeding and yolk sac absorption in *C.gariepinus*. In the current study, at 0ppt, the survival of yolk sac larvae increased with increasing temperature. These results are in contrast with those by Nwosu and Hertzlohner (2000), who reported that survival of larvae of *Heterobranchus longifilis* reduced with increasing temperature. When they reared their larvae in fresh water; they obtained the highest survival of 67% at 25°C, 50% at 27°C, 33% at 29°C, and 13% at 32°C. However the results are in line with those by Fashina-Bombata and Busari (2003), who reported the highest survival of 93.7% when they reared *H. longifilis* at 27±0.5°C in fresh water. At 3 and 6ppt the survival of *C.gariepinus* yolk sac larvae decreased with increasing temperature, however the decrease was more pronounced at high temperature. These results are in line with those reported by Borode et al. (2002) who reported no significant difference when they reared *C.gariepinus* yolk sac larvae at temperatures of 26-27°C in salinities of 0, 2, 4, and 6ppt, however they did not show the exact figures for their results. Bioenergetic studies indicate a strong positive relationship between temperature and metabolism in fish (Wotton, 1995). Wotton, further noted that increased metabolic rate increases the levels of food intake in order to maintain growth and survival rates. In the current study, the observations show a positive relationship between the average rate of growth in length per day and survival of yolk sac larvae with temperature. This relationship could have been due to the increased metabolic rate which increased yolk utilization hence maintaining high growth and survival rates of *C.gariepinus* yolk sac larvae. The finding of this study therefore indicates that the survival of *C.gariepinus* yolk sac larvae increased with increasing temperature in freshwater.

Salinity affected average rates of yolk absorption per day and yolk absorption period at a given salinity in that, at all the three incubation temperatures (25-31°C). The average rate of yolk absorption per day decreased with increasing salinity, while the Yolk absorption period increased with increasing salinity from 0-6ppt. Similar results were reported by Borode and Akin-James (2005), who reported that the yolk absorption decreases progressively with increased salt concentration for the crosses of *C.gariepinus* x *H. longifilis*. They found out that yolk absorption rate was highest in the control, but it decreased with increased salinity. High rate of yolk absorption means small yolk size and low rate of yolk absorption means large yolk size. These results are in agreement with those reported by Borode et al. (2002), who reported that yolk size was highest at higher salt concentrations than at lower salt concentration. They recorded the yolk size of 0.3mm³ at 0ppt, 0.4mm³ at 2ppt, 0.3mm³ at 4ppt and 0.7mm³ at 6ppt. Rice (1990) explained that this inverse relationship could have been due to the decrease in metabolic rate at high salinities, He further reported that, factors which reduce metabolic rate like high salinity may be accompanied by a decrease in yolk consumption. Therefore in the present study, the inverse relationship between average rates of yolk absorption per day and yolk absorption period and salinity might have been due to the decreased metabolism at high salinities.

Salinity affected the rate of growth in length per day and larval survival in that, at all the three nursing

temperatures ranging from 25 to 31°C, the average rate of growth in length per day and larval survival decreased with increasing salinity. These results are in line with those reported by Borode and Akin-James (2005) who reported that mean body lengths and survival of the larvae of a hybrid *C. gariepinus* (f) x *H. longifilis* (m) decreased with increasing salt concentration. They further explained that this was as result of the predominance of *C. gariepinus* (f) over *H. longifilis* (m). *C. gariepinus* is a freshwater stenohaline fish known for increasing maintenance requirements at higher salinities and consequent weight loss which resulted into reduced growth and survival (Borode and Akin-James, 2005). These results are also in agreement with those reported by Britz and Hecht (1986) who reported decreasing growth rate with increasing salinity for *C. gariepinus* larvae. Furthermore the results obtained in this study agree with those reported by Fashina-Bombata and Busari (2003), who reported the highest survival of 93.75% at 0ppt when they reared yolk sac larvae of *H. longifilis* at 27±0.5°C in water at different salinities. They further reported that, the yolk sac-larvae which were hatched in water at high salinities and transferred in water at lower salinities highest survival of 93.75% at 0ppt. The finding of the current indicated a negative relationship between the average rate of growth in length per day and survival of yolk sac larvae with salinity. This could have been due to the characteristic nature of *C. gariepinus* as a freshwater stenohaline fish which increases maintenance requirements at higher salinities resulting into weight loss, reduced growth and survival (Borode and Akin-James, 2005).

The interactions of both temperature and salinity on average rate of yolk absorption per day and yolk absorption period revealed that, the highest average rate of yolk absorption per day and the least yolk absorption period were obtained at the highest incubation temperature (31°C) in freshwater. These results are in line with those reported by Borode et al, (2002), who nursed *C. gariepinus* larvae at different water salinities and reported that rate of yolk absorption was delayed in saline water than in fresh water. They found out that the rate of yolk absorption was significantly faster in the control and 2ppt salt treatments, but slower in 4, 6, and 8ppt. This could have been as a result of the decrease in metabolic rate at high salinities (Rice, 1990) and the high metabolic rates at high temperature (Nwosu and Hertzlohner, 2000).

In a conclusion, this study showed that both temperature and salinity and their combinations had a significant effect on yolk absorption period, average rate of yolk absorption per day, average rate of growth in length and larval survival of *C. gariepinus*. The temperature and salinity combination of 31°C and 0ppt is better for the nursing of *C. gariepinus* as evidenced by having least yolk absorption period, high average rate of development in length and high survival of *C. gariepinus* larvae, therefore it is recommended that *C. gariepinus* larvae should be nursed at a temperature-salinity combination of 31°C and 0ppt.

Acknowledgement

Special thanks goes to the National Aquaculture Centre (NAC) at Domasi for availing their institutional facility for this research and Icelandic International Development Agency (ICEIDA) for providing the first author with the postgraduate scholarship in Malawi.

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Biogas production from potato peelings using an anaerobic phased solid (APS) bioreactor

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Abstract

The consumption of potato chips is increasing in Malawi and large quantities of potato peelings are generated each day. The disposal of the peelings is becoming an environmental problem that needs urgent attention. The peelings have properties that make them ideal for anaerobic digestion. Unfortunately, the digestion of readily degradable feedstock like potato peelings in conventional single-phase reactors often results in accumulation of acids which leads to digestion failure. Two-phase digestion has shown to be successful on wastes similar to potato peelings. This study evaluated the potential for processing potato peelings into biogas through a two-phase laboratory-scale anaerobic phased-solid (APS) bioreactor. The bioreactor consisted of a batch-fed solid-phase hydrolysis reactor (SPHR) and four batch-fed liquid-phase methanogenic reactors (LPMRs) operated at ambient temperature. Potato peelings fed into the SPHR were hydrolyzed and acidified into high organic strength liquor with a total solid (TS) concentration between 42,243 and 54,917 mg/L and a pH between 3.72 and 4.56. The liquor was fed into the LPMRs and the biogas yield was measured as a function of feed TS load. Stable operation of the LPMRs was observed throughout the study. The average specific biogas yield was 0.98 m³/kg TS of liquor or 0.14 m³/kg of potato peelings. The SPHR managed to hydrolyse the peelings except the outer skins. The skins represented about 2% of the entire mass of the peelings and were the only solids that required disposal after the hydrolysis process. The APS bioreactor managed to reduce the amount of waste to be disposed while producing biogas as a renewable source of energy.

Keywords: anaerobic digestion; two-phase digestion; hydrolysis; methanogenesis; potato peelings; biogas.

1. Introduction

Over the past few years Malawians in both the urban and rural areas have become major consumers of potato chips and this has led to a proliferation of potato chips processing stands all over the country. These stands are a common site along streets and roads throughout the country. The processing of chips involves cleaning the potatoes and then peeling them using a knife. The peeled potatoes are sliced and soaked in water before frying. Large quantities of potato peelings and wastewater are generated each day. Potato peelings just like other potato processing wastes are highly degradable and should be quickly disposed off before they generate odour and excessive leachate. Unfortunately, the potato peelings are simply disposed in the open along roads where they become a major source of environmental and health problems (Palamuleni, 2001; Elango et al., 2007). The odour from the decomposing peelings is not only a nuisance but also attracts flies which are major carrier of disease-causing microbes. The leachate from the decomposing peelings may percolate into the ground and contaminate both the soil and groundwater or may flow into rivers and other surface water sources thereby making the water unsuitable for human and livestock use. The decomposing peelings also produce gaseous products that include carbon dioxide, methane and other noxious gases like hydrogen sulfide. These gases not only pollute the environment but also contribute to the problem of climate change since most of them are greenhouse gases (GHGs).

Anaerobic digestion (AD) is among the oldest and most widely used waste treatment process that can be used to deal with the problem of disposal of the potato peelings. AD results in the decomposition of complex organic materials into simple compounds in a chain of groups of microorganisms, in the absence of oxygen, to produce biogas which is a mixture of methane and carbon dioxide under ideal conditions. The biochemistry underlying AD is complex and has been described as a sequential process of four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Aldin et al., 2011; Khalid et al., 2011; O'Flaherty et al., 2010; Deublein and Steinhauser, 2008; Monnet, 2003; Kossman et

al., 1997). Due to the strong link of the phases in each stage, the type of substrate, pH, temperature and other operating and design parameters will affect the activity and the growth rate of the different bacteria. The major advantage of AD is the production of biogas and a nutrient-rich stabilized effluent. Biogas is a renewable source of energy that can be used for cooking and for power generation. The use of biogas can reduce the dependency on firewood and petroleum fuels as sources of energy and can effectively reduce the problem of climate change. The effluent can safely be disposed in crop fields as source of nutrients without posing any serious environmental problems (Elango et al., 2007; Verma, 2002; Pavlostathis and Gossett, 1988).

Potato peelings are ideal substrate for AD due to their high soluble organic matter content. They have characteristics that are not significantly different from those of the whole potato. On average potatoes consists of 78 – 84% water, 18.4% starch, 2.2% protein, 1% ash and about 0.4% crude fiber, 0.1% lipid (Lister and Munro, 2000, 2013; Schieber and Saldana, 2009; Schieber et al., 2001). In addition, the estimated carbon-to-nitrogen (C/N) ratio of 25 (Kossman et al., 1997; Kaltwasser, 1980) is within the range for AD process. There has been considerable interest in studying the behavior of potato processing waste including potato peelings under AD (Lucas, 2014; Singh, 2014; Fang et al., 2011; Kryvoruchko et al., 2009; Monou et al., 2008; Zhu et al., 2008; Linke, 2006; Kaparaju and Rintala, 2005; Parawira et al., 2004, 2005). The results from most of these studies have indicated that anaerobic digestion of potato processing wastes including peelings as a single substrate in single-phase reactors is a challenging process. Their high biodegradability often leads to the rapid and strong acidification inside the reactor with a consequent inhibition of the methanogenic bacteria activity.

In order to achieve successful and sustainable digestion of potato processing wastes including peelings, a number of strategies have been evaluated. These strategies include co-digestion of the potato processing waste with other substrates, digestion under mesophilic and thermophilic conditions and pre-treatment of the potato peelings through grinding

or milling and chemical hydrolysis using alkaline solutions, acids and enzymes. The results on co-digestion indicate that potato peelings and other potato processing wastes are suitable material for co-digestion with livestock manure and a number of food and vegetable wastes (Kryvoruchko et al., 2009; Monou et al., 2008; Parawira et al., 2004). The results on high temperature digestion of potato processing wastes have shown that these wastes were suitable materials for biogas production under mesophilic or thermophilic conditions as long as there was a tight control of the process parameters such as loading rate (Pistis et al., 2013; Linke, 2006; Parawira et al., 2005). In his study involving chemical pre-treatment of potato processing wastes, Lucas (2014) noted that alkaline pre-treatments resulted in a significant improvement in the biogas and methane yield compared to acid pre-treatments.

Another strategy that has widely been applied to the digestion of readily digestible waste like fruits and vegetable wastes is two-phase digestion using an anaerobic phased-solid (APS) system. The two-phase APS system uses separate reactors for the hydrolytic/acidogenic phase and for the methanogenic phase and has shown to be successful on a variety of readily digestible wastes (Liu, et al., 2012; Zhang and Rapport, 2011; Zhu, et al., 2010; Konwinski and Zhang, 2008; Paven et al., 1999; Zhang and Zhang, 1999; Liu and Ghosh, 1997; Weiland, 1992). Although wastes similar to potato peelings have successfully been digested using the two-phase APS system, not much work has been done to study the potential for processing potato peelings as a single feedstock under ambient conditions using the two-phase APS system. This study was therefore conducted with the objective of evaluating the potential for processing potato peelings into biogas in a two-phase APS bioreactor system operated under ambient temperature without heating. The specific objectives of the study were to (1) estimate the quantity of peelings generated from a unit mass of potatoes, (2) determine the total solid (TS) concentration and pH of the hydrolyzed/acidified potato peelings liquor, (3) determine the biogas yield as a function of feed TS load and (4) estimate the reduction in the amount of waste accomplished through the APS bioreactor when processing potato peelings.

2. Materials and methods

2.1 Quantity and characteristics of potato peelings

Potatoes were bought from Lilongwe Central Market for estimating the amount of peelings generated from a given quantity of potatoes. The potatoes were a combination of Violet and Rosetta varieties and were placed into three size categories of small, medium, and large. Samples of the potatoes from each size category were weighed and then peeled manually using a knife. The peelings were weighed and then the mass fraction of the peelings was determined before and after oven drying. The mass fraction of the outer skin of the potatoes and peelings was determined by weighing the skins that were peeled after the potatoes and peelings had been boiled.

2.2 Laboratory-scale APS bioreactor

The laboratory-scale APS bioreactor system was constructed using one 5-litre plastic bucket and twelve 2-litre soda bottles. The 5.0-litre bucket was operated as a solid-phase hydrolysis reactor (SPHR) while four of the 2-litre soda bottles were operated as liquid-phase methanogenic reactors (LPMR). The remaining 2-litre soda bottles were used as collectors for gas and for displaced water. The water

displacement method as described by Manhokwe et al. (2009) was used for measuring the daily biogas production. All the reactors were designed to be fed manually. The SPHR and one set of the LPMR are shown in Figure 1.

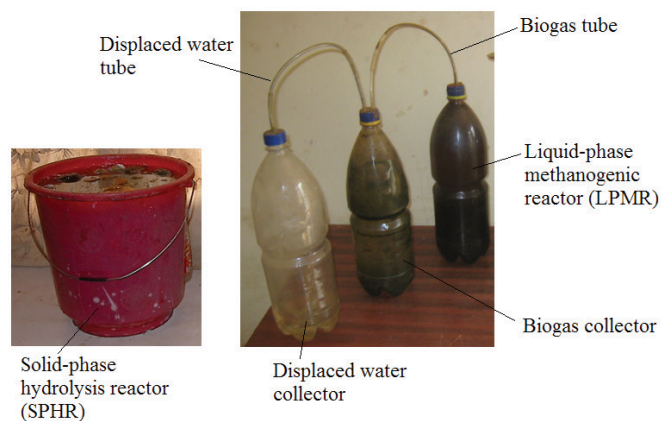


Figure 1. Pictorial set-up of one set of the LPMR and the SPHR

2.3 Laboratory operation of the APS bioreactor

The four LPMRs, identified by the letters A, B, C and D, were initially inoculated using digestate from an anaerobic digester for cattle dung to provide the starting population of bacteria. The reactors were then taken through a start-up period in which they were fed with hydrolyzed/acidified potato peelings liquor derived from the SPHR. The start-up period ensured that there was complete substitution of the initial cattle dung digestate with hydrolyzed/acidified potato peelings liquor. The hydrolyzed/acidified potato peelings liquor was obtained from the hydrolysis of potato peelings collected from restaurants within Bunda College. In order to produce the liquor, samples of peelings were mixed with water and fed into the SPHR. The mixing proportion was one part peelings to two parts water by mass. The amount of water was enough to completely immerse the peelings in the SPHR. After the hydrolysis was complete, the liquor was collected and fed into the LPMRs. The residual solids were removed from the SPHRs and dried and a new batch of potato peelings and water was added into the SPHR.

Feeding of the LPMRs involved removing a given volume of clarified effluent from the LPMRs and then adding an equal volume of feed. This procedure ensured that the LPMRs were operated as sequencing batch reactors (SBR) with maximum biomass retention. Biogas production was monitored and measured daily using the water displacement method. A new batch of feed was added to the LPMRs whenever the biogas production for five consecutive days was negligible. The potential for the APS bioreactor to process hydrolyzed/acidified potato peelings liquor was evaluated by subjecting the reactors to different loading rates of the liquor. The different loading rates were obtained by using different combinations of liquor TS concentrations and liquor volumes. The loading rate was measured in terms of TS and was obtained as the product of TS concentration and the volume of liquor. All the reactors were operated at ambient temperature without any means for heating.

2.4 Data collection and analysis

Samples of the potato peelings were analyzed for TS and VS using a drying oven and a muffle furnace. The TS represent the solids that remain in the sample after the water has evaporated by drying at $105 \pm 1^\circ\text{C}$. The VS

represent a fraction of the TS that undergo volatilization at a temperature of 550 ± 50 °C, constituting a mainly organic fraction. Samples of hydrolyzed/acidified potato peelings liquor, effluent from the LPMRs and the residual solids from the SPHR were also collected and analysed for their TS using the procedure described in Standard Methods (APHA, 1998). The pH of the hydrolyzed/acidified liquor and the effluent from the LPMRs was determined using an IQ Scientific Instrument pH meter Model IQ150. The total biogas yield was obtained by summing up all the daily biogas measurements for a given reactor loading. In order to establish the relationship between the biogas yield and the feed TS load, a linear regression analysis was performed.

3. Results and discussion

3.1 Quantity of potato peelings

The average quantities of potato peelings and outer skins for the Violet and Rosetta potatoes that were assessed in this study are presented in Table 1. The table shows that the mass fraction of peelings ranged from 18.4 to 22.9% while that for the outer skin ranged from 0.44 to 0.49%. On average, the results indicate that the mass of peelings and outer skins represented, respectively, 19% and 0.46% of the total mass of potatoes. The outer skins represented about 2% of the total mass of peelings. The findings from this study are in agreement with results reported in a number of studies. Singh (2014) reported that in India, the amount of peelings from potato processing industries represented about 25% of the potato input while in university cafeterias the figure was around 13.6%. Scieber et al. (2001) reported that the quantity of peelings from a number of potato processing industries in Europe ranged from 15 to 40% of the potato input depending on the procedure used in peeling the potatoes. Peeling methods that are used in European industries include steaming, abrasion and lye peeling.

Table 1. Average quantities of potato peelings and outer skin

Parameter	Size of potato		
	Small	Medium	Large
Mass of potato (g)	30.6	64.3	125.9
Mass of wet peelings per potato (g)	7.0	13.8	23.0
Mass of dry peelings per potato (g)	1.3	2.3	3.3
Mass fraction of wet peelings (%)	22.9	21.5	18.4
Mass of wet outer skin per potato (g)	0.15	0.29	0.55
Mass fraction of outer skin (%)	0.49	0.45	0.44

3.2 Characteristics of potato peelings

An analysis of the potato peelings collected from restaurants around Bunda College indicated that the peelings had an average TS of 14% (i.e. a water content of 86%) and an average VS of 86% on TS basis. These results are in agreement with those obtained from similar studies on

potato peelings. For example, Pistil et al. (2013) determined that the average moisture content of potato peelings ranged from 75% to 85% (i.e. a TS ranging from 15 to 25%) with a VS on TS basis ranging from 82% to 90%. Singh (2014) determined the moisture content of potato peelings in his study in India to be 85.7% (i.e. TS of 14.5%) and a VS of 85.5% on TS basis. Lucas (2014) in his study obtained the TS and VS of potato peelings to be 12% and 93.4% on dry matter basis, respectively. The VS of 86% on TS basis obtained from the current study indicates that in theory about 86% of the dry matter in the peelings can be degraded by bacteria and consequently be transformed into biogas. However, in practice, this does not happen because not all VS can be degraded and be converted into biogas by bacteria (Hamilton, 2012).

3.3 The hydrolytic/acidogenic phase

The hydrolysis of the potato peelings was carried out in water in the SPHR and resulted in the breakdown of the potato peelings into a high organic strength liquor and outer skins. The liquor had a consistency and appearance of milk and was largely composed of suspended and colloidal solids. It took 4 – 8 days for the inner part of the peelings to completely breakdown to leave only the outer skins. Each sample of 720 g potato peelings plus the 1.44 litres of water that was added produced about 2.0 litres of hydrolyzed/acidified liquor and about 14 g of outer skins. The outer skins could not be broken down even at extended retention periods and were the only large chunks of solid remaining after the hydrolysis/acidification process. The results show that water can effectively be used to facilitate the breakdown of potato peelings. Singh (2014) studied the production of leachate from potato peelings without the addition of water and noted that the process was slow and only produced a small quantity of leachate. The results showed that 1 kg of peelings produced about 282 ml of leachate over a period of 25 days.

3.4 Characteristics of the hydrolyzed/acidified potato liquor

The hydrolyzed/acidified potato peelings liquor consisted of a large fraction of suspended and colloidal solids and produced a strong odour. Due to lack of suitable laboratory equipment, no chemical analysis was performed to determine the composition of the liquor. However, its TS and pH were determined and the results for the 720-g samples of peelings gave TS values ranging from 42,243 to 54,917 mg/l and pH values ranging from 3.72 to 4.56. The TS concentration was dependent on the solid content of the peelings and on the amount of water used in the SPHR. The pH was expected to be low because of the acidification process that took place inside the SPHR in addition to the hydrolysis process.

3.5 The methanogenic phase

The four LPMRs were fed with the hydrolyzed/acidified liquor from the SPHR at different loading rates. Throughout the study, all the four LPMRs had a stable operation and did not fail. At the end of each feed cycle the effluent pH for all the four LPMRs was above 7.0 indicating that the digestion process was stable despite feeding the reactors with acidic liquor with pH values below 5.0. Since the hydrolysis/acidification process occurred outside the LPMRs, there was no build-up of acids inside the LPMRs. The methanogenic bacteria were not overloaded with

substrate in the LPMRs. Feeding the LPHRs with already hydrolyzed/acidified liquor, avoided the drop in pH that would have occurred had the peelings been fed directly into the reactors. Singh (2014), in his study involving the digestion of potato peelings in a single reactor reported that the pH was dropping to levels below 5.0 and had to use potassium hydroxide (KOH) to bring back the pH to neutral.

3.6 Biogas production

The performance of all the four LPMRs in terms of biogas production was similar. In all the tests, biogas production started immediately after feeding the LPMRs. The daily biogas production was high within the first two days after feeding and then began to drop until it became negligible when all the readily degradable fraction of the liquor was exhausted. Figure 2 shows the daily biogas production for four cycles of feeding the digesters.

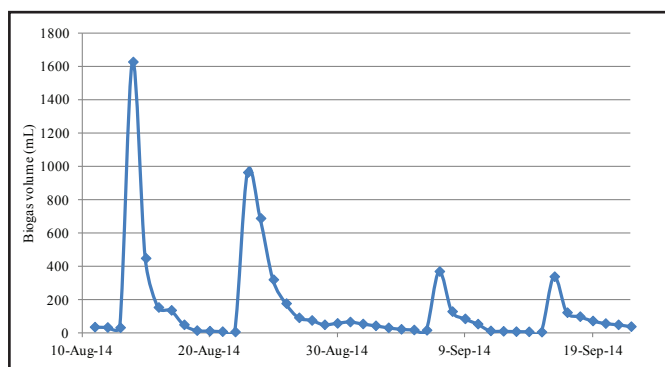


Figure 2. Daily biogas production during four digester feeding cycles

The methane and carbon dioxide content of the biogas was not measured due to lack of equipment. However, each day samples of the gas were ignited and the blue colour of the flame was a good indicator of a high methane content. Biogas is only flammable when its methane content is above 45% (Deublein and Steinhauser, 2008). The gas was almost odorless indicating the absence of hydrogen sulfide. Potatoes have a low content of sulfur-containing amino acids (Markakis, 1975) and hence the digestion of the peelings is not expected to produce any substantial quantity of hydrogen sulfide. The relationship between the biogas yield and the feed TS load is presented graphically in Figure 3. The graph shows that the total biogas yield increased linearly with the feed TS load

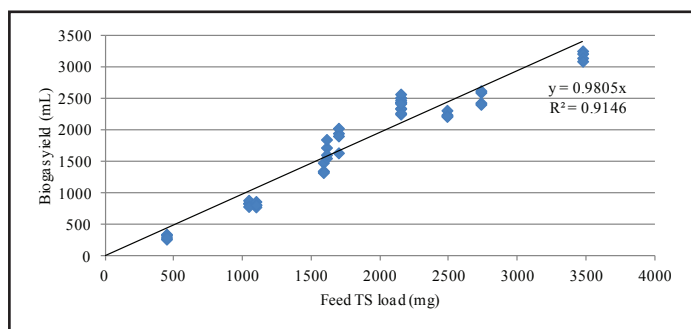


Figure 3. Total biogas yield plotted as a function of TS load

A linear regression equation of the form $y = \beta x$, where y is the total biogas yield, x is the TS load and β is the specific biogas yield was obtained for each LPMR and for the overall results using Microsoft Excel. Table 2 gives values of the specific biogas yield β , and the corresponding R^2 values

for the four LPMRs and for all the results. The specific biogas yield ranged from 0.9540 to 1.0044 ml/mg TS with an overall average of 0.9805 ml/mg TS of the feed liquor. This is equivalent to 0.98 m³/kg TS of feed liquor or 0.14 m³/kg of potato peelings. Due to differences in digestion procedures and operating conditions, values ranging from 0.1 to 0.70 m³/kg TS have been reported for the specific biogas yield from potatoes and potato processing waste in single-stage AD processes (Pistis et al., 2013; Singh, 2014; Lucas, 2014; SEAI, 2014; IEA, 2014; Moody et al., 2011; Deublein and Steinhauser, 2008; Lehtomaki, 2006). The high value of specific biogas yield obtained from this study is a clear indication of the improvement accomplished through the use of the two-phase APS bioreactor system. By allowing the hydrolysis/acidification phase to occur separate from the methanogenic phase and feeding the methanogenic phase with the hydrolyzed/acidified liquor, the stability and productivity of the AD process was improved. The two-phase APS bioreactor with water facilitating the hydrolysis process can therefore be used as a viable technology for generating renewable energy in the form of biogas from potato peelings without any digestion failure.

Table 2. Specific biogas yield

Reactor	Specific biogas yield, β (ml/mg TS)	R^2
A	0.9989	0.9139
B	1.0044	0.9168
C	0.9645	0.9199
D	0.9540	0.9190
Overall	0.9805	0.9946

3.7 Solid reduction

Effluent was removed from the LPMRs after allowing all the settleable solids to settle within the reactors. Measurement of the effluent TS indicated that the TS ranged from 1,902 to 3,740 mg/L. This gave a TS reduction of 90 to 95%. The low TS values in the effluent and the high TS reduction are an indication that the AD system was adequately removing all the readily degradable solids from the feed liquor. A material balance conducted on the solids passing through the APS bioreactor system indicated that for each kilogram of potato peelings, the solid output was about 20 g (i.e. 2% of the mass of peelings) of potato outer skins. The rest of the solids in the potato peelings were converted into biogas and bacterial biomass while a small fraction (between 1.9 and 3.7 g per litre) was contained in the effluent. Since the effluent has gone through AD process, it is stable and can safely be disposed as water for irrigation of crops without causing any threat to the environment. By using the APS bioreactor for processing potato peelings, the quantity of waste to be disposed is substantially reduced to about 2% of the original mass of peelings. The APS bioreactor can therefore be used as a technology for managing the problem of disposing potato peelings.

4. Conclusions

The study has shown that the two-phase APS bioreactor has great potential for processing potato peelings into biogas and consequently reducing the amount of waste to be disposed. Potato peelings were easily broken down into

acidified liquor in the SPHR and the liquor was an excellent feedstock for AD. The LPMRs of the APS bioreactor achieved a specific biogas yield of 0.98 m³/kg TS of potato peelings liquor or about 0.14 m³/kg of potato peelings while operating at ambient temperature. The potato outer skins which constitute about 2% of the potato peelings could not be degraded by the hydrolysis reactions and were the only solids that needed to be disposed. The APS bioreactor can therefore be used as a technology for managing the problem of disposing potato peelings and at the same time as a technology for generating renewable energy in the form of biogas.

5. Acknowledgements

The author is acknowledging the Agricultural Engineering and Animal Science Departments at the Lilongwe University of Agriculture and Natural Resources (LUANAR) for providing laboratory space and equipment for the study.

6. Conflict of interest

The author declares no conflict of interest in this work.

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Productivity and marketing efficiency of small scale dairy enterprises in Malawi: A case study of Dwale and Emfeni extension planning areas

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Abstract

A study was conducted in Thyolo and Mzimba Districts to assess the technical efficiency (TE) of small scale milk producers in production and marketing. The aim of the study was to contribute to knowledge on challenges to growth of small scale dairy enterprises in Malawi through assessment of efficiency levels in production and marketing of milk. A deterministic Cobb-Douglas frontier production function was used to estimate TE indices. Overall, 76% and 21% of farmers, with purebred and crossbred cows, respectively, had technical efficiencies below the sample mean. The calculated degree of homogeneity provided evidence of increasing returns to scale, for the enterprises. However, despite farmers possessing inherent bargaining power, dependency of farmers on processors, for acquisition of capital assets, contributed to farmers being the price takers. This paper concludes that an improvement in feeding and farm labour management would enhance productivity, and hence growth of the enterprises, without necessarily increasing the number of cows. The study recommended that to regain some bargaining power, a change in attitude towards self-reliance, is required for dairy enterprises management to use retained profits to acquire capital assets, as opposed to relying on processors.

Key Words: Efficiency; Dairy enterprises; Malawi; Marketing

Introduction

Dairy farming in Malawi is characterised by low productivity, due to poor management practices, low dairy cattle population and use of local breeds among others (Department of Animal Health and Livestock Development (DAHLD), 2012). This contributes to low milk consumption, which is at 4kg/capita/year (FAO, 2014). However, although dairy cow population in Malawi (estimated at 2% of human population), compares favourably with Zambia (1%) and Tanzania (2%), Malawi's milk consumption is low compared to Zambia (8 kg) and Tanzania (23 kg) (Tambi et al., 2001). Dairy production involves both government and the private sector, at both large and small scale production systems (Banda et al, 2012). The sector is further categorized into formal and informal operators. The formal sub-sector caters for milk produced, and sold using the conventional marketing channels, for example, through milk bulking groups (MBGs). The informal sub-sector caters for milk produced, and progressively sold raw to local consumers or consumed at household level (Revoredo-Gihaet al, 2013).

According to DAHLD (2012), the low milk supply could partly be due to the low cattle population of about 1,131,000, of which an estimated 6% are dairy cows. Other reported other challenges to dairy production include lack of foundation stock, low farm-gate milk prices, high labour demands, inadequate grazing land, poor nutrition, low fertility, inadequate extension, health and breeding services (Mangisoni, 1989, Banda et al., 2012). However, the theory of production encompasses both effectiveness and efficiency and most studies on dairy production concentrate on effectiveness (Mangisoni, 1989, Banda et al., 2012; Tambi et al., 2001; DAHLD, 2012), with minimal attention to efficiency. Efficiency is concerned with how well the processes are carried out to convert resources into goods. Efficiency is influenced by the technology used, level of utilisation of production resources, level of wastage of materials and management processes being deployed Chavas and Cox (1988) in Sidik (2006). Therefore, if farmers are not producing more with the available resources, improving efficiency would be more cost effective than introducing more new technologies. This forms the background to analysing farm level efficiency (Bravo-Ureta and Evanson,

1994). Therefore, this study was undertaken to assess the efficiency of small scale dairy production and marketing, in order to identify key areas that need strengthening for improved dairy production and marketing in Malawi.

In Malawi, a few studies have been done on production and marketing efficiency of agricultural produce. For example Edriss (2003) measured the TE for smallholder groundnut farmers; Nzima and Dzanja (2014) analysed the efficiency of the soybean market; Tchale (2009) and Chirwa (2007) looked at the technical efficiencies of maize production. To fill the gap in literature, this study assessed technical efficiency of milk production and consumption. Historically, intensive dairy productions commenced in 1969 when processing plants were installed in Blantyre. These developments followed in Lilongwe in 1973 and Mzuzu in 1974, through the initiative by Malawi government, under Malawi Milk Marketing (MMM), in order to collect and process milk, to meet a growing urban demand (Land O Lakes, 2003). After a collapse of this establishment, in 1997, the privatization of Malawi Dairy Industries (MDI) factories represented a major policy change. Since then, other small dairy plants mushroomed serving both formal and informal sub-sectors. There are currently an estimated 9,584 dairy farmers, most of which are smallholder farmers, and with 61% located in the southern region of Malawi (Revoredo-Gihaet al, 2013).

Materials and Methods

The study involved interviewing farmers from Dwale and Emfeni Extension Planning Areas (EPA) in Thyolo and Mzimba Districts, located in the southern and northern regions, respectively. The farmers were categorized as belonging to either the formal or informal sector, based on whether they formally sold milk to milk bulking groups (MBGs) or sold the milk locally. Farmers from Emfeni represented the informal sector, because there were no operational MBGs at the time of the study, while farmers from Dwale represented the formal sector. A sample size of 33, with power level of 0.5, was determined for the formal sector, using stata. However, as many respondents as practical were interviewed, and hence a total of 111 (formal) and 77 (informal) farmers were randomly selected and interviewed using structured questionnaire. The sampled farmers from Dwale EPA belonged to Bvumbwe, Chandamale, Dwale and

Thunga MBGs.

Data collected included farmer demography, type and quantity of feeds per cow per day, type and number of breeds used for milk production, quantity of potential farm labour, total variable and fixed costs in milk production, and the selling price. Data were analysed using STATA software. The analysis used descriptive statistics, and the deterministic production frontier model (DPFM), to measure technical efficiency (TE). DPFM was used because it allows a simultaneous estimation of the technical efficiency (TE), and the technical inefficiency component (-) of the individual dairy farmer (Barthwal, 2007). Lerner's index model was used to estimate market efficiency, which represents monopoly power (Lerner, 1934). Three separate analyses were done to estimate production efficiencies for dairy farmers with purebred dairy cows, crossbred cows and Malawi Zebu cows. Milk production was a dependent variable in all the three DPFM functions.

There are a number of different functional forms used to model production functions, and Cobb-Douglas (linear logs of outputs and inputs) is one of them. Production (output) is a function of inputs, and hence, for dairy cattle, feeding and management practices, would be of paramount importance in this equation. Therefore given the diversity of feeding and management practices, the question of technical efficiency needs to be addressed, especially for developing countries like Malawi. As pointed out by (Greene, 1990), a producer is considered efficient if a higher output cannot be obtained from a given set of inputs and technology. Technical efficiency is defined as a factor by which the level of production is less than its frontier output (Battese, 1992). Using the estimated deterministic Cobb-Douglas (C-D) function, TE indices were therefore estimated.

The Cobb-Douglas stochastic function was utilized because stochastic frontiers approach performs better than data development analysis, especially, with agricultural data, because of measurement errors, particularly if the data is from developing countries (Battese and Coelli, 1995). The Cobb-Douglas production function used in the analysis is in equation 1.

$$\ln y_i = \beta_0 + \beta_1 \ln x_{i1} + \beta_2 \ln x_{i2} + v_i - u_i \dots \dots \dots (1)$$

Where y_i = output indicator (milk production), β =coefficient of inputs, x_i and also indicates the production elasticities, i = sampled farmer group, v_i = random error and u_i = one sided error

In the model all the errors are assumed to be due to technical inefficiency i.e the noise is not taken into account. x_i 's are in logs and include a constant, u_i is a non-negative random variable. Therefore $\ln y_i \leq x_i \beta$. Given the frontier nature of this model, which considers only one side of the model error (u_i), production of $y_i = f(\ln x_i, \beta)$ and hence technical efficiency is assumed. However, The stochastic frontier proposed by Aigner et al. (1977) and Meeusen and Van den Broeck (1977) differs from the deterministic frontier in that the error term is decomposed into a random error (v_i), and a one sided error (u_i). The one sided error is assumed to account for technical inefficiency. Therefore the frontier changes as in equation 2.

$$\ln y_i = \ln f(x_i, \beta) + v_i - u_i \dots \dots \dots (2)$$

The frontier is deterministic because it is given by $\exp(x_i \beta)$ which is non-random. TE measure is therefore obtained from the ratio of y_i to the maximum achievable

level of output y_i^* , which lies on the frontier ($u_i = 0$). TE among farmers with purebred, crossbred Malawi Zebu cows was therefore defined as:

$$E = \frac{y_i}{y_i^*} = \frac{f(x_i, \beta) \exp(v_i - u_i)}{f(x_i, \beta) \exp(v_i)} = \exp(-u_i) \dots \dots \dots (3)$$

Results and Discussion

A descriptive analysis of the socio economic variables indicated a significant difference in the variables between formal and informal dairy farmers (Table 1). The results show the mean household sizes of 5 and 6 for formal and informal, respectively, which were slightly higher than the national average of 4.2 in the southern region and 4.9 in the northern region (NSO, 2008). A correlation analysis revealed that, contrary to the informal sub-sector, household size was positively correlated with milk production in the formal sub-sector, which implies that large sized households had capacity to produce more milk compared to smaller households.

Table 1 Key Socio Economic Variables

Variable	Category of Dairy farmer				t-value
	Formal		Informal		
	Mean	SE	Mean	SE	
Household size	5	0.20	6	0.27	-2.98**
Land holding size (ha)	0.66	0.06	2.26	0.17	-10.23**
Age (years)	44.5	1.33	49.0	1.09	-2.07**

****Significant at 0.05**

Mean land holding sizes were 0.66 and 2.26 hectares for formal and informal milk producers, respectively (Table 1), and these were significantly different ($p < 0.05$). Farmers in the northern region have more land compared to those in the southern region, due to population. However, despite land availability, milk producers had no pasture establishment. 70% of farmers in the informal sub-sector, kept livestock for dowry and did not invest in pasture management.

1. Technical Efficiency analysis

Three models were fitted to estimate technical efficiencies of farmer's productivity compared across three breeds. The fitted model results from producers with purebred, cross breeds, and Malawi Zebu are indicated in Table 2. The results indicate that 1 % change in quantity of roughages fed to purebred cows per lactation period would result into 59% variation in milk production per lactation period. Similarly, one percent change in quantity of maize bran fed, per lactation period, contributes to a 17% variation, and one percent increase in the number of purebred dairy cows per lactation period contributes to 88% variation in milk production per lactation period.

The deterministic production frontier models showed mean TE of 0.57. This means farmers who had purebred cows were 57% efficient in production; representing 43% below production possibility frontier. One of the properties of C-D function according for single output technologies, is homogeneity (Binger and Hoffman, 1988). The results showed that the sum of β for explanatory variables is 1.68, which is evidence for increasing return to scale for farmers with purebred cows. Returns to scale are technical properties

of the production function. Increasing returns to scale indicates that doubling inputs more than doubles the output.

Table 2 Technical Efficiencies per Breed

Variable	Coefficients		
	Purebred	Crossbred	Malawi Zebu
Intercept	-0.71 (1.22)	-2.49 (4.02)	3.71 (0.71)
Log (roughages, kg)	0.59** (-0.18)	0.27 (0.57)	
Log (maize bran, kg)	0.17** (-0.14)	0.25** (0.38)	
Log (Number of cows)	0.88** (-0.1)	1.13** (0.69)	0.27** (0.11)
Log (labour, man-days)	0.04** (-0.1)	0.46** (0.46)	
Degree of homogeneity	1.68	2.11	0.27
Mean Technical efficiency	0.57	0.99	
Model diagnostics			
R squared	0.53	0.56	0.08
Breusch-Pagan test	0.08	0.14	
Tolerance	0.49	0.36	
N	111	111	77

Note: standard errors are reported in brackets, **refers to significance at 5%

Regarding the farmers who were rearing crossbreds the results indicated that DFPM was deterministic although roughages were not significant at 0.05. However, a 1% change in roughages fed would vary milk production by 27%, while a 1% change in number of crossbred cows affected over 100% variation in production. Maize bran and labour were significant at 0.05, and hence a 1% change in quantity of maize bran and labour varied production by 25% and 46%, respectively. The degree of homogeneity of 2.11 indicates that farmers rearing crossbred cows have more increasing return to scale than dairy farming with purebred cows (1.68). The results suggest that enterprises with crossbred cows are relatively more rewarding than the similar business with purebred cows under the prevailing small scale farming system.

The study was however challenged by lack of data among farmers in the informal sub-sector. Free grazing in open access resources made it difficult to estimate the quantity of feed and labour inputs. However, the study managed to capture number of Malawi Zebu cows per farmer that has been used to make a rough estimate of DFPM. From the 77 households interviewed, the average number of cows was 6 that ranged from 5 to 8. The significance of Malawi Zebu at 0.05 means that one percent change in the number

of Malawi Zebu cows, affects 27% variation in production levels. However, more data is required in this regard.

A Ramsey reset test using powers of the fitted values of log of observed values of milk production of ($p > F = 0.02$) and ($prob > F = 0.046$), for pure and crossbred models, respectively, indicated that the model had no omitted variables. The TE results were further confirmed by an analysis of the distribution of farmers according to the TE index levels. Table 3 displays the results of this analysis. Table 4 highlights a summary of TE indices and degree of homogeneity for farmers owning purebred, crossbred dairy and Malawi Zebu cows.

Table 3 Distribution of Technical Efficiencies

TE Index	Formal		Informal
	Purebred cows	Crossbred cows	Malawi Zebu
0.38-0.40	2.67	N/A	65.10
0.41-0.50	5.33	N/A	23.88
0.51-0.60	68.00	N/A	11.02
0.81-0.90	N/A	6.89	N/A
0.91-0.95	N/A	13.80	N/A
0.96-1.00	N/A	79.31	N/A
Total	100	100	100

Source: Research data, (2008)

As in Table 3, 76% of farmers owning purebred cows were below a mean TE index of 0.57, indicating high technical inefficiencies. Similarly, 21% of the farmers rearing crossbred cows were below a mean TE of 0.95 which is pointer that 79% of the farmers attain TE above average TE that is generally higher than farmers with purebred and Malawi Zebu.

2. Factors Determining Technical Efficiency

Given the deterministic C-D frontier production function, type offeds, number of cows and labour were investigated to establish which factor might cause inefficiencies. OLS regression model in equation 4 was used to investigate whether the mentioned factors might cause inefficiencies. Parameter estimates were generated and are summarized in Tables 4, 5, and 6

$$TE = f(FR, FM, \text{Cow breed and } L) \dots \dots \dots (4)$$

The test of quality, in the tables 4, 5, and 6 showed that estimated coefficients on TE were not statistically significant at 0.05. However, all variables had positive relationship with TE. This means that a change in any of the explanatory variables affected TE levels with varying magnitudes. The findings tally with determinants of production efficiencies obtained when estimating C-D production function. The technical inefficiencies among 76% of farmers (purebred cows), technical inefficiencies among 21% of farmers (crossbred cows) and almost all Malawi Zebu famers (informal) were associated within adequate feeding, number of cow breed and labour. Intuitively, productivity efficiency

can be improved by increasing feed and farm labour, without necessarily increasing the number of dairy cows.

Table 4 Parameter Estimates for Purebred cows

Variable	Coefficient	Standard error	t-value
Log (roughages, kg)	0.006	0.007	0.085
Log (maize bran, kg)	0.003	0.006	0.57
Log (number cows)	0.001	0.008	0.04
Log (labour, man-days)	0.003	0.006	0.47

R² = 0.07 Note: Estimated coefficients were not statistically significant at 0.05.

Table 5 Parameter Estimates for Crossbred Cows

Variable	Coefficient	Standard error	t-value
Log (roughages, kg)	0.001	0.025	0.050
Log (maize bran, kg)	0.004	0.021	0.170
Log (number cows)	0.003	0.027	0.013
Log (labour, man-days)	0.002	0.028	0.70

R² = 0.09 Note: Estimated coefficients were not statistically significant at 0.05.

Table 6 Parameter Estimates for Malawi Zebu

Variable	Coefficient	Standard error	t-value
Log (number of cows)	0.303	0.059	0.014

R² = 0.09 Note: Estimated coefficients were not statistically significant at 0.05.

3. Measurement of Lerner’s Degree of monopoly power among milk producers

The producers’ monopoly power was estimated using Lerner’s index as defined in equation 5.

$$L = \frac{P - M}{P} \text{ (Lerner, 1934).....(5)}$$

The index identifies the degree of monopoly power as the difference between the price (P) and its marginal cost (MC) at the profit maximizing rate of output. The bigger the difference between price and marginal cost the greater the monopoly power (Lerner, 1934). The Mean Lerner’s Index (L) for farmers in the formal sector was 0.66 with a

ranged from -1.76 to 0.98, while for the informal sector was 0.79. An equality test of L between the two categories of farmers was statistically significant at 0.05. Despite statistical insignificance of the relationship between monopoly power (L) and milk prices, there was positive correlation between the variables. Over 75% of farmers in all categories have the monopoly power as evidenced by low percentage of famers below L of 0.5. The results are summarised in Table 6. The results indicate that, all MBGs had the bargaining power for their products yet sentiments about dissatisfaction with price were common. Farmers failed to effectively reach desired selling prices due to lack of record on production costs and had no basis for negotiation.

Table 7 Monopoly Power for Milk Bulking Groups

MBG	Mean Lerner Index	Standard error
Bvumbwe	0.71	0.42
Chandamale	0.70	0.34
Dwale	0.26	0.95
Thunga	0.77	0.15
Mean for MBGs	0.66	

4. Determinants for ineffectiveness of monopoly power among farmers in formal sub-sector

The ineffectiveness of monopoly power among farmers transcends to dependency on handouts for capital equipment, and other outreach programmes which cripple ownership of bulking groups. In addition, milk marketing lacks legal framework to protect local producers.

(a) Ownership of MBGs

The relationship between milk buyers (processors) and MBGs was not that of a pure buyer-seller type. MBGs benefited from outreach programmes and financial support from processors. For example, on site assessment showed that withdrawal of cooling tanks, which were installed with funding from processors, would result into immediate collapse of Thunga and Dwale bulking groups, which served 350 and 228 dairy farmers respectively. The ownership of financial capital assets means that MBGs were “owned” by buyers or processors. Dairy farmers and processors had a form of “tenant-landlord” type of business relationship. This partially explains the failure of farmers to exercise the monopoly power as measured by Lerner’s Indices above (Table 6). Processors were in strategic position of influence on prices despite farmers’ monopoly power and therefore do not effectively negotiate the prices.

Generally, trend of milk prices was increasing. However, the study revealed that the pattern of prices increases was not a reflection of bargaining power of farmers. Rather, there was an increase in number of processors on the market. In November, 2008, Lilongwe Dairies started purchasing fresh milk from MBGs. The new entrant increased number of processors, resulting into price increases from MK45 to MK65. The study further noted that mushrooming of MBGs was not a reflection of increased dairy farmers. It is the antagonistic competition among processors which facilitated the split of MBGs. For instance, Chandamale and Dwale were splits from Bvumbwe and Thunga respectively. Although it was a positive externality for bulking groups, it

did not translate to farmer ownership of bulking groups.

(b) Legal framework

One of the six pillars of Malawi government development agenda is agricultural production and food security (GOM, 2008). However, the contribution of livestock production to food security is too insignificant (Mtimuni, 2001), and hence Malawi may not be considered a livestock country. This partially explains the lack of legal framework to protect the interest of local small scale milk producers. In the circumstances, MBGs, Southern Region Milk Producers Association (SHMPA), Central Region Milk Producers Association (CREMPA), Mpoto Dairy Farmers Association (MDFA) and other stakeholders would take a central role in lobbying to the government for protection. Malawi government has proactively facilitated the setting and enforcing of minimum prices for tobacco and cotton. A similar approach would make a difference in dairy.

Break-even point in dairy enterprises

To understand the profit margins for the enterprises, a break-even point analysis was conducted. A break-even point is when gross income is equal to total costs. It assumes fixed costs are constant while variable costs vary at a constant rate (Total cost(TC) = variable + fixed costs) and there is only one selling price (Pm) is mathematically expressed as in equation 6, where Qm is the output, milk quantity.

$$Q_m = \frac{TC}{P_m} \dots\dots\dots(6)$$

Mean breakeven milk quantity of 1540 litres and 83 litres per lactation period in formal and informal sub-sectors was found, respectively. A two sample t-test of $Pr(|T| > |t|) = 0.0000$ was evidence that mean breakeven of the two categories were statistically significant. However, mean milk quantity delivery to MBGs of 8 litres per cow per day and 7 litres per cow per day with 7.7 and 7.3 standard deviations, were found for dairy enterprises owning purebred and crossbred cows, respectively. Mean lactation period of 224 days and 184 days for purebred and crossbred cows respectively were observed, which are lower than the recommended 305 days.

A cost analysis, as concluded in (Lockie et al., 2009) showed that at least 58% of administrative cost for Bvumbwe MBG was due to losses associated with power or ESCOM blackouts and 11% (Chandamale) 36% (Dwale) and 20% (Thunga) which reduced net profits significantly. In view of favourable return on investment(ROI) of over 50% and positive return on sales(ROS) (Bvumbwe 55%, 5.0; Chandamala 65%, 2.8; Dwale 103%, 3.8; Thunga 117%, 3.9). In such a scenario, prioritising investments in alternative power supply using retained earnings, in line with the pecking order theory of investment, would be a priority. On the contrary, bulking groups were waiting for the processors to help with such investments, and hence incurring avoidable losses in the long run.

Conclusion and Recommendations

This study concludes that dairy farmers could increase income through efficient use of recommended feeding practices and adequate farm labour, to increase milk production, and hence incomes, without necessarily increasing the number of cows. Although pure bred cows are

genetically superior and hence produce more milk than cross breeds, this study established that enterprises using crossbred cows had higher increasing returns to scale when compared to those using purebreds, largely because of low level of management at household level. Farmers have a monopoly power in the marketing of milk. However, dependency of farmers on processors for capital assets creates ineffectiveness of their monopoly power of marketing their product. MBGs need to use retained profits for capital expenditures, so as to re-gain the bargaining power. MBGs are encouraged to acquire their own capital assets like cooling equipment and stand-by generators to avoid being “owned” by processors and unnecessary loss of milk, respectively. Cost of milk losses due to blackout is directly shouldered by the individual farmer proportionate to the amount of milk delivered to bulking groups. This study recommends a policy strategy of intensifying efficient utilisation of already existing guides to successful dairy business, to improve technical efficiencies, as opposed to increasing number of cows owned.

It is recommended that to regain some bargaining power, a change in attitude towards self-reliance, is required for dairy enterprises management to use retained profits to acquire capital assets, as opposed to relying on processors. In addition the Malawi Government could proactively facilitate the setting and enforcing of minimum prices for the dairy sector similar to the approach in tobacco and cotton sectors.

Acknowledgements

Special gratification is extended to the dairy farmers for their time, and exceptional cooperation during the growing season of 2007/2008. We acknowledge the technical and financial support rendered by NORAD through ARDEP, Bunda College (University of Malawi).

Author Contributions

The study was conducted as part of an MSc dissertation, and a project. All authors were either part of the supervisory team, or project implementers, and hence contributed to the paper.

Conflict of Interest

The authors declare no conflict. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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Mass Fish kills in aquatic ecosystems: A review of the dynamics and their potential relevance for Lake Malawi

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Abstract

Sudden fish mortalities of varying magnitudes occur in both natural and man-made aquatic impoundments, often as a culmination of diverse interacting environmental and biological factors. Although there have been reports of unexplained dead fish of several genera in some parts of Lake Malawi recently, the exact causes of such fish kills have not yet been conclusively established. In this short review comprising over 60 studies from Google Scholar dealing with fish kills from across the world, we demonstrate that mass mortalities of fish populations may be driven by non-infectious pollutants like Lead, Cyanide salts or pesticides, fungal, viral or bacterial infectious agents as well as certain physical-chemical upheavals of the aquatic habitat. The role of enhanced primary productivity associated with increased nutrient enrichment is also discussed in the light of harmful algal blooms that potentially release ichthyotoxins into water bodies. Some fish from the recent Lake Malawi fish kill had haemorrhaging eyes and pelvic fins while close-to-dead fish swam erratically and were an easy catch by hand. Although some of these signs are seemingly consistent with pathogenic infections, this possibility is at best conjectural until confirmatory studies are conducted. Based on global experiences, the review highlights possible causes of fish kills in order to provoke interest in research towards understanding which factors are relevant for unexplained fish deaths in Lake Malawi. In the long run, sustained monitoring studies of this aquatic ecosystem may lead to a holistic and better understanding of the dynamics of its unusual fish mortalities.

Key words: Lake Malawi; Fish kill; pathogens; ichthyotoxins; environmental pollutants; upwelling

Introduction

Fish kills are the occurrence of sudden and unexpected mass mortality of wild or cultured fish at varying spatial scale and magnitude, sometimes involving thousands or millions of fish (Lugg and Fisheries, 2000, Stauffer et al., 2012). Mass fish kills are often a culmination of an interaction among diverse natural and anthropogenically-driven physical-chemical and biological factors of the fish's aquatic ecosystem as well as the biology of concerned fish species itself (Eissa et al., 2013, Stauffer et al., 2012). Such incidents may implicate a variety of environmental pollutants like Mercury, Cadmium, Lead, Cyanide salts or pesticides (Ullrich et al., 2001, Barber et al., 2003, Eissa et al., 2013), infectious agents including septicaemic bacterial (e.g. *Pseudomonas* spp.) and viral (e.g. Haemorrhagic Septicaemia virus) pathogens (Wakabayashi and Egusa, 1972, Nyman, 1986, Olson et al., 2013) as well as physical changes that upset the chemistry of the aquatic habitat to the detriment of the survival of the resident ichthyofauna (Swingle, 1968, Stauffer et al., 2012, Morgan, 1972, Hoyer et al., 2009). Some fish kills have involved species of high value sport and commercial natural fisheries (Meyers and Winton, 1995, King et al., 2001, Olson et al., 2013) as well as aquaculture, occasionally wiping out entire fishery stocks (Ogawa, 2002, Bertin et al., 2012). Certain toxins from algal blooms that are associated with mortalities of aquatic fauna may also cause mass poisoning and lead to acute illness and fatalities in other wild animals and livestock (Stewart et al., 2008) including sheep, pigs, horses, dogs, ducks and cattle (Nehring, 1993, Francis, 1878). Consumption of fisheries products dying from algal bloom poisoning may also lead to acute illness, hospitalisation and occasional death in humans (Teen et al., 2012). Mass mortalities of fish and fisheries resources therefore hold high ecological and socio-economic significance.

Although unexplained mass fish mortalities tend to be temporally and spatially restricted (Stauffer et al., 2012), their frequency, magnitude and range of environments in which they occur has tended to increase globally in recent decades (Stauffer et al., 2012, Olson et al., 2013). In the late 1960s, mass mortalities of *Tilapia* and *Barbus* species associated

with mud re-suspension and depleted oxygen occurred in Lake Chilwa, Malawi (Morgan, 1972) while in recent times two episodes (1999, 2013) of fish deaths have occurred in Lake Malawi, with no definite explanation of the factors driving these mortalities. In the Malawian summer of 2013, we visited and physically verified the occurrence of mass fish kills at Thumbi west, Cape Maclear, in the southern part of Lake Malawi, where this incident was verbally reported. In this short review, we summarise what is known about fish kills as documented in 85 studies of fish kills in other aquatic ecosystems from around the world and juxtapose this information with our observations of the recent (2013) fish kill event in Lake Malawi. We finally suggest potential research directions and urge for more monitoring studies in this lake for a better understanding of the dynamics underlying such occurrences in this water body.

Causes of mass Fish kills

Mass fish kills in aquatic ecosystems often result from the interplay of a diversity of both anthropogenically- and naturally-driven factors in the fish's habitat (Stauffer et al., 2012, Eissa et al., 2013). Among the main causes of fish kills are environmental pollutants (Barber et al., 2003, Eissa et al., 2013), infectious bacterial and viral agents (Foo et al., 1985, Olson et al., 2013) and physical-chemical changes of the aquatic habitat that compromise the survival of resident fishes (Swingle, 1968, Morgan, 1972, Monteiro et al., 2008, Stauffer et al., 2012). We used the phrase 'fish kill' in combination with words 'algal bloom', 'pollution', 'bacteria', 'fungi', 'viruses' and 'upwelling' to search for relevant literature on Google Scholar and identified 84 authorities applicable to this subject. Among these publications, physical factors were involved in 19 studies of fish kills, chemical pollution was implicated in 11 studies, fungal agents in 6 studies, bacterial pathogens in 8 studies, viral pathogens in 15 cases while 19 studies reported on the role of algal blooms and their associated ichthyotoxins in fish kills. We could not get any further new causes of fish kills beyond those reported in the cited works of this review. We briefly discuss how each of these factors, singly or in concert with other elements, drives fish mortalities before we comment on their potential

relevance to the Lake Malawi ecosystem's fish mortalities.

Pathogen-driven mass fish kills

Viruses have been known to underlie several cases of mass fish mortalities. Viral Haemorrhagic Septicaemia virus (VHSV) is one of the most serious fish pathogen and one of the most important finfish disease agent in the northern hemisphere (over 80 species affected), responsible for both marine and freshwater fish kills (Hedrick et al., 2003, Faisal et al., 2012). For instance, Viral Haemorrhagic Septicaemia (VHS) has been known to infect and to lead to massive fish kills of a diversity of fish in the Midwestern United States since 2005 including that of the yellow perch, *Perca flavescens* (Mitchill), a freshwater species of high sport and commercial fisheries value (Olson et al., 2013). VHSV particles can thrive for up to two weeks in water (Hawley and Garver, 2008).

These viruses are transmitted from place to place by boating, ballast water, fishing tackle and animals (e.g., crustaceans, leeches, and birds) mostly through fish waste, reproductive fluids and skin secretions (Faisal and Schulz, 2009, Faisal and Winters, 2011, Goodwin and Merry, 2011).

The VHSV pathogens have a wide host range and acute clinical signs, (Olson et al., 2013) including moderate-to-severe haemorrhages at the pelvic and pectoral fin bases as well as exophthalmia, erratic swimming, distended abdomens and extensive external/internal bleeding (Wolf, 1988, Winton and Einer-Jensen, 2002, Kim and Faisal, 2011). Although the VHSV reported to ravage both farmed and wild fish species in North America and Eurasia is of marine origin (Meyers and Winton, 1995, Raja-Halli et al., 2006, Lee et al., 2007), a new freshwater strain (VHSV-IVb) has recently been isolated from at least 31 fish species in North America where it has been associated with extensive fish kills (Grocock et al., 2007, Faisal et al., 2012, Olson et al., 2013). The occurrence of this new strain has been recorded in ever-increasing number of host environments (Olson et al., 2013).

Septicaemic bacterial pathogens like *Pseudomonas fluorescens*, *Aeromonas hydrophila*, and *Streptococcus iniae* are also associated with mass mortalities in wild aquatic environments (Wakabayashi et al., 1980, Berthe et al., 1995, Sahu et al., 2007). The biological invasion of pathogenic *P. fluorescens*, has been linked to mortalities of Tilapia and catfish in Egypt (Eissa et al., 2013). The involvement of fungal infections in fish deaths has likewise been reported in association with catfish, salmon and zebra fish (Ross and Yasutake, 1973, Bly et al., 1992, Khoo, 2000, Chao et al., 2010). Pathogenic *Candida albicans* has also been implicated in the mass mortalities of juvenile Nile Tilapia (*Oreochromis niloticus*) and sharp-toothed catfish (*Clarias gariepinus*) in Egypt (Czeczuga and Woronowicz, 1993, Eissa et al., 2013). The epizootic ulcerative syndrome caused by the oomycete *Aphanomyces invadans*, a serious fish pathogen in Japan, south-east Asia, Australia and the USA since the 1970s, was recently identified for the first time in the Zambezi River system where it devastated a variety of species populations (Andrew et al., 2008).

Mass fish kills underlain by toxic algal blooms

In some aquatic ecosystems, mass fish kills have been closely linked with hazardous compounds produced by certain strains of algae. Increased growth of harmful microalgal species consistent with algal blooms is a widespread natural phenomenon in both marine and freshwater environments (Burkholder et al., 1992, Teen et al., 2012).

Seasonal toxic algal blooms may wipe out entire fishery stocks in hatcheries and aquaculture facilities (Burkholder et al., 1992, Glibert et al., 2002, Bertin et al., 2012). Increased growth of *Microcystis*, *Anabaena*, *Trachelomonas* and *Gyrodinium* (*Gyrodinium*) that forms scums of these species on surface waters may be a primary cause of fish kills, particularly in ponds (Swingle, 1968, Padmavathi and Prasad, 2007). Blooms of microscopic flagellated algal species *Cochlodinium polykrikoides* and *Pfisteria* sp. or the haptophyte *Prymnesium parvum*, for instance, may produce ichthyotoxic compounds (Glasgow Jr et al., 1995, Miller and Belas, 2003, Mulholland et al., 2009, Stauffer et al., 2012).

Across the world, extensive growth of *P. parvum* has been reported to cause fish and aquatic animal kills (Edvardsen and Imai, 2006, Roelke et al., 2010). The suite of ichthyotoxic compounds produced by *P. parvum* includes primary fatty acid amides myristamide, palmitamide, stearamide, oleamide, elaidamide, linoleamide, erucamide and a hydroxamic acid, linoleylhydroxamic acid (Bertin et al., 2012). Although most of what is known about the effects of fatty acid amides is based on work in mammalian systems, the diverse biological impacts of these compounds suggest that their high concentrations during *P. parvum* blooms may have extremely deleterious effects on aquatic life (Bertin et al., 2012). Sleep-inducing Oleamide and Linoleamide lipids, for instance, have both been known to depress body temperature and locomotor activity in rats and humans (Huitron-Resendiz et al., 2001, Leggett et al., 2004). Some of the toxic fatty acid amides produced by *P. parvum*, inhibit the activity of important enzymes for fatty acid metabolism and this functional disruption may lead to several pathological states (Butovich and Reddy, 2002, Butovich and Lukyanova, 2008). Cyanobacterial blooms may also produce hepatotoxins and neurotoxins that potentially cause acute illness and fatalities in wild animals, livestock and humans (Nehring, 1993, Stewart et al., 2006, Stewart et al., 2008). Following an algal bloom of *Alexandrium minutum* and consumption of the contaminated clams in Malaysia and the Philippines, for instance, a number of people were hospitalised and some deaths were reported (Lim et al., 2004, Teen et al., 2012).

In recent times, incidences of toxic algal blooms are increasing across a number of geographical locations including North America, Africa, Europe, Asia and Australia (Bertin et al., 2012). Increasing utilization of coastal areas and associated human activities like industrialization, urbanization and commercial agriculture that enhance nutrient run-off and enrichment may lead to eutrophication of aquatic ecosystems conducive to occurrences of algal blooms (Teen et al., 2012). The effects of eutrophication may thus cascade through enhanced phytoplankton growth to result in toxic compounds that may synergistically combine to drive fish mortality events (Stauffer et al., 2013).

Fish kills driven by physical-chemical changes

a. Physical changes

Rapid and substantial reduction of dissolved oxygen in surface waters fuelled by an incursion of upwelled low-oxygen water from deeper layers may lead to distress and ultimately cause fish mortality events (Swingle, 1968, Stauffer et al., 2012). Depressed dissolved oxygen concentrations related to substantial organic load led to the death of 5000 fish belonging to 18 species in the Katherine River system in Australia (Townsend et al., 1992). Upwelling of deeper oxygen-deficient waters may be facilitated by cold air masses

and heavy winds (Swingle, 1968, Patterson et al., 2000). The 1966 mass mortalities of *Tilapia* and *Barbus* species in Lake Chilwa, Malawi, occurred following days of very strong “Mwera” winds which resuspended a 0.3m liquid mud layer from the lake bed, causing depletion of dissolved oxygen in the water column (Morgan, 1972). In Lake Victoria, over 400,000 fish weighing in excess of 2400 tonnes died in 1984 when violent storms resuspended nutrient-rich bottom mud (Ochumba, 1990). Similar overturns of mud leading to de-oxygenation have been the cause of *Tilapia* mortalities in a number of shallow tropical water bodies in Africa including Lake George (Uganda), the Nampongwe River (Zambia), Lake Victoria and Mweru-wa-Ntipa (Zambia) (Fish, 1956, Hickling, 1961, Tait, 1965, Bruton, 1985). Changes in upwelling regimes and processes can thus cause hypoxia-driven fish mortalities (Stauffer et al., 2012).

De-oxygenation of water driven by nocturnal respiration of algae and its decomposition during the day (related to phytoplankton blooms) may also result in extensive fish mortalities (Ruelo, 1976, McInnes and Quigg, 2010, Hecky et al., 1994, Mhlanga et al., 2006). Dense concentrations of algal growth may also absorb heat from sunlight and cause a sharp rise in temperature of surface waters, resulting in shallow stratification and lack of sufficient dissolved oxygen to support fish life in deeper waters (Swingle, 1968, Townsend et al., 1992). Such anoxic conditions at depth may result in fish kills (Townsend et al., 1992). Souring temperatures may also exceed tolerance limits of some species and lead to seasonally localized fish mortalities (Hoyer et al., 2009, Stauffer et al., 2012). The mass mortalities of *Tilapia* and *Barbus* in Lake Chilwa (Malawi) occurred during calm hot weather when the lake was at its lowest level for 17 years (Morgan, 1972).

b. Chemical pollution-driven fish mortalities

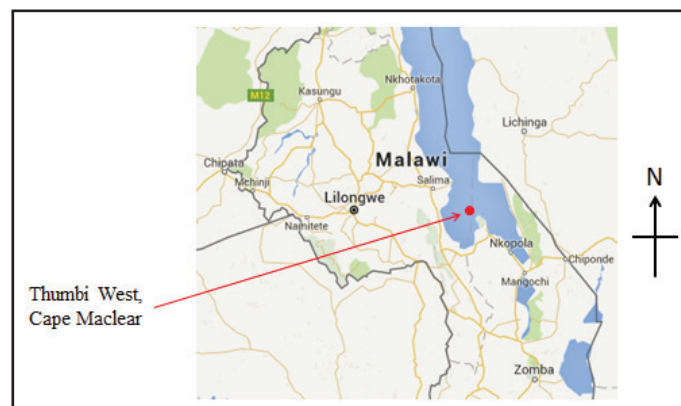
Non-infectious agents comprising a variety of environmental pollutants like Mercury, Cadmium, Lead, Cyanide salts and pesticides may also cause mass mortalities among wild fish populations (Ferrando et al., 1992, Barber et al., 2003, Kuivila and Jennings, 2007, Pinto et al., 2009). Such environmental pollutants may have direct damaging effects on gills, skin and fins and may result in the breach of the skin's ability to function as an immunological barrier, thereby leaving the fish prone to a combination of bacterial dermatropic toxins and secondary infection by pathogenic fungi (Eissa et al., 2013). Several mass fish deaths across the globe have been linked to chemical pollution (DouAbul et al., 1997, Abdelaziz and Zaki, 2010). The cause of mass fish kills of Nile tilapia (*Oreochromis niloticus*) and sharp toothed catfish (*Clarias gariepinus*) in water courses of the Mariotteya drainage in Egypt was found to be multifactorial but linked to the dumping of improperly treated organic and inorganic chemicals from factories and municipal sewage that led to increased levels of ammonia, phenol and polycyclic aromatic hydrocarbons (Abdelaziz and Zaki, 2010, Eissa et al., 2013), which potentially cause fish kills (Austin, 1998, Alam et al., 1998, Austin, 2007).

The recent Lake Malawi Fish Kills

In the summer of 2013, there were several reports of floating dead fish in some parts of the Shire River and Lake Malawi including the north as well as south eastern arm and the south western arm of the lake. Efforts to establish the exact causal agent of such a seemingly widespread fish kill have not yet yielded definite answers. Some preliminary

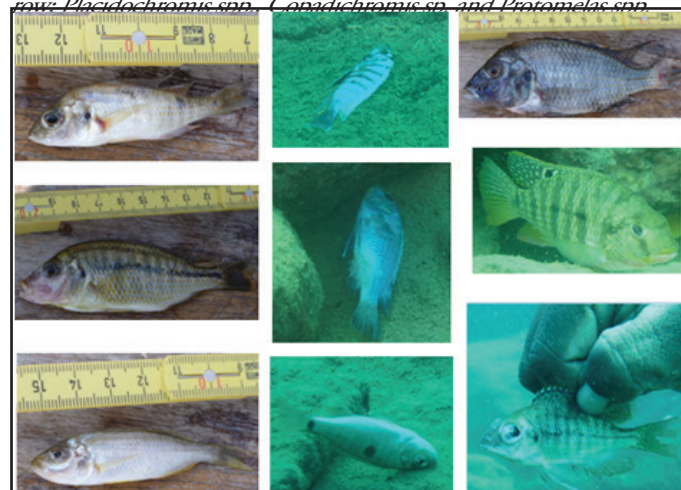
laboratory analyses of the dead fish samples from Lake Malawi have found no signs of chemical poisoning (Gumulira, pers. com). However, low oxygen concentrations were reportedly rampant in the surface waters at the sites of the kills, especially early in the morning (Ngochera, pers. com). In the lower Shire River, the re-suspension of bottom silt associated with dam operations upstream may have played a part as most dead fish had silted up gills (Ngochera, pers. com). We conducted a site visit for field observations in southern Lake Malawi at Thumbi West, Cape Maclear, to verify rumours about the incidence of fish kills in this water body (Figure 1).

Figure 1. Part of the map of Lake Malawi, showing the site at which some of the fish kills were observed in 2013 at Thumbi West, southern part of the lake.



We confirmed that some fish had died inexplicably at this site, including those of the genera *Nyassachromis*, *Protomelas*, *Otopharynx*, *Alticorpus*, *Placidochromis*, *Copadichromis*, *Tilapia* and *Maylandia*. Some of the symptoms observed on the dead fish included blooded eyes and blooded pelvic fins and disintegrating eyes (Figure 2).

*Figure 2. Some of the genera that died in the south western arm of Lake Malawi during the fish kills of 2013. From left to right, top row; *Otopharynx* spp., *Maylandia zebra*, *Alticorpus* spp., middle row; *Nyassachromis* spp., *Maylandia callainos*, *Tilapia rendalli*, and bottom row; *Placidochromis* spp., *Copadichromis* sp., and *Protomelas* spp.*



Other close-to-dead fish behaved erratically and swam daringly close to divers and were easily caught by hand. Other dead fish were clean of any obvious physical anomalies. A few of these clinical signs like exophthalmia (bulging eyes), haemorrhaging at the bases of the pelvic and pectoral fin bases, erratic swimming and external/internal bleeding are consistent with viral infections (Winton and Einer-Jensen, 2002, Olson et al., 2013). In recent times, some new strains of freshwater viruses have been recorded in an

ever-increasing number of host environments where they have caused extensive fresh water fish kills (Faisal et al., 2012, Grocock et al., 2007, Olson et al., 2013). In Malawi these viruses have not been recorded and their existence can only remain speculative.

There is a formidable relationship among wind patterns, upwelling and fish kills, with major fish kills sometimes occurring after strong and persistent winds (Marti-Cardona et al., 2008). It is interesting that Lake Malawi fish kills were mostly reported in summer, following extensive south easterly 'Mwera' winds (pers. observ.). Phytoplankton productivity seasonally increases on Lake Malawi around June–July in direct response to intensified lake mixing driven by physical climatic factors, notably temperature and wind regimes, that lead to higher available nutrient supplies (Twombly, 1983, Patterson, 1993, Irvine and Waya, 1999, Patterson et al., 2000). Localised algal blooms were sighted in some parts of Lake Malawi at the same time the fish kills were observed in this lake (Ngochera, pers. com.). Deteriorated limnological conditions, particularly depressed dissolved oxygen driven by high oxygen demand from a decomposing algal bloom die-off may lead to fish deaths (Mhlanga et al., 2006). Phytoplankton blooms may also produce and release into the water ichthyotoxic compounds that could trigger a fish kill (Mulholland et al., 2009, Stauffer et al., 2012). Another consequence of strong winds is that they may facilitate the upwelling of hypolimnetic water at the upwind end of a water body, potentially leading to the de-oxygenation of substantial portions of the water column and its enrichment with Hydrogen Sulphide and Ammonium (Marti-Cardona et al., 2008). Although a few fish species (e.g. *Hoplosternum littorale* or *Poecilia mexicana*) are adapted to surviving in water that is hypoxic, acidic and rich in Hydrogen Sulphide (Brauner et al., 1995, Tobler et al., 2006), most fishes and other aquatic organisms do not generally survive for long in environments that have high Hydrogen Sulphide concentration (Luther et al., 2004). An accumulation of toxic gases like hydrogen sulphide, methane and carbon dioxide may thus lead to fish kills (Bagarinao and Lantin-Olaguer, 1998, Luther et al., 2004). While the exact factors driving fish kills on Lake Malawi remain a mystery, we contend that sustained studies in areas that focus on fish pathogens, lake chemistry and environmental pollution, primary productivity and the determination of dominant algal strains, especially those that abound during upwelling seasons and phytoplankton blooms and the role of winds and other physical forces may be key to understanding the potential roles of the exact chemical, physical and biological factors that interact to culminate into fish kills on Lake Malawi.

Conclusion

This review has described the possible causes of fish kills based on published evidence from over 80 studies from across the globe. The nature of fish kills is potentially complex; multiple anthropogenically- and naturally-derived agents and processes may be involved. Some fish kills are underlain by environmental pollutants while others are triggered by infectious bacterial, fungal and viral agents. In some instances, drastic physical-chemical changes of the aquatic habitat may endanger the survival of resident fishes. In any particular context, the task of pinpointing the actual causative factors implicated is not an easy one. This is certainly the case with Lake Malawi where not much, if

any, detailed ecological or disease diagnostic studies of fish kills exist. This paucity of information for such a world-renowned water body as Lake Malawi should provoke some research towards understanding factors associated with unusual fish deaths in this water body. From the reviewed literature and personal communications on fish kills in this water body, it may be the case that opportunistic upsurge of disease pathogens, upwelling events and their effect on phytoplankton blooms and on Oxygen and temperature profiles in the lake may offer clues in this regard. In the long run, concerted monitoring studies of this aquatic ecosystem may lead to a better and holistic understanding of the dynamics of its mass fish mortalities.

Acknowledgements

We wish to acknowledge the support of Chancellor College that facilitated our field visit to the lake upon hearing reports of the fish kills. We would also like to acknowledge our fruitful exchanges of information with Maxon Ngochera of the Senga Bay Fisheries Research Station, Salima, and Innocent Gumulira of Monkey Bay fisheries research station regarding algal blooms in some parts of Lake Malawi at the time of the recent fish kill. Dickson Mazibuko, Wilbert Chitaukali and two anonymous reviewers offered their helpful insights and comments which greatly improved an earlier version of this manuscript.

Author contributions and conflicts of interest

Bosco Rusuwa conceived and drafted the manuscript, Jonas Mwatsetedza organised the field visit and helped in the drafting of the manuscript while Wisdom Changadeya participated in the field visit and reviewed the manuscript. The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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