Culturing African Lungfish (*Protopterus sp*) in Uganda: Prospects, Performance in tanks, potential pathogens, and toxicity of salt and formalin

by

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Abstract

Culturing species resilient to drought and stressful water quality conditions may be a significant part of the future of African aquaculture. Air breathing fishes potentially have a role in low-management culture systems for small farms because dissolved oxygen does not threaten the fish crop. The African lungfish (*Protopterus* sp) is advantageous because it is: an indigenous fish with good flesh quality, an air-breather, and a biocontrol agent against schistosome vector snails. Wild lungfish stocks are declining and national strategies to protect its natural population are lacking.

Lungfish is highly valued as food, has certain nutraceutical benefits and supports livelihoods of many communities in Uganda. A variety of lungfish products on markets include fried pieces (54%), cured/smoked fish (28%), whole fresh gutted fish (10%) and soup (8%). Lungfish products are increasingly found alongside tilapia and Nile perch in rural and urban markets with cured products being exported to Kenya, DRC and Southern Sudan. Its fingerlings are now sold as bait in the Nile perch fishery. Women not only consume lungfish but are actively engaged in its trade. However, some countervailing sociocultural beliefs continue to deter some fish consumers from eating lungfish.

Culture performance of wild caught African lungfish fingerlings (9.58 ─ 9.95g) fed at three commercial diets was evaluated. Experimental fish accepted exogenous sinking pellets but marginal increase in average body weight were observed. Mean (± SE) final weight (15.86 ± 0.80g) for fish fed the commercial diet-3 was significantly higher ($p < 0.05$) than fish which fed

on diet-1 and diet-2. Specific growth rates (SGR) for diet-2 (0.50 ± 0.06%/d) were significantly higher (p< 0.05) than diet-1 (0.27 ± 0.03%/d), and marginally more ($p < 0.05$) than diet-2 (0.37 ± 0.04%/d). Feed conversions were similar for fish fed diet-1, 2 and 3. Survivals after an 11-week culture period were relatively low (< 60%) but generally increased ($R^2 = 0.667$, P = 0.0071) with increasing dietary proteins. Diet-3 (57.50 ± 2.85%) had a significant higher survival rate (p < 0.05) than diet-1 (45.83 ± 3.44%) and diet-2 (40.84 ± 2.10%). All water quality parameters were within recommended aquaculture ranges. Poor growth and high mortalities experienced in this study may be due to i) sub-optimum dietary protein levels, ii) cannibalism, iii) disease infections, iii) density, iv) contaminants in the feed and, iv) wrong management protocols. Unless these factors are adequately addressed this fish is not a good aquaculture candidate. Additional studies will be needed to assess the culture potential of African lungfish due to high rates of mortality that occurred in the present study.

Common diseases encountered include; bacteria (*Aeromonas* sp., *Flavobacterium columnare* and *Pseudomonas sp.*), fungus (*Fusarium spp., Aspergilus sp and Saprolegnia sp)* and parasite (*Dactylogurus sp*, *Trichodina sp.*, *Tetrahymena sp*, *Heterorchis sp.* and Cestodes). However, about 60% fungal infections mostly occurred compared to monogenes (9%), tapeworms (25%) and bacteria (6%). Moribund fish were infected with fungal and bacterial infections in the liver, spleen, dermal layer and gastro-intestinal tract. Skin erosion and dermal mycosis were evident in most infected fish but with some indication of regeneration. African lungfish fingerlings (7.78 ± 1.47 g) appear to be sensitive to saline conditions having a LC50 of 2.59 and 1.84 for 24 and 96h, respectively. Lungfish behaves normally at low salt concentrations (0 ─ 1.6 g/L) but become lethargic within 4h when concentrations reach 4 g/L. Juveniles are tolerant to formalin having LC50 of 220.8 and 193.8 mg/L for 24 and 96h, respectively.

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Introduction and Literature Review

1.0.0 Introduction

Globally, rising food prices have shifted 44 million people into extreme poverty. Uganda has nine million people facing an acute food shortage with 38% of its children chronically malnourished in the rural areas (WFP 2009; FAO 2010, 2012; World Bank 2010; Shively and Hao 2012). Aquaculture is an alternative source for food security and livelihood improvement in the Sub-Saharan African region (Brummet and Williams 2000). Uganda's natural fisheries stocks are continuing to decline and aquaculture production (subsistence and commercial oriented) is less than 20% of total national production (Isyagi et al. 2009; FAO 2012).

Climate changes manifested in shifting rainfall and temperature regimes are bringing new challenges to management of aquatic systems that are important to aquaculture production (Milly et al. 2005; Hansen et al. 2006; Klein and Petschel-Held 2007; Daw et al. 2009; Brander 2010; Ramasamy 2011; Coumou et al. 2013). Hence, culturing fish species that are tolerant to drought and stressful water quality conditions would be a significant future for African aquaculture development (Allison et al. 2007; Daw et al. 2009; Wagle et al. 2011; Williams and Rota 2011). Air breathing fishes would be suitable candidates in stressful conditions because of their ability to obtain and utilize atmospheric oxygen to meet all or part of their metabolic demands (Myers 1986; Pethiyagoda 1991; Graham 1997; Thomson 2013).

Air breathing fishes like snakehead (*Channa striatus*) and lungfish (*Protopterus sp*. or *Neoceratodus sp.*) are potentially more productive in aquaculture systems because dissolved oxygen is not a limiting factor. In Uganda the air-breathing Clarias catfish is very popular fish species that can also tolerate low dissolved oxygen concentrations but products usually from small-scale farmers are of low quality (Ssebisubi et al. 2012).

African lungfish (*Protopterus aethiopicus and Protopterus amphibious*) culture would be advantageous since; i) it is indigenous to Ugandan waters (Greenwood 1958, 1986), ii) it has good quality flesh, iii) it is a good bio-control agent for schistosome vector snails (Daffalla et al. 1985), and, iv) it is highly valued, acceptable, demanded, and v) communities are deriving livelihoods and healthy proteins from this fish (Walakira et al. 2012).

Wild stocks of African lungfish in Uganda are rapidly declining mainly due to overexploitation, environmental degradation and large-scale conversion of wetlands to agricultural land (Goudswaard et al. 2002; Balirwa et al. 2003). However, Uganda lacks i) technologies to domesticate this fish and, ii) policy guidelines to protect this fish in the wild environment. Initiatives to culture lungfish involve collection of wild nestlings that are raised in earthen ponds. This practice is not sustainable, environmentally and economically and yields are usually low (Walakira et al. 2012).

Understanding the indigenous and scientific culture practices of African lungfish present opportunities to domesticate this fish in the region. Anecdotal evidence reveals how farmers gather wild nestlings of lungfish and stock small water bodies but documentation on management practices and yields to substantiate these findings is not available (Walakira et al. 2012). Literature on African lungfish mainly examines its ecology, fishery, biology, physiology but little is reported on diseases and its use as food fish in aquaculture (Greenwood 1958, 1986; Baer et al. 1992; Mlewa and Green 2004).

Attempts in Kenya to grow wild-caught marble lungfish (*P. aethiopicus*) in earthen ponds encountered low survival rates as most fish burrowed through pond-banks and disappeared. In addition, there were indications of earlier maturation of cultured fish compared to wild populations (Mlewa et al. 2009). Lungfish can breed naturally in ponds in presence of water hyacinth (*Eichornia crassipes*) plants, providing a simple technology for propagating this species (Joss and Joss 1995).

Efforts to culture lungfish have demonstrated how this fish can be domesticated but more research is needed to develop propagation technologies while increasing its productivity and profitability. Furthermore, culture techniques should also address handling and harvesting issues associated with lungfish which have sharp plate-like teeth that snaps if disturbed. This fish can burrow through pond-banks leading to loss of stocked fish, hence, the need to use other production systems (e.g cages and tanks).

1.1.0 Problem Statement

A substantial population vulnerable to climate change effects is facing an acute food shortage in Uganda. Aquaculture can improve food security and livelihoods at the household level but is challenged with water quality issues caused by environmental changes. Hence, we need favorable aquaculture species that are resilient to stressful water quality conditions, preferably, air-breathing fish (e.g African lungfish). However, African lungfish natural stocks in Uganda are declining with no clear national policies to protect it. Successful attempts to raise wild-caught African lungfish in earthen ponds usually produce low yields. This is mainly attributed to its burrowing habits through pond banks and cannibalism but these facts have not been proven, scientifically.

Therefore, the problem is as if, i) information to grow African lungfish as an alternative aquaculture species in the sub-Saharan African region is non-existent, ii) exogenous feeds to raise this fish in captivity is a challenge, iii) low pond yields are caused by cannibalism, escape tendencies and diseases, and iv) information on effects of salt and formalin as future prophylaxis agents is non-existent.

1.2.0 Objective of the study

The overall objective is to generate appropriate aquaculture technologies and information for culturing African lungfish as an alternative livelihood source for fish farmers. This will also directly contribute to food security for those vulnerable communities in Uganda. This was first achieved through field interviews to determine prospects of culturing African lungfish in Uganda which was consequently developed for public consumption. Subsequently, on-station experiments were conducted on wild-caught African lungfish fingerlings to determine performance in tanks when fed on commercially available artificial fish-feed. Information produced would eventually facilitate the propagation and promotion of African lungfish in Uganda and entire region.

1.2.1 Supporting objectives

The study's specific objectives were to;

1. Assess the prospects and potential of the African Lungfish (*Protopterus sp*) in Uganda.
2. Determine the performance of African lungfish fingerlings fed on three commercial diets.
3. a. Assess fish pathogens associated with culturing African lungfish.

   b. Evaluate the effects formalin and salt doses exposures to African lungfish juveniles in aquaria.

1.2.2 Research questions

1) <u>Potential and prospects.</u> What is/are a) the status of fish farming of African lungfish, b) the indigenous knowledge and practices associated with culturing this fish, and c) socio-

economic conditions shaping the culture of African Lungfish including prices, demand, and public perceptions, in Uganda?

2) Artificial diets. Which commercial feed diets produce significant growth, survival and food conversion ratios when raising this fish in tanks?

3) a) Diseases. What are the common fish diseases encountered when culturing African lungfish under experimental conditions?

   b) Prevention strategies: Can we use the available chemotheraputants (formalin and salt) as prophylactic agents against disease outbreaks to improve productivity? What is the standard measure of toxicity (lethal dose-50) of salt and formalin that kills half of fish population?

1.3.0 Significance of the study

This is the first study conducted in Uganda to evaluate the culture of wild-caught African lungfish fingerlings using available production technologies adaptable to fish farmers. Results will enhance the knowledge base on water resource use that work best for African lungfish culture. The study identifies best management practices (BMPs) and enterprise development aspects which contribute to Uganda's aquaculture development, and the sub-Saharan African region. These technologies will positively impact on the national food security and livelihoods of those dependent on this fish. Diversification of culture species in Uganda is an added advantage to producers and fish feed manufactures. Pressure on lungfish's natural fish stocks is anticipated to be reduced thereby maintaining or enhancing threatened wetland biodiversity. Researchers and planners will have a basis on formulating policy guidelines and to develop conservation programs that ensure protection of this declining fish.

2.0.0 Literature Review

2.1.0 Fisheries

World Fish production (capture fisheries and aquaculture[1]) in 2010 was 148 million tons valued at US$ 217.5 billion, growing at 3.2% per year (1961 ─ 2009) and overtaking the global population annual rate of 1.7 % (FAO 2012). Capture fisheries and aquaculture contribute 68% and 32% of world production, respectively, while China alone contributes 35% (Brander 2007; FAO 2012). Fisheries sector augments economic development in the sub-Saharan African region through foreign exchange revenue from regional export markets (Ponte et al. 2007).

Capture fisheries production remains stable at 90 million tons, and inland fish catches increased by 2.6 million tons in the period 2004 - 2010 (FAO 2012). Asia is the largest inland fisheries producers (≈ 70%) while the Great Lakes (Uganda and United Republic of Tanzania), Nigeria and Egypt dominate fish production in Africa (FAO 2012). The increasing global demand for fish, while wild populations are plummeting, calls for innovative solutions to increase fish production and this can be through aquaculture production (Brunner et al. 2009; Muir 2013).

About 85% of global fish production is utilized for food consumption and world per capita food fish supply increased from 9.9 kg (live weight equivalent) in the 1960s to 18.6 kg in 2010 (FAO 2012). Fish is an important source of protein that contributes 42% of animal protein intake for 2.6 billion people, and considered a human health asset in developing countries (Brunner et al. 2009; FAO 2012). However, fish prices are increasing as demand increases,

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[1] *Aquaculture* is the farming of aquatic organisms that include fish, mollusks, crustaceans and aquatic plants, with interventions (e.g stocking, feeding) to enhance production, and these farmed aquatic organisms are either owned individually or by a corporate body during the culture period. Hence, aquatic organisms that are harvested by an individual or corporate body contribute to aquaculture and those exploitable by public as common resource are harvests of fisheries (FAO 1997, 1999).

limiting poorer people to access wild stocks and what is available in markets (Muir 2013). Promoting direct consumption of small pelagic fish may ensure global food security but this fish species is important in animal feed production (Tacon and Metian 2013). About 54.8 million people derive a livelihood from this sector, employing 4.2% of the 1.3 billion people engaged in broad agriculture (FAO 2012).

By 2004, 70% of world fish stocks were either exploited, over-exploited or under restoration (FAO 2004; Brander 2007). Inland waters are the most over-fished resources due to anthropogenic factors like population pressure and environmental degradation (FAO 2012). The International Union for Conservation of Nature (IUCN) 2008 Red List data revealed freshwater species as being under threat; Europe (50%), Mediterranean and Madagascar (40%), East Africa (30%) and 20% in Southern Africa (2010).

2.2.0 Global Aquaculture

Aquaculture is the fastest growing sub-sector with average annual growth rate of 8.9% compared to 1.2% and 2.8% for capture fisheries and terrestrially farmed meat-production, respectively (Brink 2001). Aquaculture production increased 12 times during the period 1980 to 2010, and currently produces 79 million tones (including aquatic plants and non-food products) valued at US $ 125 billion (FAO 2012). Brander (2007) estimates that by 2030, aquaculture production will equate that of capture fisheries. This is only possible if dependence on fish-based feeds is reduced and culturing fish species that feed low on the aquatic food chain are promoted (Muir 2013).

Aquaculture is practiced intensively and extensively in 190 countries with 57% (i.e 33.7 million tons) of global production coming from fresh water fishes (FAO 2012). Land-based culture systems (e.g earthen ponds) are most common and are easily integrated in existing broad agriculture farming systems. Other aquaculture facilities in production include cages and tanks that are designed, technologically, to suit a particular environment (e.g marine or freshwaters).

Aquaculture accounts for over one-quarter of fish for human consumption (Naylor et al. 2000) and 30% of world population is engaged in aquaculture production (FAO 2012). It enhances livelihoods of rural poor, especially in Asia and Africa, where rural development programs focus on alleviating poverty and under-nutrition (Hishamunda and Ridler 2006; Edward 2009). Furthermore, aquaculture can reduce pressure on wild fish stocks if its production is sustainably increased to meet the current demand (Naylor et al. 2000). Increasing farmed fish on markets will directly compete with fish from the wild, thus reducing fish prices and gradually fishing efforts (Naylor et al. 2010). However, aquaculture is also constrained by disease outbreaks that continue to impact on profits by creating losses to fish farms, and directly or indirectly threaten natural fish populations (FAO 2012).

Aquaculture is instrumental in fish stock enhancement programs (i.e. fish conservation initiatives) and strategies are available to improve its efficiency (Brown and Day 2002; Collares-Pereira and Cowx 2004). Consequently, an attempt to domesticate endangered or declining native fish species has been successful and wild fish stocks are protected. For example, culturing large hybrid catfish is anticipated to protect the endangered giant catfish of Mekong River (Bartley et al. 2000). Furthermore, protecting declining or threatened fish populations can also be achieved when public-private partnership is emphasized (Collares-Pereira and Cowx 2004).

About 600 aquatic species are farm-raised with majority cultured in freshwater environments (FAO 2012). Common aquaculture freshwater species include tilapia (*Oreochromis spp.*), catfish (*Clarias spp.)* and dominated by carps that comprise 72% of total farmed fish (FAO 2012). Criteria for selecting aquaculture species favor those that are;

acceptable to consumers, fast growing on low-cost feed, easily bred in captivity, resilient to culture conditions, and compatible to production systems (Webber and Riordan 1975). Most farmed fish are omnivorous (e.g African catfish) but carnivorous species (2.6% of fresh water species), for example snakehead (*Channa striata*) and striped bass (*Morone saxatilis*) (FAO 2012) have also been domesticated. Reliance on exotic species in aquaculture development impacts negatively on indigenous biodiversity especially developing countries (Brummett 2007; Silva et al. 2009). Hence, use of native species in developing aquaculture in Africa would be more sustainable and economical.

Air-breathing[2] fish species have attracted the attention of aquaculturists and researchers with six genera of fish (i.e. *Clarias, Channa*, *Heteropneustes*, *Pangasius* and *Dormitator*) globally cultured (Graham 1997) under research. The air-breathing feral catfish (*Heterobrachus bidorsalis*) can survive and grow well at high densities because of their ability to utilize low dissolved oxygen in culture systems (Fagbenro et al. 1993). The Indian air-breathing teleost, singhi catfish (*Heteropneustes fossilis*) is a suitable aquaculture candidate since it has capacity to tolerate high ammonia and low oxygen concentrations (Saha and Ratha 1998). Threatened *Clarias batrachus* and *Parachanna obscura* are promoted for income, food and conservation purposes because of good growth and efficient food conversion rates (Argungu 2013; Kpogue et al. 2013). It is also profitable to culture Pirarucu (*Arapaima gigas)* in cages than using traditional management practices (de Oliveira et al. 2012). These examples demonstrate how air-breathing fishes have a potential in aquaculture development.

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[2] *Air-breathing* fish directly exchange gas with the aerial environment for respiration purposes; an adaptive response when the fish is exposed to areas with little or depleted dissolved oxygen (Graham 1997).

2.2.1 Aquaculture in Africa

Africa has low-income food-deficit countries and remains the lowest fish consumer in the world. About 9 million tons of fish (i.e. 9.1 kg per capita) were consumed in 2009 compared to 85.4 million tons (i.e. 20.4 kg per capita) consumed in Asia but this gap is narrowing (FAO 2012). Endowed with a rich biodiversity of native fishes and water resources, Africa has a potential to increase fish productivity to solve its food deficit and poverty challenges (Jamu and Ayinla 2003). About 37─43% of land surface in Africa is suitable for small-scale and commercial aquaculture (Aguilar-Manjarrez and Nath 1998). Thirty-five percent of Africa's fish need in 2010 could have been supplied by 0.5% of 9.2 million Km^2 of land that is identified to be potentially productive for aquaculture (Kapetsky 1994, 1995).

Aquaculture has potential to ensure food security and livelihoods for the rural poor in Sub-Saharan region (Brummet et al. 2008). About 200,000 people are directly engaged in fish farming (Béné and Heck 2005; FAO 2012). Currently, Nigeria, Uganda, Kenya, Zambia and Ghana have tremendously improved their aquaculture production in the region through adoption of appropriate innovative technologies (e.g. feed-based aquaculture) and Egypt continues to lead aquaculture production (FAO 2102).

Species commonly grown in Africa include Tilapia (*Oreochromis spp*.), African catfish (*Clarias gariepinus*) and carps but efforts are now focusing on domesticating threatened and high value indigenous species like Nile Perch (*Lates niloticus*), *Labeo victorianus* and *Barbus altianalis*). Management systems include monoculture (tilapia or African catfish), poly-culture (mainly tilapia and catfish) and integrated models (e.g. crop-fish-livestock) that are subsistence in nature (Dadzie 1992).

Aquaculture in Africa is largely small-scale subsistence oriented but commercial enterprises producing significant volumes of farmed fish are emerging (Brummet and Williams 2000; Brummet et al. 2008; Brummett 2011). However, FAO Limbé statement concludes that aquaculture development in sub-Saharan Africa (SSA) is at crossroads (Moehl et al. 2005). For the last decades aquaculture development has generated marginal contributions to economic development and food security in Africa (Brummett and Williams 2000). Strategies to spearhead aquaculture in Africa through group fish farming also face economic and sociological challenges (Molnar et al. 1985).

Therefore, enhancing a profitable aquaculture industry in Africa calls for inclusive polices and infrastructure development (i.e industrial) that protects vulnerable groups while promoting resilience to climate change adaptation (Hishamunda et al. 2002; Hishamunda and Ridler 2006; Page 2012; Muir 2013). Furthermore, efforts to alleviate poverty and food insecurity through aquaculture should not compromise Africa's natural biodiversity and fish genetic resources (Lind et al. 2012).

2.2.2 Fisheries and Aquaculture in Uganda

Globally, rising food prices have shifted 44 million people into extreme poverty while Uganda has nine million people facing an acute food shortage (World Bank 2010). More than 1.2 million people depend on fisheries as a source of income and livelihood (OPM-PEAP 2008). Fisheries resources in Uganda continue to decline and aquaculture is producing less than 20% of total national production since 1940s (Dadzie 1992; Isyagi et al. 2009; FAO 2012). Current national fisheries production is 475, 000 tones (capture and aquaculture) but with a potential to produce 800,000 t if fish farming can produce at least 300,000 tons more, annually (MAAIF 2011). The fisheries sector contributed 3.1% (U Shs 1,201 billion) to Gross Domestic Product

(GDP) in 2011 (MAAIF 2012). Uganda produced 95,000 tons of farmed fish in 2010 mainly from African catfish (*C. gariepinus*) which has gradually replaced farmed tilapia (FAO 2012), and can increase through development pathways, especially in densely populated areas with a low-wage-rate labor (Jagger and Pender 2001).

Aquaculture started in 1941 with introduction of exotic carps but the subsequent establishment of Kajjansi Fish Experimental Station in 1947 was a milestone in Uganda history. Support from FAO stimulated subsistence fish farming during 1959 ─1968 period using 11,000 fish ponds. In 1999, about 4500 functional ponds produced 285 tons and by 2003 efforts from Government of Uganda (GoU) and development partners helped to increase yields to 15,000 tons produced from 20,000 fish ponds, averaging 500 m^2 (Mwanja 2007). Land-based culture systems (i.e pond and tanks) dominate aquaculture facilities but cage farming is gradually increasing. Recently, more land has been identified to be suitable for aquaculture development using GIS technologies (Ferreira et al. 2012). The north-eastern part of Uganda and shores of Lakes Victoria and Kyoga are 98% suitable for fish farming compared to other regions (Ssegane et al. 2012).

Eight species are cultured in Uganda; Nile tilapia (*O. niloticus*), African catfish (*C. gariepinus*), Mbiru (*O. leucostictus*), Zilli tilapia (*Tilapia zilli*), common carp (*Cyprinus carpio*), Ningu (*L. victorionus*), freshwater prawn (*Macrobrachium rosenbergii*) and crocodiles (*Crocodylus niloticus*). Nile tilapia is more popular than carps and is mostly poly-cultured with African catfish which is added to; i) increase productivity, ii) control over-crowding and iii) reduce stuntedness in ponds (De Graaf et al. 2005; Saiti et al. 2007). Exotic carps, *T. zilli* and *T. rendalli* are not commonly cultured in Uganda since they are less known or acceptable to local consumers.

Ugandan scientists are now spearheading research to domesticate high value indigenous species and those under threat. Technologies to propagate the endangered ‘Ningu’ (*L. victorionus*) are now available at the National Fisheries Resources Research Institute (NaFIRRI), and farmers can access the seed. Domestication of Nile perch *(L. niloticus)* is on-going at NaFIRRI but undocumented information indicates some farmers have successfully raised wild-caught fingerlings to market size. Attempts to culture other indigenous fish species under threat of extinction include ‘Ssemutundu’ (*Bagrus docmac*), ‘Kisinja’ (*Barbus altianalis*) and now, African lungfish (*P. aethiopicus*) (Walakira et al. 2012).

Aquaculture in Uganda is largely small-scale, marginally profitable, and its development challenged with; expensive feeds, quality fish seed, credit accessibility and predators such as otters (Hyuha et al. 2011). Low aquaculture productivity is also caused by weaknesses in input supply and delivery (Timmers 2012). Most farmed fish products are either sold locally in restaurants, on-site (fish farms) or regionally to neighboring countries like Rwanda, Democratic Republic of Congo (DRC) and Kenya. Unfortunately, most commercial aquaculture establishments may not access international markets since Uganda’s National Food Control System does not meet international requirements (Bagumire et al. 2009). Furthermore, most aquaculture operations in the country do not use formulated manufactured feed which raises food safety concerns (Bagumire et al. 2009).

Fish diseases are emerging concerns for aquaculture development in the East African region, hence emphasis is placed on strengthening the aquatic biosecurity at all levels (Akoll and Mwanja 2012). Intensive aquaculture of *O. niloticus* and *C. gariepinus* raised in earthen ponds or cages is reported to be vulnerable to parasite infestations (Akoll et al. 2012; Akoll, Konecny et al. 2012). Furthermore, threats from climate-change effects may augment disease outbreaks which will undermine productivity and development aquaculture in the SSA region (Timmers 2012).

2.3.0 Climate change

Defined as change of climate attributed to anthropogenic activities that alter composition of global atmosphere and the natural variability observed over comparable period of time (UN 1994). Notable variables of climate change include high global temperatures, changes in rainfall patterns, decreasing snow cover, and extreme weather patterns like El Niño and prolonged droughts. Climate change phenomenon and its impact on global atmosphere has attracted global discussions in various forums that focus on future challenges on earth. For example, Thomas et al. (2004) postulates that 15 ─ 37% of terrestrial species studied will be 'committed to extinction'. While, Xenopoulos et al. (2005) developed a global hydrological model that portray 75% of local fish biodiversity in rivers with reduced discharge being under threat of extinction due to combination of climate changes and water consumption. Hence, reduction of greenhouse emissions and carbon sequestration strategies should be implemented to prevent future calamities caused by climate change.

The average global surface temperature has increased by 0.6 ± 0.2 C since the 1860s and increased by about 0.2 C per decade in the past 30 years (Houghton et al. 2001; Hansen et al. 2006). Elevated temperatures have led to global warming mainly through greenhouse emissions, and land surface temperatures increased more than sea surface during 1950 to 1993 (Houghton et al. 2001). As global warming exceeds 1 C relative to year-2000 then the earth will experience "dangerous" climate changes characterized by extinction of species (Hansen et al. 2006). Currently, extreme monthly temperatures are five times more than expected and global monthly heat records are predicted to be over 12 times high by 2040s (Coumou et al. 2013).

Changes in global climatic patterns will directly influence mean run-off, ground water recharge and floods (Milly et al. 2005; Klein and Petschel-Held 2007; Ramasamy 2011). Freshwater resources are also under pressure were the ratio of water use to accessible supply increased by 20% per decade (Millennium Ecosystem Assessment 2005; Ramasamy 2011). Consequently, a majority of the world population is experiencing water scarcity and demand for water has offset the greenhouse warming (Vörösmarty et al. 2000). Hence, over three billion people may experience water stress conditions by 2025 and 1.8 billion people are likely to reside in water–scarce environments by 2080 (UNDP 2006, 2008; Ramasamy 2011).

Recently, parts of Africa and Asia have been characterized by severe and frequent drought periods that is evidenced by the global drought severity index (DSI) evaluated in 2000 ─ 2011 (Mu et al. 2013). Subsequently, availability of water for agricultural activities, a major livelihood for developing nations, is under threat. By 2050, rainfall in the SSA region may drop by 10%, reducing drainage by 17% in areas receiving 500 to 600 mm year $^{-1}$, thus depending on a region's precipitation surface drainage, will drop by 30 ─ 50% (De Wit and Stankiewicz 2006). Furthermore, 30 ─ 90% of world's wetlands have been destroyed and these are important for providing i) habitat to world's biodiversity, ii) livelihoods, iii) buffering the hydrological cycle and iv) reducing organic carbon (Junk et al. 2013).

Climate is a critical factor affecting food security in Africa; therefore, adverse changes in climate will negatively impact on food production systems (Gregory et al. 2005; Verdin et al. 2005; Challinor et al. 2007). Surprisingly, regardless of being the least contributor to global accumulation of greenhouse gas emissions, the SSA region is more vulnerable to impacts of climate change than any other region of the world (Kula et al. 2013).

2.3.1 Climate change: Fisheries

Fisheries (capture and aquaculture) production in tropical areas is vulnerable to climate change effects, mainly high temperatures and inadequate rain supply (Change 2001; Ramasamy 2011; Chessman 2013). Consequently, communities reliant on fisheries for livelihoods will become less stable when fish supply decreases. Brander (2007) attributes to changes in fish species distribution and productivity to climate variability (e.g. El Niño-Southern Oscillation). Fish losses are experienced in fresh water ecosystems located in poor developing countries (Xenopoulos et al. 2005) therefore sustainable use of fisheries resources can mitigate this problem.

Fish production in high altitudes may increase when ice cover decreases but production can also decline when reduced nutrient cycling emanates from reduced vertical mixing of the water strata (Brander 2007). Increased fishing efforts coupled with climatic changes (i.e. precipitation) have resulted into changes in fish demography, distribution and biodiversity in inland aquatic ecosystems (Brander 2007).

Anthropogenic factors like over-fishing, land reclamation and pollution, continue to threaten wetlands that are important for fisheries (Mitchell 2012; Junk et al. 2013). For example, the fishery of Logone Valley (a tributary of Lake Chad) was reduced by 90% when dam constructions blocked drainage into the surrounding wetlands (Odada et al. 2006; Mitchell 2012). Fishing operations also contribute to greenhouse gas emissions, estimated at 40 – 130Tg CO_2, when boats and trawls burn fossil fuels (Daw et al. 2009).

Natural recruitment of exploited fish stocks is challenged by climatic change (Brander 2007) and low water discharge in tropical or sub-tropical fresh water ecosystems has exposed endemic fish to extinction risks, prompting governments to reduce on water consumption

(Xenopoulos et al. 2005). However, there is still little evidence to relate climate change to reduction in natural fish stocks. According to Marshall (2012), decrease in fish catches from Lake Kariba is caused by impact of fishing effort other than changes within the lake ecosystem. Conversely, long-term periodic droughts are reported to maintain the diversity of freshwater ecosystems (Everard 2010).

Climate change directly affects fish physiology, behavior, growth, development, natural recruitment, mortality and distribution, while indirectly changing the productivity, structure and composition of the aquatic ecosystem (Brander 2007). Evidently, physiological responses (i.e reduction in fitness) to climatic irregularities (i.e temperature changes) have been observed in marine populations and were tolerant species are predicted to morphologically respond to these changes (Bartolini et al. 2013). Changes in water ecosystem may also result in stressful conditions leading to disease outbreaks. Mass mortalities of aquatic species, like the Eastern oysters (*Crassostrea virginica*) are linked to climatic change despite inadequate data to substantiate this fact. Kundzewicz et al. (2007) predicts an increase in aquatic pathogen load in SSA areas receiving heavy precipitation events and with poor sanitation infrastructure.

Climate change increases poverty, droughts, degradation of environmental and natural resources (Dixon et al. 2003). Consequently, the SSA region may face increased economic hardship since fish provides 27% dietary protein besides the income it generates for vulnerable communities (Allison et al. 2009). Uganda, Democratic Republic of Congo (DRC) and Tanzania are some countries whose national economies are highly susceptible to climate-change impact (Allison et al. 2009). Episodes of water loss in Lake Chad and its surrounding during extreme hot conditions is a clear indication of climate change on fisheries resources (Coe and Foley 2001). Likewise, Regina Ndebele-Murisa et al. (2012) reveals a decline of Kapenta fish production from Lake Kariba when temperatures rose by 3.17 ─ 3.42 C and rainfall decrease by 27.46%.

Appropriate scientific or aquaculture innovations will enhance and ensure sustainable fish production in this climate change era (Cochrane et al. 2009; De Silva and Soto 2009). Additionally, fisheries policies on resource management should embrace a balanced welfare and wealth-based approach that attracts political support and strengthens provision of services to fisheries communities (Nunan 2013). Conserving or protecting the age and geographic structure of resilient fish populations that are at risk of collapse is important instead of implementing only management approaches (Brander 2007; Merino et al. 2012).

Climate change affects aquaculture production when farmed fish are stressed by; prolonged high temperatures, low dissolved oxygen concentrations, erratic and unreliable rain supply, increased aquatic disease episodes, high incidence of toxic substances, limited supply of fish meal from capture fisheries, and extreme weather events (Brander 2007; Ficke et al. 2007; Timmers 2012). Furthermore, increased demand for fish oil from wild caught fish contributes to decline of natural fish stocks (Merino et al. 2012). Nevertheless, aquacultural returns are improved when temperature increases; that is through increased growth rates and food conversion efficiency (Handisyde 2006; Brander 2007). Other potential impacts from climate change on aquaculture include *i)* loss of fish resulting from frequent intensive storms that damage culture facilities, eventually introducing diseases and predators, *ii)* prolonged droughts that cause poor water quality conditions, *iii)* water stress resulting from prolonged evaporation rates and decreased precipitation, *iv)* water conflicts resulting from reduced natural water supply, and *v)* limited fish production and increased cost of production per unit area (Handisyde 2006).

Culturing fish species resilient to prolonged drought and water stressed conditions may be a significant future of African aquaculture. Air breathing fishes such as Marbled African Lungfish (*P. aethiopicus*) will be suitable candidates since they can obtain and utilize atmospheric oxygen to meet all or part of their metabolic demands. Lungfish (*Protopterus spp*.) have evolutionary survived through prolonged drought seasons by digging burrows and aestivate in prevailing stressful conditions for months or a few years (Ilves and Randall 2007; Otero 2011). Air breathing fish have an advantage of utilizing atmospheric oxygen when water ecosystems are deprived of dissolved oxygen (< 1 mg/L). Hence, lungfish are potentially more productive in culture systems because dissolved oxygen is not a limiting factor.

2.4.0 Biology of African Lungfish

Lungfish are members of the lobe finned fishes together with coelacanths[3]. African lungfish is an extant dipnoan ("double-breathing") organism belonging to class Sarcopterygii, and is endemic to freshwater habitats of Africa (Helfman et al. 2009). In nature, aestivating lungfish remain buried in cocoons relying solely on atmospheric air to survive during drought periods lasting several months.

Lungfish are locally important food fishes exploited from lakes, wetlands and reservoirs using a variety of gear including gillnets, baskets and long lines. Africa has four species and seven sub-species of *Protopterus*: *P. annectens* (Owen 1839), *P. amphibious* (Peters 1844), *P. aethiopicus* (Heckel 1851), and *P. dolloi* (Boulenger 1900). Poll (1961) lists the sub-species as *P. aethiopicus (P. a. aethiopicus, P. a. congicus, P. a. mesmaekersi)*, *P. annectens* (*P. a. annectens, P. a. brieni*), *P.dolloi and P.amphibius*. These are widely distributed in lentic (static),

[3] Order: Ceratodontiformes, Australian, S. American and African species Family: Protopteridae; Genus: Protopterus; Species: *P. aethiopicus (P. a. aethiopicus, P. a. congicus, P. a. mesmaekersi), P. annectens (P. a. annectens, P. a. brieni), P.dolloi and P.amphibius*.

lotic (flowing) and swampy aquatic environments (Garner et al. 2006; Helfman et al. 2009; Greenwood 1958, 1986, 1996) as shown in figure 1. This information identifies sources of lungfish brood-stock (parent fish) that can be used for captive breeding programs.

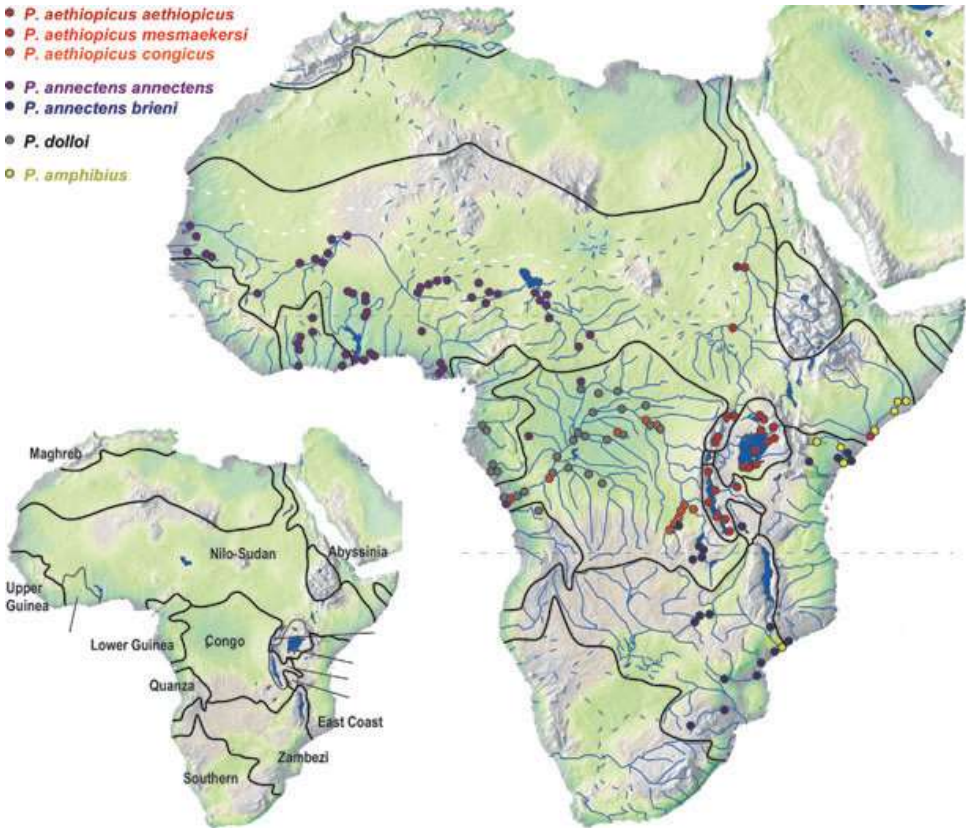


Figure 1. 1. *Distribution of Protopterus species in Africa (Greenwood 1986; Paugy et al. 2008; Froese and Pauly 2009; Otero 2011).*

Two species, *P .dolloi* and *P. aethiopicus*, lack functional gills in their adult stage (Greenwood 1986; Reid et al. 2005; Tokita et al. 2005), therefore, utilize 90% of air through the pulmonary system for respiration purposes (Lomholt 1993; Helfman et al. 2009). Their ability to survive hypoxic environs characterized with low dissolved oxygen, aids them to aestivate in cocoons for several months or years, during dry seasons when water levels fall

(Janssens 1964; Fishman et al. 1986; Brauner et al. 2007; Mlewa et al. 2007; Helfman et al. 2009).

Lungfish are important socially and economically in Africa. Its significance to African communities is listed below;

1) **Food fish**: Lungfish is a major food consumed among the poor, therefore, contributing to national food security in developing countries of Africa.

2) **Livelihoods**: Fisher-folk communities and fish traders (especially women) derive income from selling lungfish.

3) **Omega 3-PUFA:** The liver of African Lungfish has high contents of useful oils that are essential for human health (Masa et al. 2011).

4) **Medicinal**: African lungfish is locally used for treating kwashiorkor, breast ailments, backache, gonorrhea, and revitalize the immune system for sick persons especially those living with HIV/AIDS (Kayiso 2009).

5) **Cultural value**: Some sections of the African tribes for example, the Baganda in Uganda, use the African lungfish as a totem. Furthermore, women in some African communities are forbidden to consume lungfish as it is referred to as a "sister fish" (Bruton 1998; Kayiso 2009).

6) **Disease control**: African lungfish feeds on snails that are known to transmit schistosomiasis, hence, it can be used as a biological control agent in infested areas (Daffalla et al. 1985).

7) **Losses in ponds**: Its burrowing habits create leakages through which other stocked fish may escape. It may cannibalize on fry or fingerlings stocked in ponds.

Demand for lungfish is increasing while its natural populations are decreasing (Goudswaard et al. 2002; Pomeroy et al. 2006). Degradation, conversion of wetlands (breeding grounds) to agricultural land, pollution and mal-fishing practices all threaten its habitat (Goudswaard et al. 2002; Balirwa et al. 2003; Brunner et al. 2009; BakamaNume 2011). Its introduction in Lake Baringo (Kenya), resulted annual catches up to 99 metric tons in 1970s, and emerged in the 1980s (Mlewa et al. 2005; Mlewa and Green 2006).

African lungfish is dioecious (separation of sex organs) and a nest or substrate spawner (Greenwood 1958). Males are responsible for constructing spawning nests (Fig. 2) and reaching maturity at larger sizes than females (Okedi 1971; Greenwood 1986; Mosille and Mainoya 1988). Size (i.e total length) at maturity in males varies among species: *P. aethiopicus* (180 cm), *P. dolloi* (130 cm), *P. annectens* (82 cm) and *P. amphibious* (44 cm) (Greenwood 1986; Mlewa et al. 2010).

Mlewa and Green (2004) found that females and males of *P. aethiopicus* attain their first maturity at total lengths (TL) 70 ─ 76 cm and 82 ─ 88 cm, respectively. Small mature male *P. aethiopicus* measuring 72.9 cm (about 1.0kg) and mature females weighing 2.5 kg have also been reported (Mosille and Mainoya 1988). Dunbrack et al. (2006) used growth trajectory studies to show that *P. aethiopicus* reaches maturity in the wild after three years. However, Mlewa et al. (2009) observed early breeding behavior of *P. aethiopicus* cultured in earthen ponds compared to wild lungfish.

Females are oviparous (egg-laying) and more than one female may spawn in one nest since different stages (eggs and larvae) of African lungfish are found in nests (Greenwood 1966; Baensch and Riehl 1991). Mosille and Mainoya (1988) reports *P. aethiopicus* as a multiple spawner due to absence of spent gonads observed over a 12-month period and presence of different egg sizes in its ovaries. Mlewa and Green (2004) concluded that female *P. aethiopicus* are iteroparous (i.e. having multiple reproductive cycles) with mature individuals containing at least two classes of eggs. However, other sources describe the female of *P. aethiopicus* as a partial spawner with presence of oocytes in the ovaries (Greenwood 1958; Okedi 1971; Mosille and Mainoya 1988; Mlewa and Green 2004).

Natural breeding in African lungfish occurs when the rain season begins: Peak breeding season occurs during November-April period when rainfall is plenty and protracted (Greenwood 1958; 1986). However, *P. dolloi* has capabilities of reproducing in the dry season as evidenced in Stanly Pool in Uganda (Greenwood 1958; Brien et al. 1959). Reproduction is triggered by high rainfall (Mosille and Mainoya 1988), and as water levels rise, mature adults make spawning migrations to shallow inshore swampy areas or mashes where breeding occurs (Greenwood 1958, 1986).

Water temperatures in these breeding areas range from 17 to 25 C with a diurnal range of 0–5 C (Greenwood 1958, 1986). General environmental conditions that prevail in these wetlands or marshes are summarized in Table 1. These parameters (especially dissolved oxygen) are reasonably stressful for other aquatic organism but the African lungfish has evolved to survive in such conditions (Reid et al. 2005). The behavior of African lungfish or other protopterids to select areas that are relatively anoxic (i.e less dissolved oxygen) for breeding is a protective strategy against predators (Mlewa et al. 2010).

Table 1: *Water quality parameters existing in the natural habitat of African lungfish.*

Parameter	Range
Temperature (C)	24 – 28
pH	7 – 9
Ammonium nitrogen -NH_4-N (mg/L)	0.30 – 0.8
Nitrate-nitrogen $N0_3$-N (mg/L)	0.22 – 0.03
Total phosphorus (mg/L)	0.28 – 0.40
Electrical conductivity (mS/cm)	5.37 – 7.58
Total suspended solids (TSS) (mg/L)	44.7 – 190
Alkalinity $CaCO_3$ (mg/L)	122.1 – 199.7
BOD_5 (mg/L)	2.92 – 12.13
Dissolved Oxygen (mg/L)	1 – 3

Source: *(Greenwoods 1958; Kipkemboi et al. 2010; Muyodi et al. 2011).*

Broods (parents) of African lungfish usually inhabit temporary floodplains, swamps, marshes and backwaters (Greenwood 1958) in burrows. These burrows or spawning nests vary in shape, size and location; some constructed in tangled roots of papyrus or holes in mud, located in shallow grass-swamps or beneath the sand of fallen trees (Greenwood 1958, 1986; Goudswaard et al. 2002). Males construct spawning nests where mature females (from open waters) deposit eggs for fertilization.

It is difficult to visually separate the sex of protopterids as their genital structures appear similar. Mlewa and Green (2004) described the position of the cloaca (urino-genital opening) of African lungfishes being asymmetrical with its orientation not correlated to gender. However, Greenwood (1958) suggests that a protopterid male has a broad pectoral fin and snout. Also, mature female have an extended abdomen due to presence of ripe eggs that are ready to be ovulated.

The fecundity (i.e the potential reproductive capacity) of protopterids ranges from 400 to 20,000 eggs depending on the species (Bouillon 1961; Greenwood 1986; Mlewa et al. 2010). Fecundity also varies within species, for example, as the weight of *P. aethiopicus* increases from 2 to 10 kg the fecundity also increases from 4,000 to 16,000, respectively (Mlewa and Green 2004). The highest number reported is 58,422 eggs for a 91 cm size African lungfish (Okedi 1971). Overall, protopterids are considered K-strategists organisms as they use a survival and reproductive strategy characterized by low fecundity and low mortality rates (MacArther and Wilson 1967; Mosille and Mainoya 1988). Greenwood (1958) recorded the largest egg size for *P. aethiopicus* as 4.5 ─ 5.0 mm, diameter. However, mature eggs (stage III, IV and V) can range from approximately 1.0 to 5.0 mm (Okedi 1971; Mosille and Mainoya 1988).

The female deposits eggs and leaves the nest but does not return (Greenwood 1958). The male will then guard the nest for nearly eight weeks, attacking any would-be intruders or predators while constantly aerating the water (Greenwood 1966; Baensch and Riehl 1991). The male also uses its tail (‘tail lashing’) to create a current of water over the eggs and larvae for efficient gaseous exchange to occur (Greenwood 1986; Mlewa et al. 2010). This information is useful to manipulate reproductive processes in captivity and as a source of wild seed for growing in fish farms.

Eggs of *P. aethiopicus* hatch within two weeks followed by a period of parental care that extends two month (Greenwood 1958; Mlewa et al. 2010). Hatchlings or larvae of protopterids remain in the nest where they grow rapidly until they start feeding on exogenous food (Johnels and Svensson 1954; Mlewa et al. 2011). Juveniles start migrating to open waters at size 400 mm TL as a survival mechanism but majority occupy swamps, swampy lagoons and roots of floating macrophytes (e.g papyrus or water hyacinth) (Goudswaard et al. 2002; Mlewa et al.

2011). Under natural conditions, it takes 3─4 months to attain 400 mm TL, and 50 days after egg fertilization when active feeding begins (Greenwood 1986; Mlewa et al. 2011).

Protopterids are active foragers that use olfaction, sensory (i.e on pelvic and pectoral fins) and electro- receptors to locate food (Otuogbai et al. 2001; Jorgensen 1984, 2005; Mlewa et al. 2011). African lungfish is exclusively carnivorous, feeding on mollusks, aquatic insects, crustaceans, worms and fish (Mlewa et al. 2011), and rarely feed on plant material as observed with South American and Australian lungfish (Kemp 1986). African lungfish prefer eating snails with a consumption rate of 200 snails per day under aquarium conditions (Dafalla et al. 1985; Mlewa et al. 2011). Food material is sucked using hyoid and branchial arches musculature and eventually crushed by its unique tooth plates found in the upper and lower jaws (Hassanpour and Joss 2011). Cannibalism exists among wild protopterids; evidenced by fin loss which regenerate after excision (Mlewa and Green 2004; Mlewa et al. 2011).

Figure 1. 2 *Spawning nest of African lungfish. Male lungfish construct nests and provide parental protection to eggs or nestlings. Females leave the nest after fertilization or hatching of eggs.*

2.5.0 Diseases associated with Lungfishes

There is also little information on diseases or pathogens associated with African lungfish. Infectious diseases reported include leeches (family Glossiphonidae) on *Protopterus* sp (Hecht and Endemann 1998), bacteria like *Aeromonas hydrophila* and *Pseudomonas sp* (Kemp 1994) and facultative ciliates, *Tetrahymena* sp (Hoffman et al. 1975). Digenetic trematodes, *Heterorchis protopteri,* n. sp. and *H. crumenifer,* are reported in *P. annectans* and *P. aethiopicus,* respectively (Thomas 1958; Khalil 1971). Fungus *Saprolegnia sp* was isolated from eggs and embryos of Australian lungfish (Kemp 1994). Crustacean (*Argulus africanus*) was isolated from *P. aethiopicus* collected from L. Victoria (Fryer 1962). Non-infectious diseases also exist; Hubbard and Fletcher (1985) describe a tumor (seminoma and leiomyosarcoma) affecting an albino African lungfish (*P. dolloi*), while spontaneous spermatocytic seminoma (tumors of testis) were discovered in African lungfish, *P. aethiopicus* (Masahito et al. 1984). Furthermore, Ishikawa et al. (1986) found tumors (spontaneous neurinoma) developing on the skin of African lungfish (*P. annectans*).

CHAPTER II

Aquaculture of African lungfish (*Protopterus sp*) in Uganda: Prospects and Potential

1.0.0 Abstract

Culturing species resilient to drought and poor water quality conditions may be a significant part of the future of African aquaculture. Air breathing fishes potentially have a role in low-management culture systems for small scale farms because dissolved oxygen does not threaten the fish crop. African lungfish (*Protopterus sp*) is advantageous because it is: an indigenous fish with good quality flesh, an air-breather, and a biocontrol agent against schistosome vector snails. Wild lungfish stocks are declining and national strategies to protect its natural population are lacking.

Knowledge on indigenous practices to grow, harvest and marketing *Protopterus sp* raised in fish ponds is minimal or non-existent. This study assessed status and potential of lungfish aquaculture in Uganda in seven districts located in central and eastern Uganda. Through semi-structured interviews socio-economic conditions that shape the aquaculture of African Lungfish was also evaluated.

Lungfish is highly valued as food fish in eastern, northern, parts of western and broadly accepted in central region of Uganda. Certain health or nutraceutical benefits are attributed to this fish species. Most lungfish is sold fresh but smoked forms are also produced. Lungfish products are increasingly sold alongside tilapia and Nile perch in rural and urban fish markets. Nonetheless, there seems to be some countervailing sociocultural beliefs that continue to deter consumers from eating lungfish.

Fish farmers' initiative to culture African lungfish in ponds and tanks demonstrates how this fish can be raised on exogenous feed in captivity. This study identifies the applicability of

indigenous knowledge and practices to formulate a broader strategy of widespread production. The socioeconomic viability of African lungfish as a new fish species in aquaculture is beginning to be established, and its value chain delivers quality products to consumers in Uganda and across Sub-Saharan Africa.

2.0.0 Introduction

Globally, rising food prices have shifted 44 million people into extreme poverty while Uganda has nine million people facing an acute food shortage (World Bank 2010). Aquaculture provides an alternative source to food security, income and livelihood improvement, in the Sub-Saharan region population (Brummett and Williams 2000). Lake fisheries continue to decline, and aquaculture production (subsistence and commercial oriented) is less than 20% of total national fish production (Isyagi et al. 2009; FAO 2012).

Fish are an important source of protein, contributing 42% of animal protein intake for 2.6 billion people, and developing countries consider it as a human health asset (Brunner et al. 2009). Increasing demand for fish while natural populations are plummeting require urgent solutions (Brunner et al. 2009). The capita fish consumption in rural areas of Uganda is reported to be as low as 1.9 kg (Nnyepi 2006) compared to 17.4 kg expected (FAO 2012). High market prices of Nile perch and tilapia have shifted a number of fish consumers toward African lungfish consumption particularly in densely populated areas of Kampala. Uganda has the highest population growth rates in central Africa and continues to have high incidences of chronic childhood malnutrition and infant mortality, especially in rural areas (Owor et al. 2000; Nnyepi 2006; Nalwoga et al. 2010). House-holds in Uganda that raise high protein and energy food (including fish) are shown to improve child survival in rural areas (Nuwaha et al. 2011)

Aquaculture is an alternative source for food security, income and livelihood in the Sub-Saharan region (Brummet and Williams 2000). In Uganda, aquaculture production is dominated by Tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*) and common carp that has reduced scales or mirror carp (*Cyprinus carpio*), grown at subsistence and semi–commercial levels, contributing to less than 20 % of total national fisheries production (Isyagi et al. 2009; FAO 2012). Recently, shifting rainfall and temperature regimes are new challenges to management of natural water bodies and fish farming systems in sub-Saharan Africa. Therefore, culturing fish species that are resilient to drought and stressed water quality conditions will be a significant to the future of African aquaculture.

Air breathing fishes {e.g *Protopterus aethiopicus*} would be suitable candidates since they utilize atmospheric oxygen to meet all or part of their metabolic demands. Hence, air breathing fishes potentially have a role in low-management culture systems because dissolved oxygen is not a limiting factor. The African catfish (*C. gariepinus*) can tolerate low levels of dissolved oxygen but its flesh is less desirable by consumers (Ssebisubi et al. 2012). African lungfish is advantageous because; it is indigenous, has good quality flesh, obligate air breather and a bio-control agent against schistosome vector snails (Greenwood 1966; Daffalla et al. 1985). Lungfish is locally an important food fish captured from natural lakes, swamps and reservoirs using a variety of gears including gillnets, long lines and baskets.

Literature on African lungfish mainly examines its ecology, fishery, biology, and physiology but few studies treat it as food fish in aquaculture (Baer et al. 1992). Therefore, this study applies what is known about lungfish to explore its aquaculture potentials to improve food security and livelihoods in Uganda and the sub-Saharan region. The study assesses the indigenous practice and understandings about production parameters and approaches. Field work evaluates the potential paths for producer adoption using lungfish as a culture species in managed water body resource.

3.0.0 Materials and methods

To generate basic information from targeted groups a Rapid Rural Appraisal (RRA) was conducted using approaches described by Pido et al. (1996) and Townsley (1996). Semi-structured interviews were performed in May ─ June, 2011 and August ─ December 2012 in eleven districts of Uganda to understand the potential and prospects of lungfish aquaculture in Uganda. Research questions focused on a) status of farming African lungfish in Uganda, b) indigenous knowledge and practices associated with culturing this fish, and c) socio-economic conditions that influence the aquaculture of African Lungfish including prices, demand, and public perceptions.

Secondary information related to lungfish production was also obtained from government departments including the Department of Fisheries Resources (DFR), District fisheries headquarters and National Fisheries Resources Research Institute (NaFIRRI). Data was used to understand status of lungfish (aquaculture and fishery) in Uganda and the east African region, policy regulations and production. Field interviews were conducted in districts with high potential for aquaculture development and communities known to cherish African lungfish. Districts visited include: Kampala, Wakiso, Mukono, Mitiyana, Kalungu, Kumi, Busia, Soroti, Pallisa, Iganga and Jinja (Fig. 2.1).

3.1.0 Districts covered

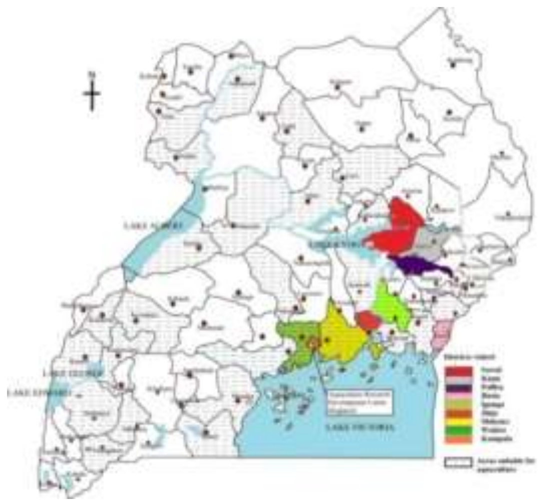


Figure 2. 1. *Map of Uganda showing districts visited that are located in areas suitable for aquaculture production.*

Table 2: *Respondents and districts visited in this study. Institutes include District Fisheries Office, National Fisheries Resources Research Institute, Department of Fisheries Resources Headquarters and boarding schools.*

District	**Fish Farmers**	**Landing sites**	**Fish Traders**	**Consumers**	**Restaurants**	**Government Institutes***
Soroti	5	1	7	16	2	1
Kumi	6	2	10	11	1	1
Pallisa	2	1	2	5	0	1
Busia	5	1	5	9	1	1
Iganga	0	0	2	0	1	0
Jinja	5	2	6	16	3	2
Mukono	2	0	1	2	2	0
Wakiso	8	2	9	11	3	3
Kampala	1	1	23	24	4	2
Kalungu	1	0	2	3	1	1
Mitiyana	1	0	1	3	1	1
Total (n)	**36**	**10**	**68**	**100**	**19**	**13**

3.2.0 Assessment of lungfish culture

Fish farmers (n=36) and residents fisher communities (n=10) were interviewed to assess the indigenous knowledge and practices associated with culturing and use of lungfish. Interactions with fish farmers reported to have cultured lungfish generated information on status of production practices, market sources and options, and constraints. Other production parameters included production system, stocking rates, source of seed, feeds, management practices, harvesting and handling procedures. African lungfish has a "beak" like that of a snapping turtle and its sharp dentition makes it hazardous to handle.

3.3.0 Socio-economics factors

Information generated from consumers (n=100), restaurant owners (n=19) and fish traders (n=68) provided the socio-economic conditions that shape aquaculture and fishery of African lungfish in selected districts. Only consumers found in fish markets or along road-side fish *kiosks* were targeted to assess lungfish consumption. Other groups interviewed include fish traders engaged in whole-sale and retail activities of lungfish. Key questions focused on prices, source, seasonal variations, demand, gender participation, and lungfish attribute perceptions.

4.0.0 Results and Discussion

4.1.0 Lungfish is popular in East Africa

Most lungfish are captured in Uganda’s natural waters (lakes and wetlands). Over 90% of lungfish caught from Lake Victoria came from Uganda. Between 1975 and 2009, a total of 404,008 tons of lungfish were caught in this region; Uganda produced 371,811 tons and Kenya captured 32,197 tons (Fig. 2. 2). No records show lungfish caught from Tanzania natural aquatic systems but unconfirmed reports indicate it is popular around the Lake Victoria region.

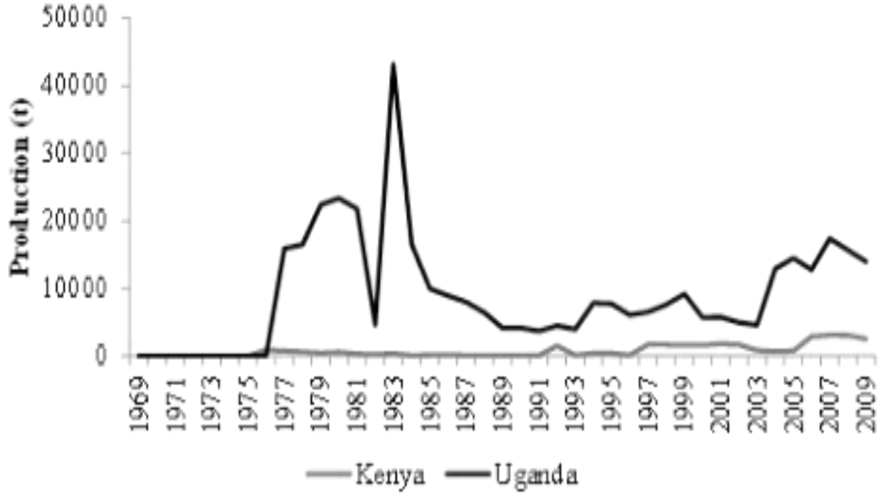


Figure 2. 2 *Production trends of African lungfish in the East African region. (Data from FAO FishStat 2012)*

Uganda had the highest production (15,000 ─ 22,000 tons) from 1976 to 1985 but declined from 1985 to 1989, and remained relatively stable at around 20,000 tons. Production from Kenya stagnated around 1000-3000 tons in the last four decades. However, since 2005, fisheries statistics indicate a decline in lungfish catches from the East African region (FAO FishStat 2012). Anthropogenic factors like wetland degradation and over-fishing (Goudswaard et al. 2002; Balirwa et al. 2003; Brunner et al. 2009) could be the primary drivers reducing wild lungfish populations.

Lungfish contributes 4% of total fish production from Uganda's natural water ecosystems with Tilapia (37%) and Nile perch (42%) continuing to dominate fish catches in Uganda (Fig. 2. 3).

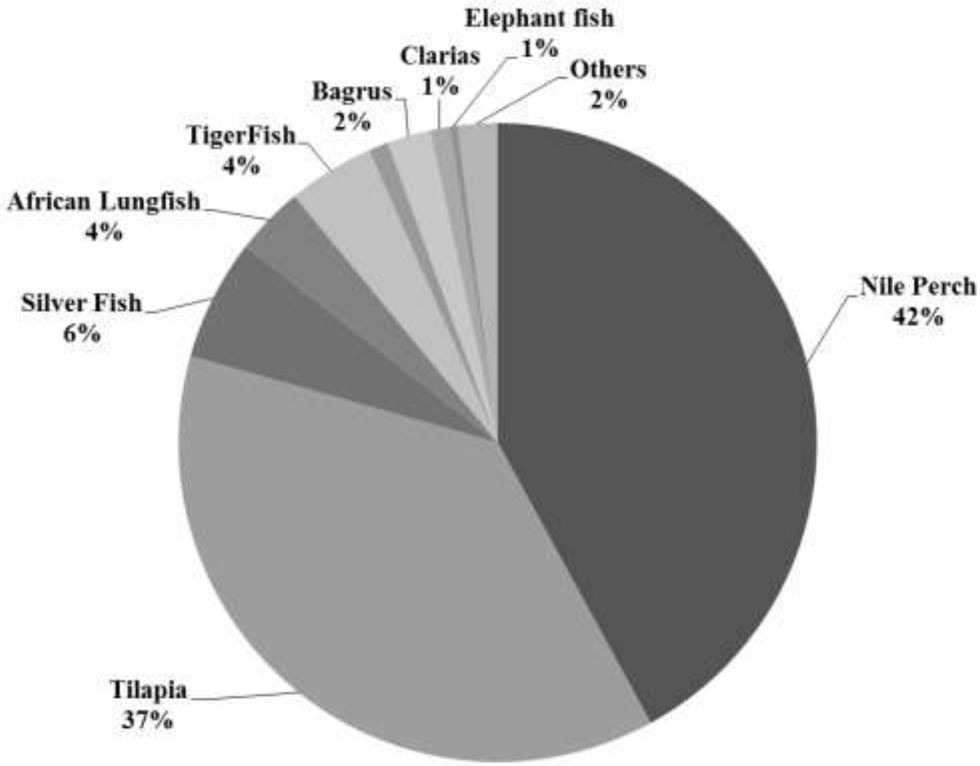


Figure 2. 3 *Types of fish caught from natural lakes and rivers in Uganda. (MAAIF 2004, 2010)*

Nile perch and tilapia production also increased from 1969 to 2006 but gradually remained steady, thereafter. Peak catches of lungfish in Uganda were recorded from 1977 to 1983 but gradually reduced for the last two decades (Fig. 2. 4). There is a decline in the Uganda fishery with production dropping from 411,800 metric tons in 2005 to 366,600 metric tons in 2010 (UB0S 2010).

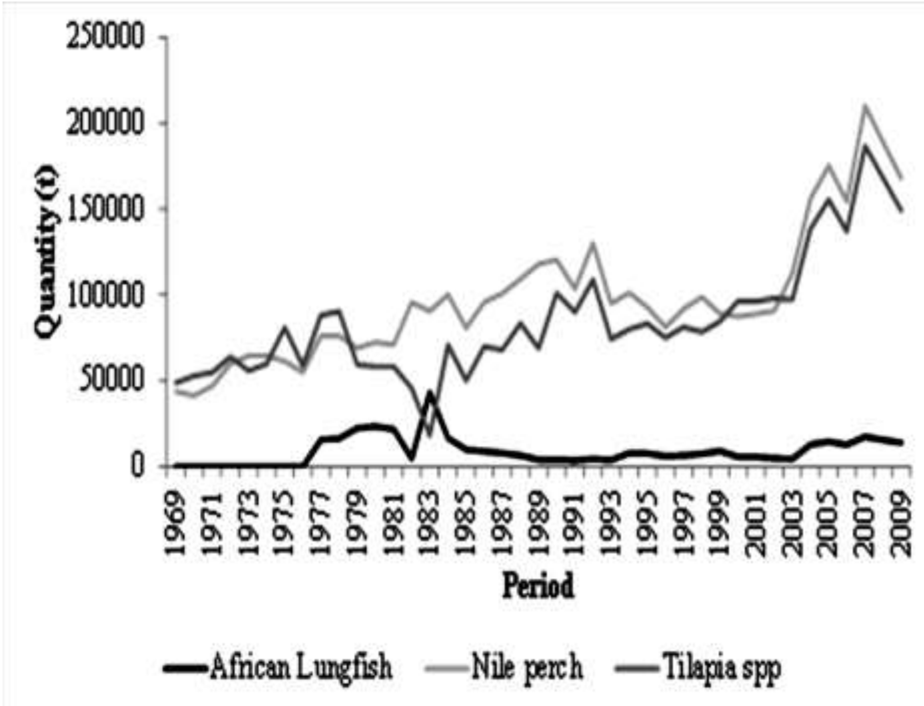


Figure 2. 4.*Trends of Nile perch, Tilapia and African Lungfish caught in Ugandan Lakes. Sourced from FAO , FISH STAT 2012.*

Demand for fish in Uganda is increasing as the population growth rates (3.2%) rises (MFPED 2010; UBOS 2010). Therefore, pressure on natural resources is increasing to meet the demand. Per capita consumption of fish in Uganda increased in 2000 but declined to 6kg/year which is below the global level of 12kg and Sub-Saharan Africa of 7kg (DFR 2011; FAO 2011). General poverty and reduced availability of fish have caused the drop in capita fish consumption in Uganda despite having a vast area of untapped aquatic resources (DFR 2011).

Lungfish catches vary among districts surveyed as part of this study, with Kumi and Soroti having the highest production from Lakes Opeta, Bisina, Nyaguo and Kyoga (Table 3). Other districts capture lungfish from shores of Lake Victoria. In Kumi district, over 50% of lungfish (locally known as *Ebileng*) is mainly from Lakes Opeta and Bisina at Ometanga and Akidea landing sites, respectively.

Table 3 .

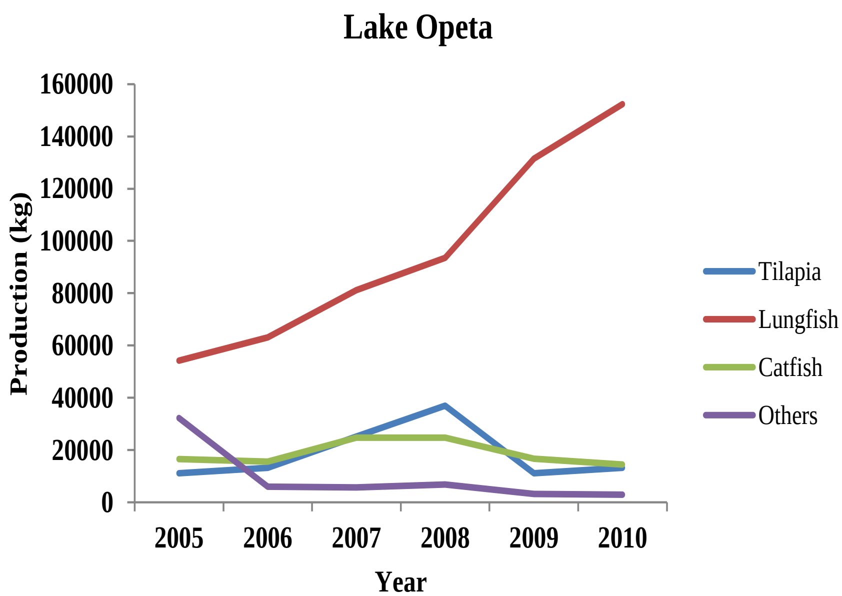
Table 3 *Fishery production from Lakes Opeta, Bisina and Nyaguo in Kumi district from 2005 to 2010.*

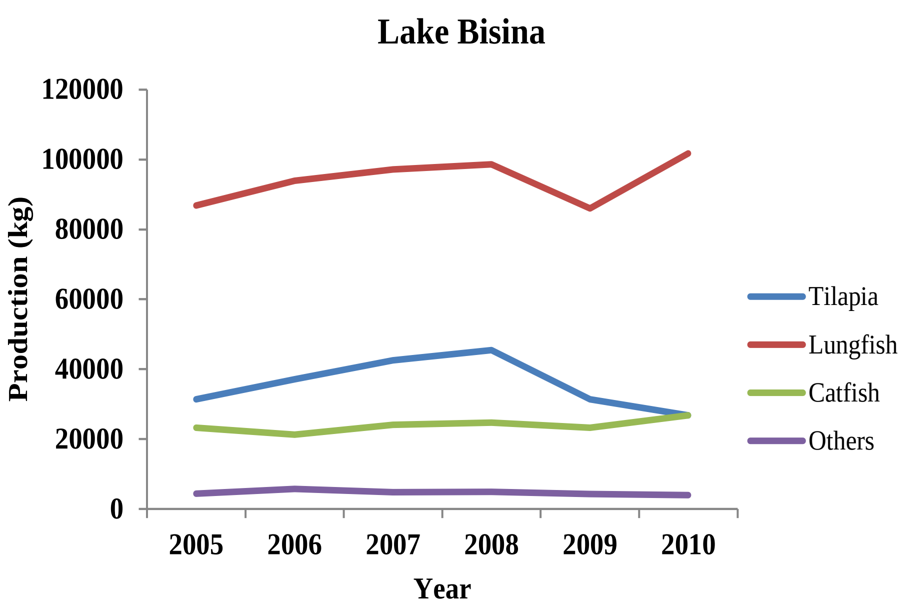
	Fish production (kg)					
Lake	2005	2006	2007	2008	2009	2010
OPETA						
Tilapia	11,196	13,200	25,136	36,994	11,196	13,273
Lungfish	54,184	63,081	81,144	93,598	131,436	152,379
Catfish	16,628	15,557	24,817	24,817	16,664	14,501
Others	32,268	6,048	5,705	6,889	3,216	2,926
NYAGUO						
Tilapia	728	1,003	2,121	3,006	720	N/A
Lungfish	467	3,745	4,734	3,517	4,680	N/A
Catfish	760	6,492	6,346	3,804	5,760	N/A
Others	542	796	803	982	588	N/A
BISINA						
Tilapia	31,392	37,111	42,553	45,447	31,392	26,834
Lungfish	86,828	93,914	97,159	98,601	86,028	101,745
Catfish	23,288	21,304	24,098	24,755	23,280	26,817
Others	4,356	5,760	4,812	4,957	4,336	4,003

N/A: *indicates data was not available. Sourced from Fisheries department, Kumi district*.

Records from this survey show that since 2005, lungfish contributes 59% of total fish harvest, while tilapia, *Clarias spp*, and other types contribute 18, 18 and 5%, respectively (Fig. 2.5). Size and number of lungfish caught, however, is progressively declining. For example, over 3,000 tons of lungfish were caught annually in 1990s but recent records show annual catches of 154 tons (Soroti and Kumi district Annual Reports 1995 to 2010). Size of lungfish harvested from L. Kyoga is relatively larger than fish caught from its satellite lakes (e.g Opeta and Bisina).

Additionally, over 400 fishing boats are utilized on these lakes, daily, thus increasing fishing efforts (Soroti and Kumi district Annual Reports 1995 to 2010).





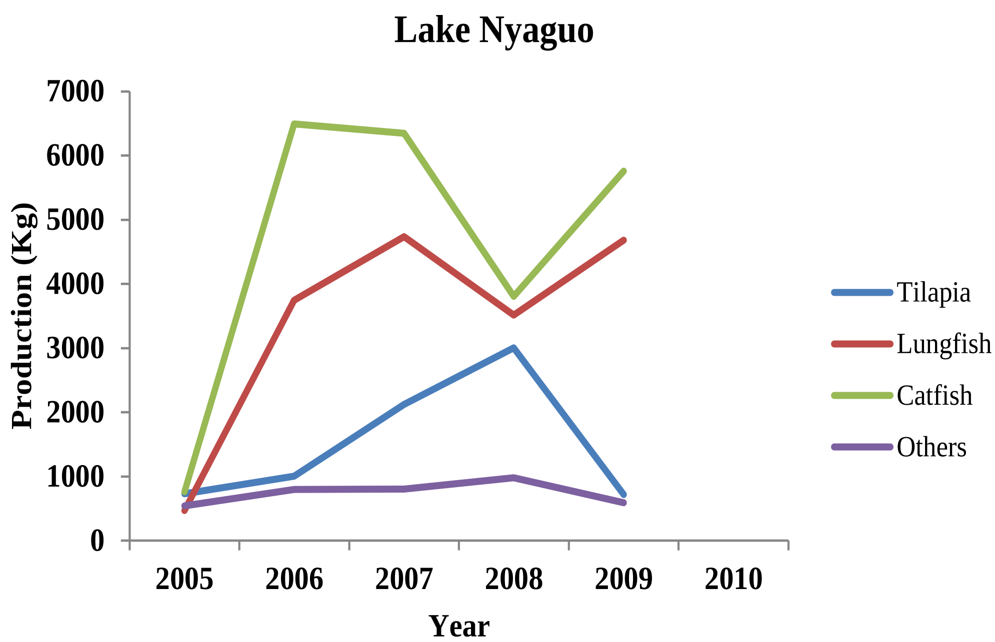


Figure 2. 5. *Trends in capture fishery production from Lakes Opeta, Bisina and Nyaguo. Unlike L. Nyaguo, lungfish dominates catches from lakes, providing livelihoods to resident communities. Sourced from Fisheries department, Kumi district.*

In this survey it was found that lungfish is mainly caught using gillnets, hooks, basket traps, or long-lines. However, these harvesting methods and gears applied are generally non-selective. High catches occur when water –levels in the lake are low and during the onset of rainy seasons. The demand for lungfish is high and they are hunted in the dry season when they are aestivating in dried swamps. In rainy seasons, parent fish and juveniles migrate through flooded swamps or wetlands and this makes them vulnerable to hunters. Consequently, immature or small sized lungfish (100-500g) are usually caught for home consumption (District Fisheries Officer-Kumi, personal communication).

4.2.0 Sources of parent (Brood) stock

Field surveys undertaken by NaFIRRI reveal that the majority of lungfish caught using gill nets, baskets and hooks are in mature stages (III-V) with size ranging 35─55 cm (Fig. 2.6, 2.7, 2.8 & 2.9). Hence, efficiency of non-selective fishing gears has improved but the fishery is under pressure.

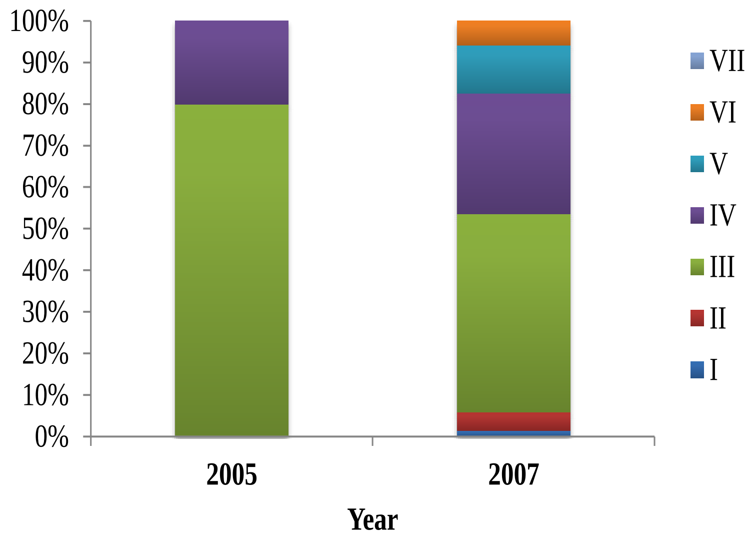


Figure 2. 6.*Gonadal state of lungfish caught from L. Victoria (NaFIRRI 2005 & 2007).*

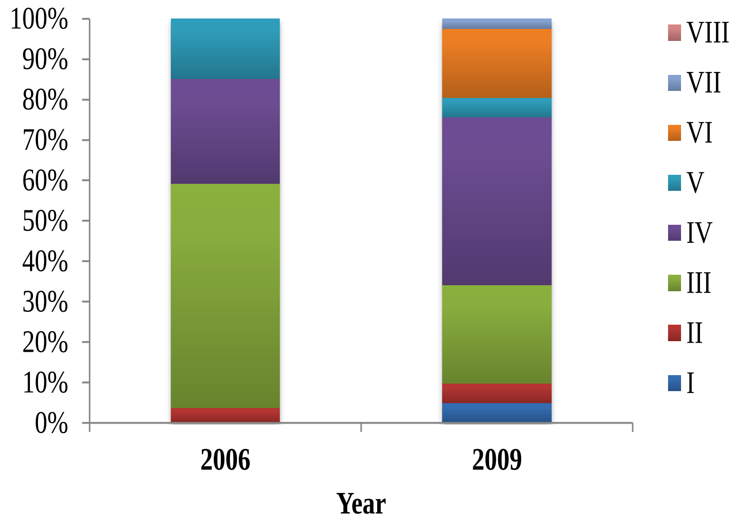


Figure 2. 7. *Gonadal state of lungfish caught from L. Kyoga (NaFIRRI 2006 & 2009).*

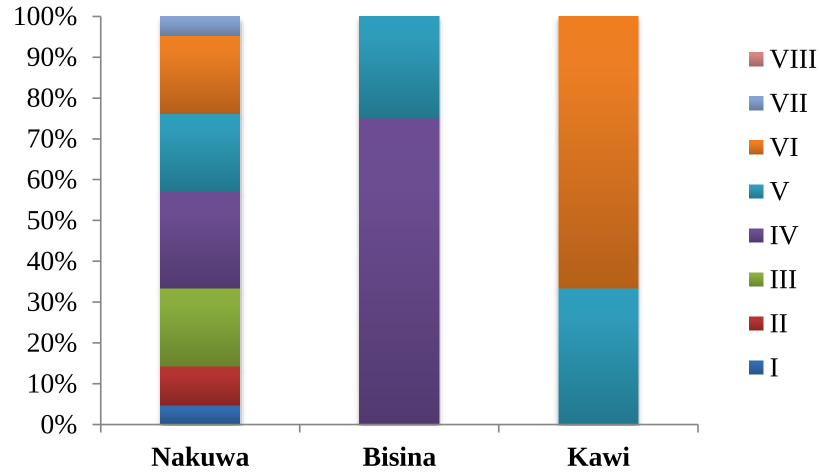
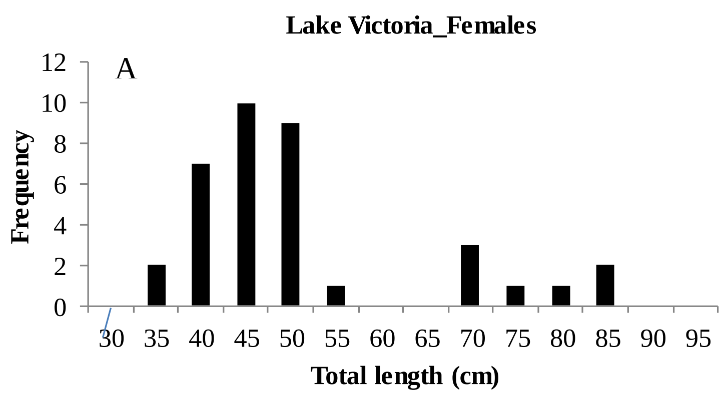
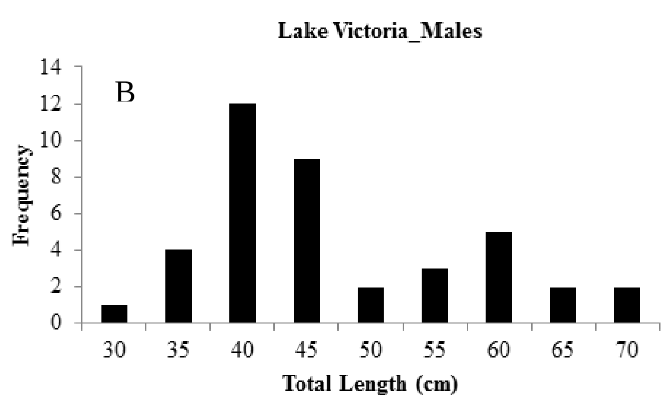
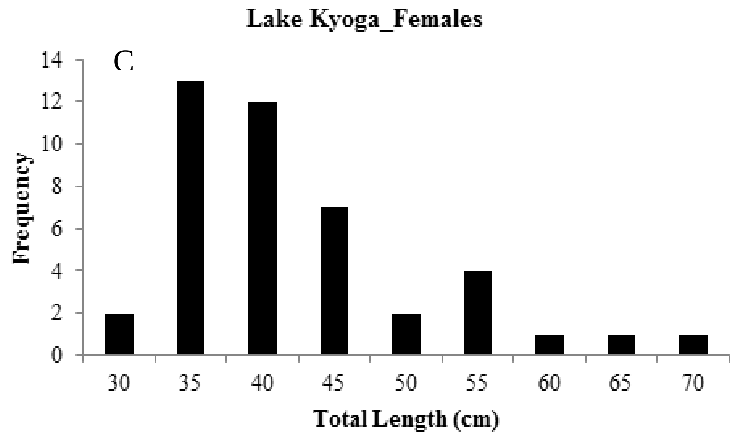


Figure 2. 8 *Gonadal state of lungfish caught from satellite lakes of L. Kyoga region (NaFIRRI 2006 & 2010).*







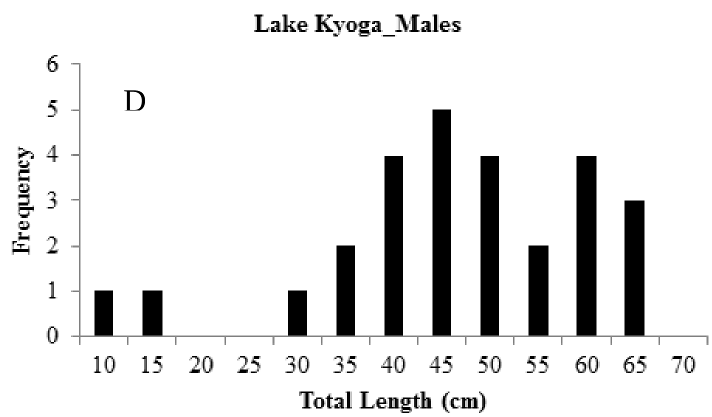


Figure 2. 9 (A,B,C and D) *Size distribution of randomly sampled lungfish caught from Lakes Victoria and Kyoga from January to April and June to August in 2005, 2006, 2007 &2009 (NaFIRRI annual reports 2005; 2006; 2007; 2009).*

Mature females and males of *P. aethiopicus* are reported to be in TL of 70 ─ 76 cm and 82 ─ 88 cm, respectively (Mlewa and Green 2004). Sizes of most mature females and males in this study are below 60 and 65 cm, respectively. Changes in lungfish maturity could be due to physiological responses resulting from overfishing and augmented by climate change effects (Pörtner and Farrell 2008). Climate changes (e.g increased temperature) and increased fishing pressures influence size reduction at first sexual maturity in fish (Heino et al. 2002; Rijnsdorp et al. 2009).

Aquaculturist or scientists planning to develop breeding programs for African lungfish will have to ascertain the maturity stages of this fish in captivity. This approach will facilitate the propagation of African lungfish. Various fish species have been domesticated with the majority of farmed fish categorized as exploited captives (Le François et al. 2010). Initially, mature lungfish will have to be collected from wild environments during the rainy season as they migrate to breed. Parent-stocks are useful for restocking programs and for initial production of seed for farmers. Fish maturing at less than expected sizes are an advantage to aquaculturists because propagation will be enhanced in short time.

Successful breeding schemes to rebuild threatened populations of White Sturgeon (*Acipenser transmontanus*) involve collection of wild gravid females that are artificially induced to spawn in hatcheries (Ireland et al. 2002). Anecdotal reports indicate lungfish can breed naturally in ponds as farmers sometime find fingerlings or juveniles in completely drained pond. Generally, it is easier to domesticate wild aquatic species than terrestrial plants and animals because; i) basic zoo-technologies are available, ii) past experience acquired in domesticating other fish species, iii) fewer human-health risks involved, and iv) rampant exploitation of wild fish populations (Duarte et al. 2007; Teletchea et al. 2009).

4.3.0 African Lungfish Aquaculture

Results of this survey are shown in the appendix B. Six fish farmers (17%) are growing lungfish in ponds, ditch and tanks. Most farmers (83%) visited grow Nile tilapia (*O. niloticus*) and African catfish (*C. gariepinus*) in earthen ponds ranging 200 ─ 6100 m^2. Fish is either polycultured or monocultured system. Some farmers from Wakiso, Mitiyana and Kalungu districts are producing an unknown amount of lungfish from ponds or reservoirs using seed collected from Lakes Victoria, Wamala and Kyoga.

4.3.1 Management of lungfish aquaculture on farms visited

In Nangabo (Wakiso district), a farmer polycultured 1,000 wild caught lungfish juveniles (15 ─ 20 cm) with mirror carp and tilapia in a 400 m^2 pond in 2003. He fed them with a prepared diet (unknown proportion) containing maize bran, blood meal and *mukene* (*Rastrineobola argentea*), once-daily. Unfortunately, he lacked reliable records to track the total amount of feed applied, daily. All fish were harvested after 1.5 years using seine nets or when ponds were completely drained. Less than 40% of lungfish were harvested with size ranging 1─ 3 Kg. All lungfish were sold to lucrative Kampala City fish markets at prices ranging US $ 2.5 ─ 4.0 per kg. Regrettably, he abandoned fish farming in 2006 when he became incapacitated to run the farm.

In 2010, two farmers in Kalungu and Mitiyana districts stocked 100 ─ 800 wild caught lungfish fingerlings (5 ─10 cm) in ponds ranging 1000 ─ 6100 m^2 and extensively raised the fish to market size (> 400 g). These ponds normally stocked with wild small tilapia, haplochromines and *Gambusia* that are prey for lungfish. Market size lungfish are harvested using hooks and basket traps after one year of growth. Fish are mostly sold on-site but traders or consumers usually make orders before collecting fish from these fish farms. Nevertheless, it is difficult to evaluate the productivity and profitability of these production systems since farmers lack appropriate aquaculture technologies and management skills.

Some fish farmers inadvertently grow African lungfish in ponds especially in areas prone to floods like Kumi and Soroti districts. Small scale farmers either enrich their house-hold diets with this fish or generate income from lungfish production. However, a few fish farmers have lost their stocked fish (e.g Tilapia and Catfish) through predation or leakages created by this burrowing lungfish. A farmer in Soroti district lost over 70% of his African catfish fingerlings and attributing this to presence of lungfish in his ponds.

About 58% fish farmers (n=36) contacted expressed willingness to invest in lungfish production while 42% were not interested. Potential lungfish farmers located in Kumi , Soroti, Busia and Wakiso districts cite good market prices, large sized fish (> 1 kg) and substantial quality products (e.g fillet size) as reasons for being interested in lungfish culture. However, the absence of aquaculture technologies has greatly challenged fish farmers to produce this fish in captivity. Those who have succeeded have largely adopted technologies used in catfish farming. Reasons for not investing in lungfish aquaculture include; i) religious (e,g Seventh day Adventists) and tribal beliefs, ii) predatory and burrowing behavior of lungfish cause losses to fish farming business, iii) lack of technologies or information, and v) it is known to harm a person if not properly handled.

A few fisher folk in Kumi district attempted to raise 15 ─ 30 cm wild caught juveniles in excavated holes (40 cm diameter; 1m deep) at shores of Lake Opeta. Using indigenous knowledge, lungfish juveniles are fed with fish fry (e.g tilapia and catfish), grasshoppers, snails and kitchen leftover, every day. After a year in captivity, lungfish are harvested using hooks. Not all lungfish are recovered since some fish are thought to be lost to cannibalism or through burrows. A fish trader in Bwaise (Kampala) raised juvenile lungfish in a concrete tank (1x 1 x 0.5 m^3) in 2009. Fish were fed with kitchen leftovers and meat by-products. He harvested the fish after seven months when the water quality started deteriorating. However, fish harvested were small (about 50 cm) which discouraged him to grow lungfish again.

Attempts by farmers to grow African lungfish in captivity have demonstrated four aspects; i) this fish can be polycultured in earthen ponds with existing fish e.g Nile tilapia and

African catfish, ii) lungfish can be raised on artificial diets (e.g food leftovers, maize bran + fish meal), iii) African lungfish can be cultured in tanks and ponds, and iv) poor water quality conditions affects growth of lungfish.

Previous research on lungfish has been conducted in tanks/aquaria and ponds using available aquaculture technologies. The South American lungfish (*Lepidosiren paradoxa*) was successfully bred in captivity using tanks under laboratory conditions (Parsons 1935). Joss and Joss (1995) were able to obtain natural spawns of Australian lungfish (*Neoceratodus fosteri*) in ponds. Wild-caught fingerlings of two African lungfish species, *Protopterus aethiopicus* and *P. amphibious* were raised in ponds and tanks, respectively (Baer and Freyvogel 1992; Mlewa et al. 2009). In addition, farmers in East Africa have applied indigenous knowledge to grow African lungfish mainly in earthen ponds, tanks and ditches. A successful research program to domesticate lungfish should integrate indigenous knowledge that generates sustainable production. Trans-disciplinary approaches that embrace indigenous knowledge can ensure effective sustainable development rather than through conventional research system (Sillitoe 1998; Aikenhead and Ogawa 2007; Brandt et al. 2013).

4.4.0 Harvesting and handling methods

Most fish farmers (50%) in this study use baited hooks to harvest lungfish from ponds. Others drain (17%) or use spears and hoes (17%) as this exposes the fish hidden in burrows. Farmers who have seine nets (10%) can efficiently harvest lungfish but only when water levels in ponds are reduced. Few farmers (6%) in this survey use basket traps that contain baits (e.g fish fingerlings) to harvest lungfish.

Fishermen interviewed at Lakes Kyoga, Victoria, Lamwa and Bisina use hooks with baits like catfish fingerlings, earthworms, meat, rats, and frogs to catch lungfish. Similarly, gill nets (4

─ 4.5″) are used to trap lungfish in lakes and reservoirs. Hunters target inactive and aestivating lungfish in extreme dry seasons using spears and hoes; an approach considered primitive. Basket traps (*Ekolo*) are commonly used in wetlands or swampy areas. Lungfish is safely handled when dead or simply avoiding mouth parts.

4.5.0 Consumers' perspective

Uganda's per capita fish consumption increased from 7.6 to 14.7 kg from 2000 to 2009 (FAOSTAT 2013) but gradually declined to 6 kg in 2011 (DFR 2011). Many respondents consider fish an expensive food item with retail prices in Uganda ranging 1─3 US $/Kg (Dickson et al. 2012; Ssebisubi et al. 2012; Timmers 2012). This has forced many to go for cheaper sources of protein like factory by-products of Nile perch (*Lates niloticus*), beef and beans (Kabahenda et al. 2011; Nuwaha et al. 2011; Tidemann-Andersen et al. 2011; Kabahenda et al. 2013). Decline in wild fish supply has resulted in high market prices particularly tilapia and Nile perch. Lungfish, however, is the emerging fish commodity in local/regional markets and is popular among the low income families.

Lungfish is popular in Kumi, Soroti, Busia Kalungu and Kampala districts. Consumer's preference to lungfish products is explained; i) cheaper compared to Nile perch and tilapia products, especially rural areas (25%), ii) less smell (7%), iii) less tissue fat (11%), iv) adequate for an average family (29%), v) less bones (8%), vi) good taste (16%) and vii) has medicinal value (4%). Ascribed benefits for human health include; treatment of women's breasts with lactation problems, treatment of alcoholism, it's an aphrodisiac and boost immune system; none has been scientifically proven.

In this study, 77% respondents frequently eat lungfish products either at home or restaurants and bars (Fig. 10). It is more popular in eastern Uganda (63%) than the central region

(37%). Ethnic groups that prohibit consumption of lungfish are mainly located in the central region. For example, some groups of Buganda consider lungfish/*mamba* as a totem (Kabahenda and Hüsken 2009).

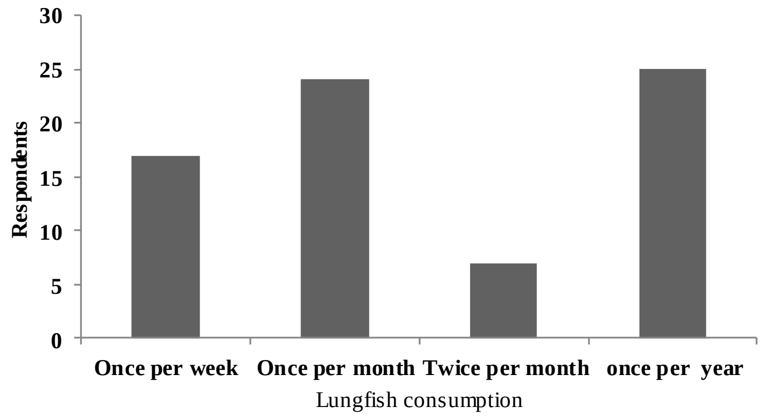


Figure 2. 10 . *Consumption frequency of lungfish products in eleven districts visited in 2012. About 73% respondents interviewed eat lungfish every month, including women***.**

Kampala city (central region) is exceptional since it is cosmopolitan. Communities resident in eastern Uganda are fish eaters and lungfish is cherished among the Teso communities. The eastern region is also endowed with seasonal wetlands and temporary pools (BakamaNume 2011) that have adequate stocks of lungfish especially during wet seasons (Greenwood 1958).

In this survey lungfish products are accepted by both women and men. About 56% of the lungfish consumers (n=73) were women who are involved in preparing it for home consumption. Kirema ─ Mukasa and Reynolds (1991), Bruton (1998) and Kayiso (2009) previously explained how women are forbidden to eat lungfish as it is referred to as "sister fish". Not all women from the central region are prohibited to eat lungfish. In this study, however, women not only consume lungfish but actively participate in its trade. Consumption of lungfish in both regions is an indication of less-resourced communities seeking for cheaper source of proteins while natural fish stocks are declining.

Only six restaurants (32%) visited had lungfish on their menu. Most of these are located in Kampala (Nalukolongo, Bwaise, Nakivubo and Nakawa) and Wakiso (Kireka and Busega) districts. Lungfish is available through-out the year since suppliers come from many areas; Lakes Victoria, Kyoga and Wamala. With a multiple source of lungfish supply, restaurant owners are able meet the increasing demand of its customers. Prices from suppliers usually range from 1 to 3 US $ per Kg and customers have to pay US $ 2 to 4.5 per Kg depending on the product. However, the price usually changes when supply decreases during prolonged dry seasons (June to September). Here an extra US $ 0.5 is usually charged to meet additional costs. Products sold in restaurants mainly include boiled source and deep fried chunks.

A range of lungfish products are available in local fish markets located in the rural and urban areas of Uganda (Fig. 13). Most consumers (59%) interviewed prefer fresh/fried pieces because they are affordable and adequate for an average family. Whole smoked (14%) and fresh fish (12%) are usually ideal for large families. Fish stalls are popular for deep fried lungfish pieces that are either sold in bar-restaurants (e.g Nalukolongo-Kampala) or bought by low-income households. Lungfish soup (10%) is a recent innovation by women selling food in restaurants. In Kumi, Soroti and parts of Pallisa, a whole fresh lungfish (Local names; *Ebileng / Nakibalo /Mamba*) is a delicacy among communities where special dishes are prepared for in-laws. Smoked lungfish pieces (5%) are rare on markets but when available low-income earners prefer them. Whole smoked forms (10%) are usually high-priced but a few products are exported to Kenya through Busia border.

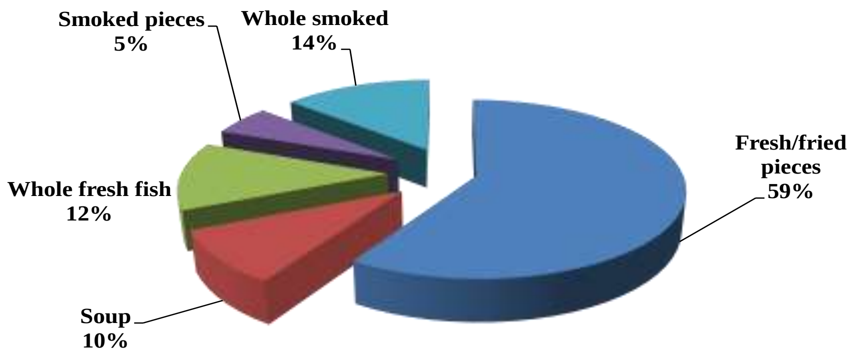


Figure 2. 11 *African lungfish products available on local markets*

4.5.0 Markets and Value chain for African lungfish

Women are primary actors in lungfish fisheries, actively engaged in hunting, post harvesting and marketing processes. Lungfish products from wild and aquaculture production systems are sold in local or regional markets and on-farm. Local markets are mostly concentrated in cities, suburbs, rural towns, landing sites and along highways. Regional markets for lungfish include Kenya, Southern Sudan and Democratic Republic of Congo (DRC). The supply chain for African lungfish products collected from the wild and fish farms is similar to Tilapia and African catfish (Fig. 2.12).

Lungfish is available in all markets (rural and urban) visited but volumes reduce during prolonged dry seasons (June to September). In most markets (>90%) lungfish is sold together with other fish species; Tilapia, catfish, Nile perch, Silver fish, *Synodontis, Bagrus docmac and Barbus sp*. Overall, lungfish is second (21%) to Tilapia (52%) of all fish sold in these markets but this is not true in all markets in Uganda. Some markets specialize in selling a few fish species and in this study emphasis was mostly on markets known to supply or sell lungfish. Prices of lungfish range from US $ 0.6 to 2.5 per Kg according to size and product (i.e. fresh or smoked).

Fresh products are popular but deep fried and smoked lungfish are also demanded. Wholesale/traders are the main suppliers (94%) of lungfish to markets but fisher folk communities can supply directly to markets located near landing sites. Markets are still challenged with a limited supply of lungfish to meet its increasing demand. This has caused traders/fishermen to market under-sized (< 200g) lungfish that that are usually sold in clusters: popular among less income communities.

Typically the same intermediary primary actors (i.e transporters, processors, wholesalers and retailers) participate in both market chains. Hence, entry of a new product may not necessarily threaten existing aquaculture or wild products as predicted in most markets (Porter 1998b; Ssebisubi et al. 2012). Consumers generate market information flow upon which quality and quantity demanded is supplied through these channels. A similar response is observed with channel catfish production in US (Jolly and Clonts 1993).

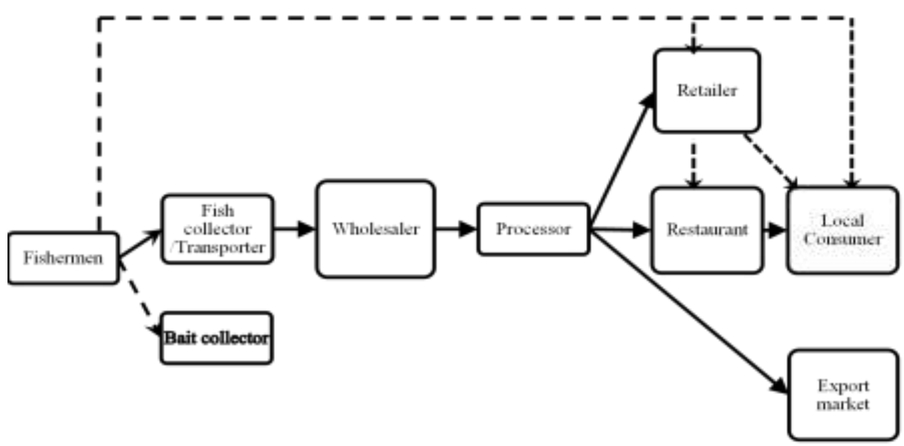


Figure 2. 12 . *General product distribution of African lungfish collected from wild environments. Lungfish bait (7-15 cm): emerging market for fishermen dealing Nile perch industry*.

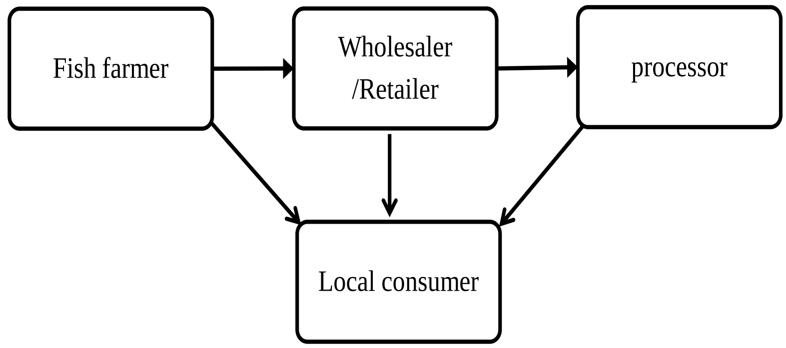


Figure 2. 13 . *General product distribution of African lungfish collected from fish farms. Low volumes of fish are sold through this channel since few fish farmers grow lungfish.*

The distribution channel for wild and farmed lungfish is similar to farmed African catfish (Ssebisubi et al. 2012) and captured fish like Nile perch (Hempel 2010; Van der Knaap et al. 2010). Wild products are sold throughout the year but fluctuations occur during prolonged dry periods when supplies decline and prices increase. It is difficult to determine the actual availability of farmed lungfish since the practice is not fully established. However, successful farmers harvest farmed lungfish after 8 to 17 months.

Lately, wild caught lungfish fingerlings (7 ─ 15 cm, long) are sold to fishermen who are engaged in Nile perch fishery. A fingerling costs US $ 0.11─ 0.18 per fish at landing sites of Lake Kyoga and its satellite lakes. About 70 ─ 100 fingerlings are packed in a 20-L plastic jerrican and transported without aeration. Lungfish baits are preferred to catfish bait because they can survive on long line hooks for more than five days. Therefore, lungfish bait competes favorably with African catfish bait in local markets. The bait industry for Nile perch fishery has not been fully exploited by hatchery operators as 50,000 bait fish per day are demanded on Lake Victoria (Ponzoni and Nguyen 2007).

Wholesale prices for fresh lungfish range between US $ 0.9 to 1.80 per kg while retail prices can go beyond US $ 2.5 per kg depending on the location. Price for cured lungfish products (e. g smoked form) range from US $ 3.00 to 5.00 per Kg. Lungfish prices in rural areas are relatively lower than in populated towns or cities.

5.0.0 Conclusion

The study assessed indigenous practices and understandings about lungfish as a potential culture species in Uganda. Fish farmers have inadvertently farmed lungfish that entered their ponds during flood periods. It is understood that they survive and grow alongside tilapia, for example, but optimal feed composition and lungfish grow-out strategies remain to be articulated. At present, growers are reliant on wild-caught lungfish fingerling for what limited culture is currently taking place.

Research must clarify the reproductive cycle of the lungfish to enable farm-based spawning and seedstock production of uniform batches of genetically advantaged fish. A clear foundation for establishing an industry, the biology and manipulation of lungfish reproduction processes are not well-understood.

An experiment program is needed to establish production parameters since little is known about the growth cycle and nutritional needs of farm-reared lungfish. For example, optimal water temperatures, salinity tolerance, and other basic parameters of the species are not known.

Farmers have developed indigenous means for handling and managing lungfish in natural water bodies and farm ponds. These are a beginning to be discovered and codified. Promoting wider levels of production of lungfish will require articulation of model production strategies and management systems that account for the burrowing and mobility of lungfish. Clearly, cage culture would overcome some of the known difficulties, but this work has not yet been accomplished.

Lungfish is a delicacy among groups in the Northern, Eastern and some parts of western Uganda. Thus the present and potential consumer demand for the species is fairly well-established. The field work assessed potential paths for producer adoption and training to use lungfish as a culture species and a managed water body resource.

Lungfish may be raised on artificial diets as all fish farms that had the fish in their ponds applied commercial pellets to catfish or tilapia stocked. Efforts to domesticate African lungfish are fundamental for the advancement of a commercial industry providing a valuable food item to people in need of affordable protein.

This study shows how initiatives to culture the fish based on indigenous knowledge and practice to formulate a broader strategy of widespread production. Future studies will explore the relative advantages of different culture systems (tanks, ponds and cages), while addressing specialized procedures for grow-out and harvest.

The socioeconomic viability of African lungfish as a new culture species is beginning to be established. This report identifies the central issues of reproduction, feeding, and management that must be addressed in order to build an industry with a value chain that delivers quality products to consumers and a sustainable return to small- and medium-scale producers in Uganda and across Sub-Saharan Africa.

CHAPTER III

Performance of African lungfish fingerlings fed three commercial diets

1.0.0 Abstract

The availability of African lungfish to many communities in Uganda is declining. Indigenous efforts to culture this fish is dependent on feeding wild-caught lungfish fingerlings with fish fry, minced meat and food leftover which usually produce poor yields. This study evaluates three formulated diets containing diet-1, 2 or 3 that were fed to wild caught lungfish fingerlings reared in in-door tanks for a period of 77 days. Experimental fish gradually accepted sinking pellets and marginal increases in average body weight were observed. Mean (± SE) final weight (15.86 ± 0.80 g) for fish fed on diet-3 was significantly higher ($p < 0.05$) than fish fed diet-1 and diet-2. Specific growth rates (SGR) for diet-3 (0.50 ± 0.06%/d) were significantly higher ($p < 0.05$) than diet-1 (0.27 ± 0.03 %/d), and marginally more than diet-2 (0.37 ± 0.04 %/d). Feed conversions were similar (p >0.05) ranging from 1.61 ± 0.26 to 2.07 ± 0.11. Survivals after an 11-week culture period were relatively low (< 60%) but generally increased (R^2 = 0.667, P = 0.0071) with increasing dietary proteins. Diet-3 (57.50 ± 2.85%) had a significant higher survival rate ($p< 0.05$) than diet-1(45.83 ± 3.44%) and diet-2 CP (40.84 ± 2.10%). All water quality parameters were within recommended aquaculture ranges. Cannibalistic engagements exposed most experimental fish to diseases; primarily fungal infections and subsequent mortalities. Regeneration of injured appendages (fins) was observed. Significant growth performance is attained with diet-3 but optimal dietary proteins beyond this level need to be determined. This study demonstrated that sinking fish feed pellets can be used to culture wild-caught African fingerlings in captivity. Poor growth and high mortalities experienced in this study may be due to i) sub-optimum dietary protein levels, ii) cannibalism,

iii) disease infections, iii) density, iv) contaminants in the feed and, iv) wrong management protocols. Additional studies will be needed to assess the culture potential of African lungfish due to high rates of mortality that occurred in the present study.

2.0.0 Introduction

Wild stocks of African lungfish in Uganda are declining (Goudswaard et al. 2002; Balirwa et al. 2003) and many communities derive livelihoods from its products (Seeley et al. 2009; Walakira et al. 2012; Van Dam et al. 2013). Policies to enhance lungfish production are not available, therefore, vulnerable groups can adopt aquaculture technologies to improve living conditions. Brummet and Williams (2000) identify aquaculture as the best alternative to increase food security and income for rural poor in Saharan African region. However, lack of appropriate technologies to promote lungfish aquaculture has derailed farmers' efforts to domesticate it.

African lungfish is an active foraging, carnivorous fish that prefers feeding on mollusks, aquatic insects, crustaceans, worms and small fish (Curry ─ Lindahl 1956; Corbet 1961; Okedi 1990; Pabari 1997; Amongi et al. 2001; Jorgensen 1984, 2005; Mlewa et al. 2011). Little information is available on the use of formulated feeds for the culture of lungfish. Current farm practices consist of raising wild caught lungfish seed on food leftovers or integrating with existing fish stocks (e.g. Nile tilapia and African Catfish) but usually attain low yields (Walakira et al. 2012). Baer et al. (1992) successfully raised *P. amphibious* on a mixture of minced, raw beef heart and minced, cooked tilapia meat but the ingredients used are expensive for an ordinary farmer.

Global attempts to domesticate new species normally begin with wild caught fish seed that is fed on formulated feed. For example, wild caught Artic charr (*Salvelinus alpinus*) and snakehead (*Channa striata*) seed were previously cultivated using artificial diets under small-scale farming systems in northern Europe or North America, and Vietnam, respectively (Jobling et al. 1993; Qin and Fast 1996; Qin et al. 1997). Similarly, wild fry/fingerlings of Florida pompano (*Trachinotus carolinus*), goby (*Pseudapocryptes elongates*) and Groupers (Serranidae: Epinephelinae), are being raised on formulated artificial diets (Lazo et al. 1998; Anh et al. 2011; Petersen et al. 2013).

Successful farming of new species depends on technologies that expedite breeding in captivity, acceptance of artificial feed, and economic, environmental and social factors that influence its production (Webber and Riordan 1975; Teletchea and Fontaine 2012). The biology and aquacultural approaches for threatened populations using sustainable technologies will enhance conservation of endangered fish species (Lorenzen et al. 2012). Fish feed development for aquaculture industry in Uganda is improving (Blow and Leonard 2007; Matsiko et al. 2010; Masette 2013) but commercial feeds not widely used by many small-scale fish farmers (Bukenya et al. 2013). Currently, Ugachick, ARDC-Kajjansi and NUVITA are major commercial fish feed producers in Uganda. Ugachick produces 4000 tons, annually, of floating fish feeds and 40% is sold to local markets (Daily Monitor 2012).

African lungfish is potentially more productive in aquaculture systems since it is an air breathing fish, and survives prolonged droughts or stressful water conditions (Greenwood 1998; Ilves and Randall 2007; Otero 2011). Efforts to nurture lungfish in captivity mostly rely on minced meat and food leftovers. Currently, commercially produced pelleted fish feed are available for farmers in Uganda. This study, therefore, evaluates the growth, survival and food utilization capacity of wild caught lungfish fingerlings fed on three commercial diet-1, 2 and 3.

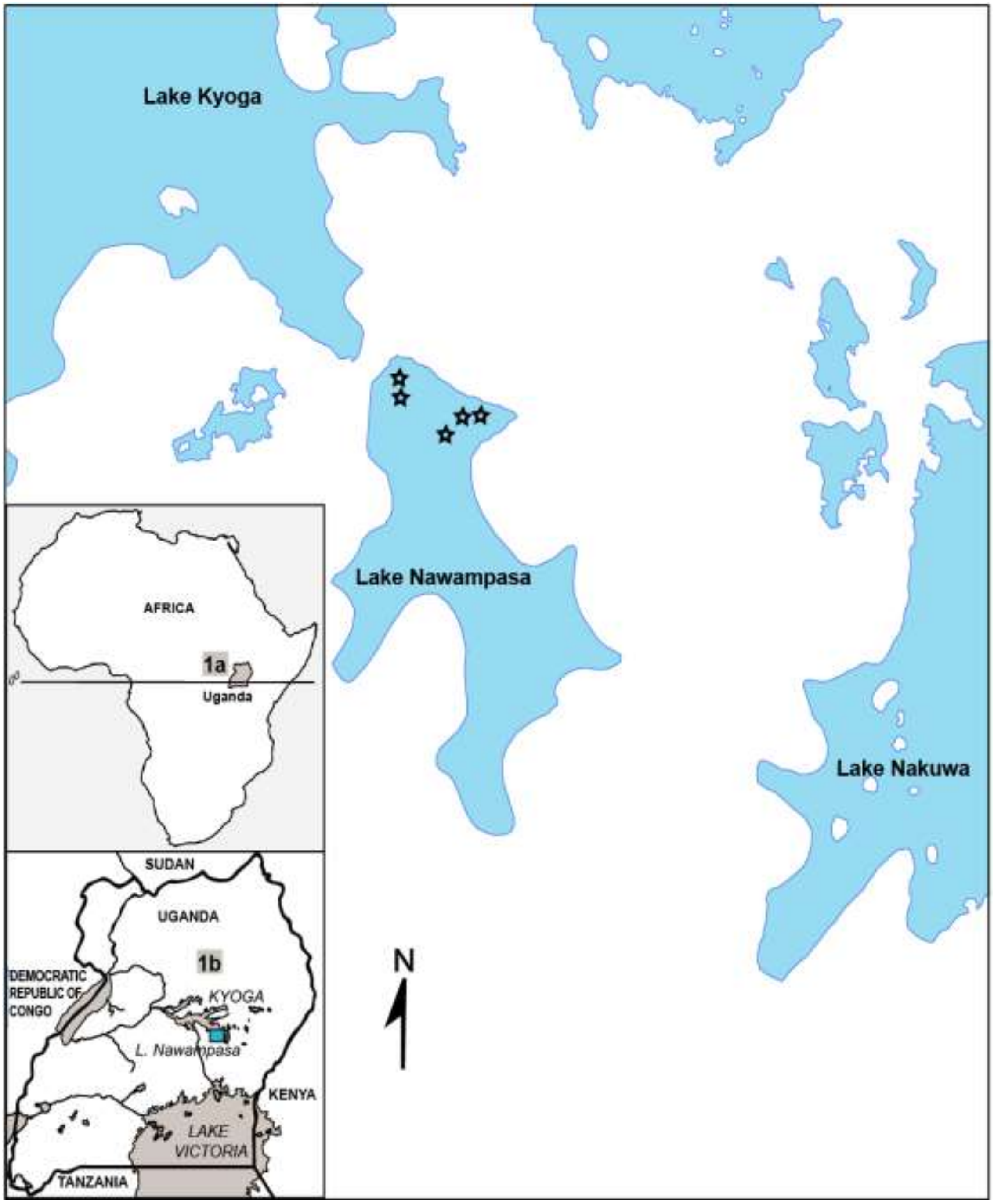


Figure 3. 1 . *Collection sites for experimental African lungfish fingerlings.*

3.0.0 Methods and materials

3.1.0 Collection of wild-caught African lungfish fingerlings

Experimental fish were collected from Lake Nawampasa, a satellite lake of Lake Kyoga, with guidance from resident fisher-folk and district fisheries officers. Lungfish fingerlings ranging (15 ─ 25 cm, Total length) were harvested using basket traps, jericans (20-L) and seine nets during rainy season (October, 2012 to January, 2013). Mean (± SD) water physiochemical parameters of targeted sites were; Dissolved Oxygen (4.69 ± 0.79 mg/L), pH (5.27 ± 0.59), Temperature (25.03 ± 0.37 C) and Secchi depth (0.52 ± 0.08 m). Mean (± SD) water depth was 0.69 ± 0.21 m and main aquatic macrophytes (floating and submerged) include; water lilies (*Nymphaea sp*), hornworts (*Ceratophylum sp*) and water hyacinth (*Eichornia crassipes*).

Fish were graded and 1600 fish were gathered in a happa (2 x 2 x4 m^3). The fish was conditioned for 24 ─ 48 h at the landing site, prior transportation to Aquaculture Research and Development Center (ARDC)-Kajjansi (Uganda). Lungfish juveniles were transported in 1000 L plastic tanks at a capacity of 100-200 g/L and oxygen monitored at 6 ─ 8 mg/L. All experiments were conducted at ARDC Kajjansi, N00.22470, E032.53395, and Elevation of 1132m.

3.2.0 Effect of commercial diet on growth performance

This investigation followed approaches by Li and Lovell (1992) but with modifications. An eleven-week experiment was conducted to evaluate the effect of different dietary protein levels on growth, feed conversion ratio, and survival of wild caught African lungfish fingerlings. Fish were fed on three commercial diets ─1, 2 and 3 that had fish meal made from Silver cyprinids or mukene (*Rastrineobola argentea*) which provided 68.32% of the total protein. These were sinking pellets of size diameter ranging 4 – 5 mm; small enough to pass through the mouth-gape lungfish juveniles.

3.2.1 Prior the experiment

Fish were fed once, daily, during day time between 1100 ─1300 h for three and half weeks. Soon after the training period, fish was conditioned for 24-hour through starvation, to empty their stomachs prior handling.

3.2.2 Stocking rate and culture systems

In-door trials were conducted in twelve Crest fibre glass tanks (60 L); 3 treatments and four replicates. Each replicate was stocked with 30 lungfish fingerlings of mean weight of 9.74 ± 0.12 g and mean length of 13.74 ± 0.33 cm since insufficient numbers were available for this experiment. Each tank was filled with 50-L of borehole water, aeration provided, and 50% of tank water was exchanged three times per week. Fish were mostly kept in a dark environment and light was used during feeding or sampling exercises.

3.2.3 Feeding

Fish fed to satiation twice daily between 0900 ─ 1100 hrs and 1500 ─ 1700 hrs. Fish were kept under 6 h light: 18 h dark photoperiod during the experimental period. Fish were mostly kept in a dark environment and light was used during feeding or sampling exercises.

3.2.4 Water quality parameters

Water quality parameters , dissolved oxygen (DO), temperature (T) and pH, were measured, daily using a Multiprobe System (YSI 556 MPS, 12L 101056, USA). Total Ammonia-nitrogen (TAN), and Total Nitrite-nitrogen were measured once per week using a FF2A Aquaculture Test Kit (Aquatic Eco-Systems, Inc., Apopka, Florida, USA) to monitor toxic ammonia build-up. Water quality parameters in all treatments were maintained to levels favorable for fish growth.

3.2.5 Sampling and mortalities

Fish sampling was done bi-weekly to measure change in weights, specific growth rates (SGR) and corresponding FCR. A random sample from each unit (30 % of population) was harvested and tranquilized using 50 mg/L of MS ─ 222 in a 10-L bucket to avoid injuring the fish. Individual fish were measured for weight (g) and Total length (mm).

Moribund fish were counted, recorded and immediately euthanized with MS ─ 222 for necropsy procedure. Dead fish in each tank were also counted daily, recorded and removed.

3.2.6 Approximate analysis of experimental feeds

Samples of experimental feed were analyzed at the nutrition laboratory of College of Agricultural and Environmental Sciences, Makerere University. Composition of crude protein, fat, moisture, ash, and gross energy in each diet was analyzed in duplicates. Feed samples were collected every week for analysis to determine any variations in quality.

3.2.7 Termination of experiment

The experiment was terminated at a point when growth curves showed differences within treatments. All fish were anaesthetized using 125 mg/L of MS ─ 222 in a bucket containing fresh water (10-L) to avoid injuries during measurements. Total number and weight and total length per fish were recorded

Average initial and final weight, specific growth rate (SGR), food conversion ratio (FCR), and survival rate (%) of fish fed various experimental diets.

i) Specific growth rate (SGR) = {[(log final body weight-log initial body weight)/time] x 100},

ii) Food conservation ratio (FCR) = dry food intake/live weight gain,

iii) Survival rate = [(initial no. of fish/final no. fish) x 100] and

iv) Weight gain (%) = {[(final weight - initial weight)/initial weight] x 100}.

3.2.8 Data analysis

This experiment used a random complete block design model ($Y_{ij} = m + T_i + B_i + R_{ij}$). Hence the assumptions were that data was normally distributed. Effects of commercial diets were analyzed through one-way analysis of variance (ANOVA). Multiple regressions were performed to account for differences in proximate analysis. Difference in treatments was analyzed using Tukey-Kramer HSD at 95 % CI using SAS 9.2 program.

4.0.0 Results and Discussion

Proximate composition of commercial diets used in this experiment is shown in Table 4. Results showed no significance difference (p= 0.0542) in percentage proteins between commercial diets 2 and 3 but differences were in other dietary components. The dry matter and gross energy was different in commercial diet -1, 2 and 3 but similarities were in ash, fat and crude fibre contents. The total ash content of diet-2 was significantly higher than diet-1 (p= 0.0110) and diet-3 (p= 0.0124).

Table 4. *Proximate composition of feeds used in this study.*

Diet	DM	Total Ash	Crude Protein (%)	Fat (%)	Crude Fiber (%)	Gross Energy (Kcal/kg)
1	90.78 ± 0.06^{a}	8.68 ± 0.10^{b}	29.12 ± 0.96^{a}	7.23 ± 0.06^{a}	5.81 ± 0.71^{a}	3964.16 ± 5.40^{a}
2	92.69 ± 0.01^{b}	9.06 ± 0.00^{a}	33.63 ± 0.88^{b}	8.11 ± 0.11^{a}	7.25 ± 0.78^{ab}	4373.94 ± 3.97^{b}
3	94.88 ± 0.14^{c}	8.69 ± 0.00^{b}	36.70 ± 0.86^{b}	10.66 ± 0.43^{b}	9.74 ± 0.56^{b}	4405.96 ± 3.69^{c}

Mean ± S.D, and values within a column with a different superscript letter are significantly different (P<0.05).

Percentage fat content of diet-3 were significantly higher than diet-1 (p= 0.0019) and diet-2 (p= 0.0045). Diet-2 had no difference in crude fibre content compared to diet-1 and 3. Collectively, components of diets 1, 2, and 3 resulted in treatment differences in terms of growth and survival.

4.1.0 Growth

A summary of growth performance of lungfish juveniles cultured in tank environments is shown in table 5. Wild caught African lungfish juveniles gradually accepted commercial diets and average body weight increased marginally with increasing dietary protein levels. Good growth performance was achieved with lungfish that fed on diet-3. Similarly, maximum growth of Tilapia and African catfish juveniles is achieved with dietary crude protein levels ranging 35 – 55% under tank conditions (Davis and Stickney 1978; Degani et al. 1989; De Silva and Anderson 1995).

Table 5. *Growth performance, feed utilization and survival of African lungfish (Protopterus sp.) reared in tanks for 11-weeks.*

	Treatment 1 (Diet-1)		Treatment 2 (Diet-2)		Treatment 3 (Diet-3)	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Initial Weight (g)	9.74 ± 0.13^{a}	(9.64-9.86)	9.72 ± 0.15^{a}	(9.62-9.85)	9.77 ± 0.15^{a}	(9.58-9.95)
Final Weight (g)	11.65 ± 0.59^{a}	(10.86-13.37)	12.92 ± 0.44^{a}	(11.73-13.87)	15.86 ± 0.80^{b}	(13.86-17.77)
Weight gain (g)	2.36 ± 0.32^{a}		3.39 ± 0.41^{ab}		5.10 ± 0.74^{b}	
SGR (%/day)	0.27 ± 0.03^{a}		0.37 ± 0.04^{ab}		0.50 ± 0.06^{b}	
FCR	2.07 ± 0.11^{a}		1.87 ± 0.22^{a}		1.61 ± 0.26^{a}	
Survival (%)	45.83 ± 3.44^{a}		40.84 ± 2.10^{a}		57.50 ± 2.85^{b}	

Means in the same row with same letter in superscript are not significantly different ($p < 0.05$) by ANOVA.

Attempts to grow *P. amphibious* juveniles in captivity only yielded good growth with meat and bone meal (Baer et al. 1992). However, Baer et al. (1992) used low stocking densities (10 fish per tank) compared to 30 fish per tank used in this study. Nevertheless, wild caught lungfish juveniles in this study generally performed poorly when cultured in tanks (Fig. 3.2).

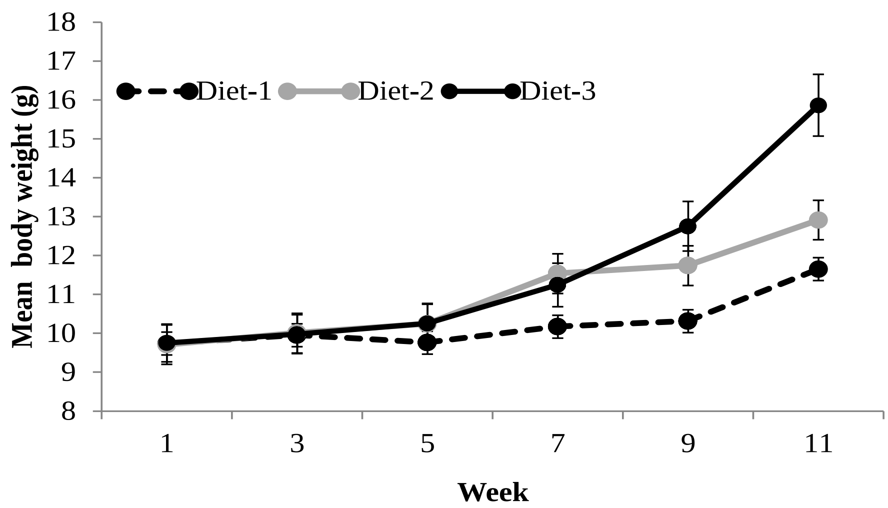


Figure 3. 2 *Trend in average body weight for the 11-week experiment. Errors bars indicate mean of quadruplicates (±SE).*

4.1.1 Final weight

Initial mean weights among treatments were not significantly different (p= 0.9743). Diet-3 produced higher final mean weights (p= 0.0027) compared to other treatments. Carnivorous fish (e.g snakehead and lungfish) require high protein diets for maximum growth (Tacon and Cowey 1985; De Silva and Anderson 1995). Several carnivorous fish species grow well with diets containing 40─50% crude proteins (Wilson and Halver 1986: Steffens 1989; Lazo et al. 1998). Snakehead fingerlings or juveniles require a minimum of 50% crude proteins to maximize growth weight under captivity (Mohanty and Samantaray 1996). Therefore, increasing dietary proteins in feed enhances weight of carnivorous fish. Data from this experiment conforms to previous studies that had same percentage protein levels with diets-2 and 3.

4.1.2 Net weight gain, Specific growth rate, Feed Conversion ratio and survival

After 11 weeks of culture, net weight gained from diet-3 (5.10 ± 0.74g) was significantly higher (p=0.0542) than those raised on diet-1 (2.36 ± 0.32g) and diet-2 (3.39 ± 0.41g). The SGRs for lungfish juveniles in all treatments were relatively low for 11-week growth period.

SGR values increased from 0.27 to 0.50%/d and diet-2(0.37 ± 0.04%/d) had similar rates with treatment 1and 3. The SGR for diet-3 (0.50 ± 0.06%/d) was higher (p= 0.0009) than diet-1 (0.27 ± 0.03%/d) but not different (p= 0.1046) to diet-2. The SGRs for diets-1 and 2 were not significantly different (p=0.2294). Low SGR values (0.048 to 0.140% per day) are also reported with African lungfish juveniles (Mlewa et al. 2009) but Baer et al. (1992) achieved a higher SGR (1.52% per day) with *P. amphibius* raised in tanks though at low stocking densities.

Feed conversion ratios did not vary significantly (p = 0.3516) among treatments. Juveniles that fed diet-3 had an FCR of 1.61 ± 0.26 while those under treatment-1and 2 had 1.87 ± 0.22 and 2.07 ± 0.11, respectively. Therefore, it costs about $ 1.92 to raise 1 kg of lungfish when using these commercial diets 1, 2 and 3. Farmers have to target premium markets to break even to pay all expenses incurred during its production. Several studies show variations in FCR values when carnivorous fish is fed different protein levels. These include largemouth bass by Portz et al. (2001); hybrid clarias catfish by Giri et al. (2003); pike perch (*Sander lucioperca)* by Schulz et al. (2007); snakehead (*Channa striatus*) by Aliyu-paiko et al. (2010); and blue gourami (*Trichogaster trichopterus*) by Mohanta et al. (2013). The FCR of cultured Nile tilapia and African catfish is usually 2.0 using locally available commercial feed (Hecht 2007).

Multiple regressions showed gross energy and fat levels did not affect the specific growth rate (SGR) and food conversion rate (FCR). Weight gain and survival rate increased linearly with rise of dietary fats and reduced linearly in weight gain with energy levels in the diet (Table 8). Energy levels and fat (%) did not influence the FCR and SGR; probably the energetic levels in the diets were not adequate to increase survival of lungfish juveniles.

Table 6. Weight gain, specific growth rate, food conversion rate and survival rate of *Protopterus sp*. juveniles presented by regression equations based on gross energy (kcal kg^{-1}) and fat (%) in the diets.

Factor of variation	Prediction equation	R^2 (%)
	Weight gain (g)	
Gross energy	$\hat{y} = -25.12 + 0.01X$	42.21
Fat (%)	$\hat{y} = 3.75 + 2.37X_1 - 1.83X_2$	69.58
	Specific growth rate (% day^{-1})	
Gross energy	ns	–
Fat (%)	ns	–
	Food conversion rate	
Gross energy	ns	–
Fat (%)	ns	–
	Survival rate (%)	
Gross energy	ns	–
Fat (%)	$\hat{y} = 48.06 + 9.44X$	66.74

ns = values are non-significant based on F-test at 5% probability.

Survival rates were consistently low but generally improved with increasing dietary proteins (Fig. 3.3). Lungfishes are known to have long captive records of more than 20 years (Genade et al. 2005) and can starve for 3 to 4 years (Coates 1937; Smith 1939; El Hakeem 1979) but in this study high mortalities occurred. Diet-3 produced higher survival rates (57.50 ± 2.85%) than fish diet-1 (45.83 ± 3.44%, p= 0.0423) and diet-2 (40.84 ± 2.10%, p= 0.0064). Survivals under diets 1 and 2 were not significantly different (p=0.4601). Survival rates 66 to 100% were achieved when snakehead (*C. striata*) fingerlings were fed on different protein diets

(35 ─ 45% Crude proteins) with low survivals attained with diets containing low proteins (Aliyu-Paiko et al. 2010a; Aliyu-Paiko et al. 2010b).

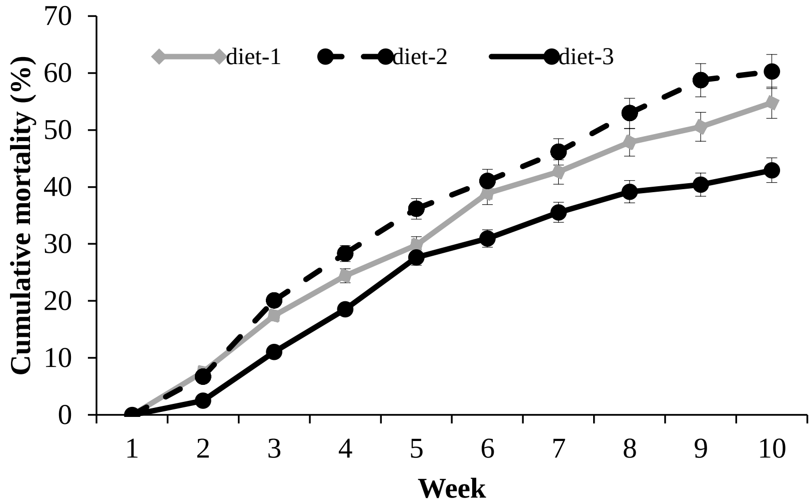


Figure 3. 4 . *Cumulative mortalities of African lungfish under experimental conditions.*

Injuries produced during cannibalistic engagements, caused most experimental fish to be susceptible to disease infections. Numerous juveniles had their caudal fin bitten off making it hard determine the Condition Factor of this experiment. Generally, the TL reduced during the first 43 days but progressively increased in the reaming period (Fig. 3.5). African lungfish has a continuous diphycercal tail (Bemis et al. 1987) and tips were mostly bitten off; distorting standard and total length measurements. Fish under diet-1 treatment were most affected than those under diet-2 and 3. Diet-1 had the lowest protein levels hence lungfish juveniles probably supplemented their energy requirement from their own tails or other fish in the tank. Increased food availability, improved quality and dark conditions may reduce cannibalism in lungfish as observed in several studies (Fox 1975; Hecht and Pienaar 1993; Qin and Fast 1996; Jesu and Appelbaum 2011). The increase in TL is explained by African lungfish's ability to regenerate its appendages after injuries (Conant 1970, 1972, 1973) through re-development of the endoskeleton structure of endochondral bones (Tamura et al. 2010).

These juveniles were trained for three and half weeks under 12h light: 12 h dark photoperiod. Response was initially slow but improved towards end of training period. The acclimatization period may be insufficient or prolonged exposure to light affected them. The dietary fish meal in the commercial feed may be inadequate to attract lungfish juveniles as response was poor. Attractive ingredients and good texture diets improve the palatability and acceptance of formulated diets by carnivorous fish (Kubitza and Lovshin 1999). However, increasing the amount of fish meal to lungfish diets will directly increase the costs of formulated feeds. This may not be cost effective for the rural poor fish farmers as prices for lungfish feed will be high.

Feeding response was also gradual during experimentation. In the first three weeks, few fish were seen to nibble on pellets soon after application but response improved slightly towards the end of the trial. Delayed response increased chances of feed pellets to dissolve in water during the day making it difficult for the fish to feed at night. Lungfish is active throughout the diel cycle in the wild but becomes intensive during the last hours of the day (Mlewa et al. 2011). Hence, the feeding protocol in this experiment may not be appropriate for this fish species. Instead, fish could have been fed in the late hours of the day. However, presence of fecal matter indicated that these diets were ingested and utilized.

Poor response or rejection of complete practical diets while voraciously accepting unbalanced diets is reported in some carnivorous fish species (Kubitza and Lovshin 1999). Kemp (1994) had also difficulty in feeding hatchlings of the Australian lungfish (*Neoceratodus forsteri*) under laboratory conditions. Baer et al. (1992) had no growth in *P. amphibius* juveniles raised in tanks and fed on pellets; feeding at 10% of the total biomass per day (dry weight), 6 days a week. Lungfish is considered an omnivorous carnivore (Greenwood 1986) that prefers snails (Daffalla et al. 1985). Snail shells and flesh have about 3 and 20% crude proteins, respectively (Fagbuaro et al. 2006; http://www.hortikultur.ch/pub/files/245.pdf). Probably, formulating a diet that has snail components would be useful for lungfish aquaculture. Freshwater snail shells are also raw materials for minerals used in animal feed (Rutaisire 2007) and are readily available in Uganda. Therefore, small scale farmers can adopt snailary that has been shown to produce 40 snail shells per 1-m3 box per year (Sonaiya 1995).

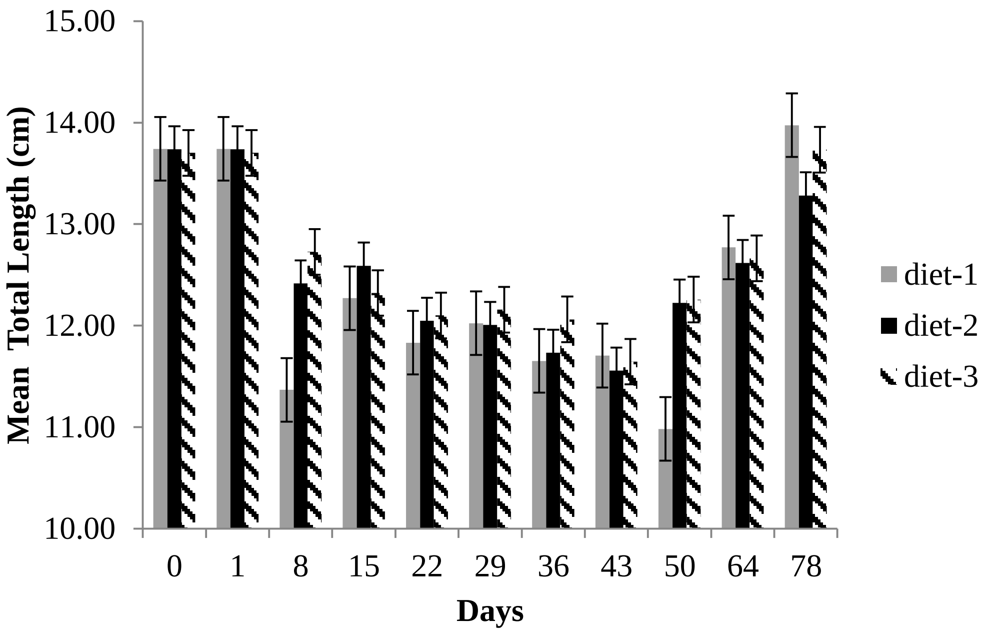


Figure 3. 5. *Mean Total length (SE) of African lungfish under experimental conditions. Cannibalism is the cause of length reduction and regeneration of caudal fin is evident as size increases.*

African lungfish is a carnivorous fish that consumes a wide variety of prey including mollusks, fish fry or fingerlings, aquatic crustaceans and insects, and worms (Curry-Lindahl 1956; Corbet 1961; Mlewa et al. 2011). Its relatives, the South American and Australian lungfish, are reported to be plantivorous (Kemp 1986; Mlewa et al. 2011). Consumption of plant materials by *Protopterus sp*. would be advantageous to small holder fish farmers and this should be explored. Carnivorous fishes require high dietary protein because of their inability to utilize dietary carbohydrates for energy requirements (Boonyaratpalin and Williams 2002; Stone 2003). This explains the good growth attained with diet-3 compared to others. Kubitza and Lovshin (1999) recommend adequate supply of food, grading fish to same sizes and stocking optimal densities to avoid cannibalism in juvenile fish. These recommendations were adopted but cannibalism occurred in this experiment. Dietary contents of experimental feed may have been low to meet optimal growth requirements of lungfish juveniles. Furthermore, fungal contamination of feeds used in this experiment is possible. Mycotoxins produced by fungus in feeds severely affect the health of cultured fish (Bacon and Williamson 1992; Goel et al. 1993).

Microbial infections resulting from cannibalism usually lead to mortalities (Kubitza and Lovshin 1999). Necropsy of moribund and dead fish revealed infections with *Flavobacterium columnare*, *Aeromonas sp*, *Pseudomonas sp* and fungal secondary infections. These pathogens are ubiquitous and opportunistic, infecting fish that has damages and when water quality conditions are poor. Disease infections were evident during third week and clinical cases continued to appear until end of the experiment. Disease occurrence could have affected lungfish growth kept in tanks. Stress related disease affect feeding in fish resulting into poor growth rates (Iwama et al. 2011; Roberts 2012).

4.2.0 Water quality

Results for the water quality parameters during the 11-week investigation are presented in Table 4. Mean water temperature (± SE) for treatment T_1 (diet-1), T_2 (diet-2) and T_3 (diet-3) were 23.91 ± 0.62, 23.88 ± 0.66 and 23.98 ± 0.61 C, respectively. Average dissolved oxygen (DO) concentrations for treatments T_1, T_2 and T_3 were 5.13 ± 0.39, 5.27 ± 0.34 and 5.09 ± 0.46

mg/L, respectively. Mean Un-ionized ammonia concentration were 0.002864 ± 0.00096, 0.003131 ± 0.00084 and 0.002406 ± 0.00111 mg NH_3/L for treatments T_1, T_2 and T_3, respectively. Mean Total alkalinity for T_1, T_2 and T_3 were 165.33 ± 1.45, 161.67 ± 1.86 and 164.00 ± 2.31 mg/L, respectively. All experimental water parameters were within acceptable levels recommended to grow warm water fish (Boyd 1982; Swann and Sea 1992).

Table 7. *Water quality means (± SD) measured during the experiments.*

	Treatment		
	Diet-1	Diet-2	Diet-3
pH	7.54 ± 0.31[a]	7.56 ± 0.41[a]	7.48 ± 0.29[a]
DO (mg/L)	5.13 ± 0.39[a]	5.27 ± 0.34[a]	5.09 ± 0.46[a]
Temperature (C)	23.91 ± 0.62[a]	23.88 ± 0.66[a]	23.98 ± 0.61[a]
Un-ionized TAN (mg NH_3/L)	0.002864 ± 0.00096[a]	0.003131 ± 0.00084[a]	0.002406 ± 0.00111[a]
Total alkalinity (mg/L)	165.33 ± 1.45[a]	161.67 ± 1.86[a]	164.00 ± 2.31[a]

Means with same superscript letter in each row are not significantly different ($p < 0.05$) by ANOVA.

Temperatures ranged from 23 to 25 C with less variation during the course of the experiment (Fig. 3.4). Recommended aquaculture temperatures for warm water fish in Africa range from 25 to 30 C (Swann and Sea 1992; Kapetsky 1994). In this study temperature was favorable for fish growth and stress-related mortalities were unlikely. Stress conditions in tropical fish are usually induced when water temperatures fall below 15 C (Boyd 1979; Summerfelt 1998). African lungfish can survive wide temperature fluctuations that prevail in weedy natural aquatic environments (Greenwood 1986; Mlewa et al. 2011).

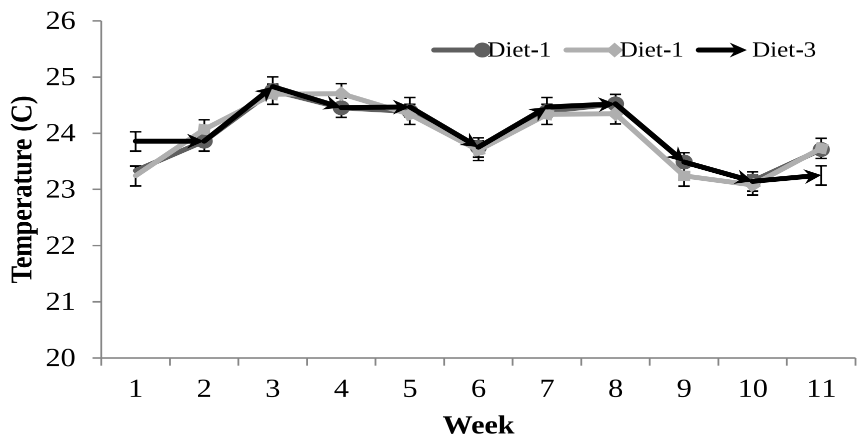


Figure 3. 6 . *Trend in temperature during the 11-week period of experimentation. Errors bars indicate mean of quadruplicates (±SE).*

A few experimental lungfish were seen swimming to the surface when low DO concentrations (<4 mg/L) lasted 2 to 3 hours (Fig. 3.6). African lungfish is an obligate air-breather hence low DO levels would not be limiting. Aerial respiration in lungfish occurs when DO levels reach lethal levels of 0─2 mg/L (Mlewa et al. 2011). Fecal and suspended material elevates system's biological oxygen demand (not measured) but this was minimized through regular siphoning of debris and water exchange. Lungfish adopts aquatic respiration to reduce energy when, i) swimming vertically in water and, ii) avoiding predators (Mlewa et al. 2011). Fingerlings or juveniles have the capacity to survive in short exposures of lethal DO concentrations (< 1 mg/L) and their utilization of oxygen per unit weight is higher than adult fish (Boyd and Tucker 1998). African lungfish (*P. aethiopicus*) juveniles can survive in hypoxic environments and this protects them against predators (Mlewa et al. 2011).

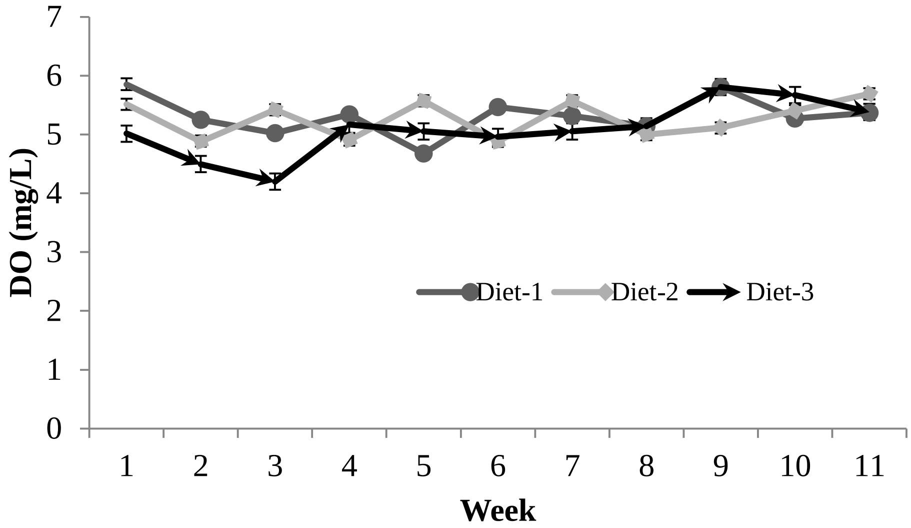


Figure 3. 7 . *Trends in Dissolved Oxygen for the 11-week experimentation. Errors bars indicate mean of quadruplicates (±SE).*

No significant differences (p= 0.9861) in pH were observed between treatments despite the occasional presence of fecal and debris that accumulated, overnight. Metabolic wastes (nitrogenous and phosphorus wastes) egested through fecal material affect the water quality, and if fish are exposed for long periods, growth and health is affected. Good growth of freshwater fish occurs when pH range from 6.5 to 9 (Boyd and Tucker 1998). The bore-hole water had high Alkalinity levels to buffer wide pH fluctuations.

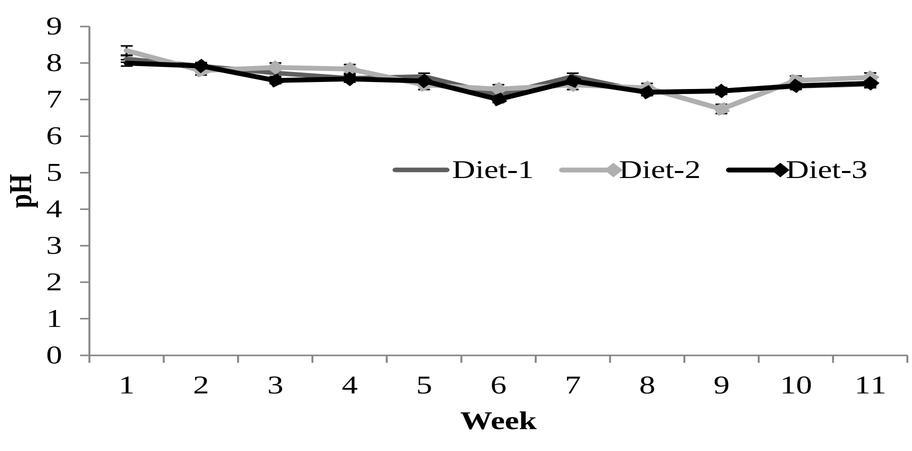


Figure 3. 8 . *Trends in pH for the 11-week experimentation. Errors bars indicate mean of quadruplicates (±SE).*

Experimental Total Ammonia-Nitrogen (TAN) levels were 0.1 mg/L (N). Calculated un-ionized ammonia based on pH and temperatures ranged from 0.00052 to 0.01171 mg/L (NH_3). Mean levels of un-ionized ammonia were moderately high in week-1 but gradually reduced towards the end of the experiment (Fig. 3.7). In week-1, the biomass of lungfish juveniles was high enough to produce substantial excretory (nitrogenous) by- products in tanks. Subsequent water exchanges, however, minimized this effect. Fish biomass also reduced in the following weeks when mortalities occurred and this reduced waste by-products in the culture systems.

Un-ionized ammonia is toxic to fish and acute toxic level for freshwater fish species is 2.79 mg NH_3 /L (Randall and Tsui 2002). Once exposed to high levels of toxic ammonia, fish produce glutamine which accelerates detoxification process (Randall and Tsui 2002). Toxicity is reliant on levels of ammonia, temperature and pH of the environment hence high concentrations reduce survival rates, constrain growth, and lead to physiological dysfunctions (Thurston et al. 1981; Tomasso 1994; Boyd and Tucker 1998). When African lungfish (*P. dolloi*) is exposed to toxic ammonia, acidification rather than detoxification through urea production is adopted (Wood et al. 2005). This process assists lungfish to thrive in anoxic water conditions.

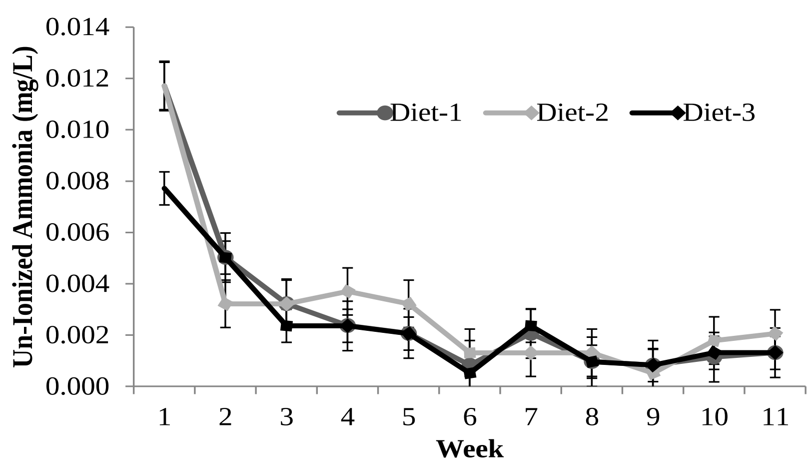


Figure 3.9 . *Trend in Un-ionized Ammonia during the 11-week experimentation. Errors bars indicate mean of quadruplicates (±SE).*

5. 0. 0 Conclusion

The hypothesis was that increase in dietary proteins promotes growth performance in lungfish juveniles. This is observed in this experiment despite the marginal growth. This study has also demonstrated that sinking commercial fish feed pellets can be applied to nurture wild-caught African fingerlings in captivity. However, poor growth and high mortalities were experienced in tank conditions. This may be attributed to i) sub-optimum dietary protein levels, ii) cannibalism, iii) disease infections, iii) density, iv) contaminants in the feed and, iv) wrong management protocols. Unless these factors are adequately addressed this fish is not a good aquaculture candidate.

CHAPTER IV

Diseases associated with culturing African lungfish (*Protopterus sp.)*

1.0.0 Abstract

Information on diseases affecting African lungfish is not sufficient to develop control strategies for enhancing African lungfish production in captivity. It is prudent for aquaculturists to understand disease communities that threaten yields of aquaculture systems. This study identified potential pathogens associated with culturing African lungfish grown in tanks. Disease flora and fauna include bacteria (*Aeromonas* sp. and *Flexibacterium columnaris*), fungi (*Fusarium sp., Aspergillus sp. and Saprolegnia sp)* and parasites (*Dactylogurus sp*, *Trichodina sp*, *Tetrahymena* sp and Cestodes). A 60% fungal incidence occurred compared to monogenes (9%), tapeworms (25%) and bacteria (6%).

Lungfish is a potential aquaculture species in Sub-Saharan Africa region but initiatives to raise it in captivity has resulted into low yields. Culturists are in need of prophylactic agents that will improve its production. This study evaluates effects of two recommended chemicals (Salt and Formalin) on African lungfish juveniles kept in aquaria tanks. Results indicate that lungfish (7.78 ± 1.47 g) appear to be sensitive to saline conditions with LC50 of 2.59 and 1.84 for 24 and 96h, respectively. Lungfish behaves normally at low salt concentrations (0 ─1.6 g/L) but become lethargic within 4h when concentrations reach 4 g/L. Lungfish juveniles are tolerant to formalin and have LC50 of 220.8 and 193.8 mg/L, respectively.

2.0.0 Introduction

African lungfish (*Protopterus sp.*) is declining in abundance in Uganda (Goudswaard et al. 2002; Balirwa et al. 2003) but continues to support many communities in the East African region. It is a resilient, air-breathing fish (Greenwood   1958, 1986) which can be raised in captivity (Baer et al. 1992) and a potential candidate for aquaculture in Uganda (Walakira et al. 2012). However, initiatives to culture this fish are challenged with low yields that are mainly caused by slow growth and their tendency to escape from pond environments (Mlewa et al. 2009).

Existing information on diseases related to African lungfish is important for aquaculturists. Infectious diseases include, bacteria *Aeromonas hydrophila* and *Pseudomonas sp* (Kemp 1994), leeches of family Glossiphonidae (Hecht and Endemann 1998), facultative ciliates of *Tetrahymena sp* (Hoffman et al. 1975), digenetic trematodes of *Heterorchis sp* (Khalil 1971) and crustaceans of species *Argulus africanus* (Fryer 1962). Non-infectious diseases include tumors; i) seminoma and leiomyosarcoma affecting albino *P. dolloi* (Hubbard and Fletcher 1985), ii) spontaneous spermatocytic seminoma discovered testis of *P. aethiopicus* (Masahito et al. 1984) and spontaneous neurinoma in the skin of *P. annectans* (Ishikawa et al. 1986).

During the previous feed experiment many lungfish juveniles were dying from disease infections. Salt and formalin are the recommended chemicals allowed for use in food fish to treat aquatic diseases (parasite and fungal infections). The United States Food and Drug Adminstration (FDA) agency considers salt (NaCl) as a drug of low regulatory priority while formalin (37% formaldehyde) is a good parasiticide for use in tank systems (Francis-Floyd 2012).

Lungfish has an aquaculture potential in the sub-Saharan region but poor yields are the major challenges affecting its development (Walakira et al. 2012). Fish farmers use prophylactic agents like salt when planning to prevent disease outbreaks, reduce stress and improve fish production (Hine et al. 2010). Salt rates of 0.5 to 1.0% are used for osmoregulatory purposes or shock prevention and 3% for treating parasites (Noga 2010). Formalin is used in all finfish to treat against parasites like flukes protozoans and fungal infections (Noga 2010; www.fda.gov/animalveterinary).

Understanding the type of pathogens that threaten yields of lungfish under culture systems will guide aquaculturists to develop control strategies in future. This study identifies pathogens that were associated with culturing African lungfish in tanks. It also evaluates the tolerance of juveniles exposed to different doses of formalin and salt.

3.0.0 Methods and materials

Wild caught African lungfish fingerlings freshly caught from Lake Nawampasa (natural habitat) and those under experimental conditions at Aquaculture Research and Development Center (ARDC)-Kajjansi (Uganda) were examined. A total of 66 asymptomatic and symptomatic lungfish were screened for presence of diseases were examined for parasitology and bacteriology; 19 fish were freshly caught from the wild-caught and 47 moribund or dead lungfish with clinical signs collected from the feed trial. Organs targeted include the skin, gills, gastro-intestinal tract (GIT) and lesions.

3.1.0 Parasitology and bacteriology

Moribund fish were euthanized with MS ─ 222 prior examination between October (2012) and April (2013). Samples were examined using necropsy procedures described by AFS ─ FHS (2010) and Noga (2010). Presumptive identification of parasites was performed at College of Natural Sciences School of Biosciences, Department of Biological Sciences, Makerere University. Gross lesions on skins of diseased fish were recorded. Wet mounts from visual skin lesions and gills were observed microscopically for presence of ecto- and endo-parasites and fungal hyphae and bacteria (e.g colonies typical of *F. columnare*). Prevalence, mean intensity and mean abundance of parasites were determined following Bush et al. (1997) approach.

Presumptive identification of pathogens was based on standard or classical biochemical test performed at the microbiology laboratory unit in College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University. Necropsy was performed on lesions, gills, liver and kidney obtained from moribund or fish with clinical signs. Samples were aseptically inoculated on to Brain Heat Infusion agar (Difco Laboratories, USA) for general growth and isolation of bacteria. Columnaris (*Flavobacterium sp.*) was detected using Hsu-Shotts selective media and Tryptone yeast extract salts (TYES) agar. Wet mounts from lesions and gills were examined microscopically for presence of bacteria (e.g colonies typical of *F. columnare*)

Inoculated plates were incubated at 26 to 28 C for 16 to 24 h, depending on growth bacteria on media. Colonies on non-selective and selective media were examined using a light microscope for morphology, motility and gram stain. Conventional biochemical identification of pathogens used include cytochrome oxidase, L- lysine, L-Ornithine, L-Cystine plus maltose, sodium chloride, yeast extract, sodium deoxycholate catalase (Difco, USA).

3.3.0 Mycology

Moribund Fish with clinical signs of fungal infections were gently cleaned twice in distilled water to remove sediments. Isolation and identification of fungus was undertaken at microbiology laboratory, School of Veterinary Medicine and Animal Resources, college of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University (Uganda).

3.3.1 Isolation of fungi

Skin scraps of sites with fungal growth were inoculated onto Potato Dextrose Agar (PDA) medium (Smith and Onions 1983; Lloyd 1994). Media was supplemented with streptomycin (66 µg /L) to suppress bacteria growth. Plates were incubated at 28 ± 1.0 C for 5 to 7 days during which fungal growth was examined and identified, microscopically.

3.3.2 Morphology

Preliminary identification of fungal infestation was done using Potassium hydroxide (KOH) mount method. Drops of KOH (10%) were added on to samples of skin scraps mounted on microscope slide using a sterile loop. Presence of fungal materials was observed using a light microscope at low magnification (X 40). Clear images of fungal hyphae or colonies were augmented using 2-3 drops of Lacto Phenol Cotton Blue (LPCB) stain (Leck 1999). After day 7 of growth, pure cultures were obtained through sub-cultures on PDA and Carnation Leaf Agar (CLA) for pink color colonies suspected to be *Fusarium sp.* (Nelson et al. 1983; Noga 2010).

3.3.3 Salt and Formalin effects

Bioassays on lungfish fingerlings were conducted in April, 2013 at ARDC-National Fisheries Resources Research Institute (NaFIRRI) Pathology Laboratory Unit to test the tolerance of African juveniles to different salt (Kensalt Ltd – Gongoni Saltworks, Malindi – Kenya) and formalin concentrations. Data were obtained using internationally accepted guidelines (OECD 1992) and procedures described by Sprague (1969) and Greenlees   (1997).

Range-finding test

Each 40-L aquarium per treatment was stocked with five fish, weighing 7.78 ± 1.47 g. A total of 60 fish were subjected to different concentrations of Salt (Non-iodized salt, Sodium chloride, 98.5%) and Formalin (Formaldehyde Solution 37─ 41%)], in static aerated water for 24 hours. Dose concentrations were increased progressively (Table. 6) with six concentrations per chemical. Fish were not fed during the assay. Mortalities and behavioral changes were recorded every 0, 2, 4, 6, 8 and 24 h. Mortalities were removed and recorded, daily and all dead fish disposed according to NaFIRRI station SOP procedures. Necropsy and pathogen identification of diseases were performed in the College of Veterinary Medicine, Makerere University (Uganda). Water quality parameters (DO, Temperature, pH and conductivity) were recorded, daily using a Multiprobe System (YSI 556 MPS, 12L 101056, USA) and FF2A Aquaculture Test Kit.

Table 8. *Concentrations of Salt and Formalin used in determining the range test in African lungfish juveniles*

	Concentration per treatment					
Salt (g/l)	0	0.001	0.01	0.1	1	10
Formalin (mg/L)	0	250	500	750	1000	1500

Definitive test

From the range-finding test a meaningful concentration was obtained to determine the tolerance level (LC50) of lungfish. Under static conditions and aeration, concentrations were applied in geometric progressions. Salt (i.e. log [salt]) and formalin concentrations were doubled, progressively. In each 60-L aquaria tank, 9 fish (mean weight=7.78 ± 1.47 g) were randomly stocked in duplicates. Mortalities were recorded after 0, 2, 4, 6, 8, 24, 48, 72 and 96 h. Necropsy and pathogen identification of diseases were performed in the College of Veterinary Medicine, Makerere University (Uganda). Water quality parameters (DO, Temperature, pH, Total Ammonia-Nitrogen and conductivity) were recorded, daily using a Multiprobe System (YSI 556 MPS, 12L 101056, USA) and a FF2A Aquaculture Test Kit.

Table 9. *Concentrations of Salt and Formalin used in determining the definitive range in African lungfish juveniles*

	Concentration per treatment								
Salt (g/l)	0	1	1.6	2.5	4.0	6.3	7.9	10	10.5
Formalin (mg/L)	0	7.5	15	30	60	120	240	480	960

Percent mortality was calculated using the following formula;

Percent mortality = (number of dead fish/number of live fish present)*100

Mortalities are presented as pooled numbers from duplicates of treatments. The LC 20, 50 and 80 for salt and formalin were estimated using King and Farrell (2002) method and data was fitted using linear models.

Basal water quality measurements from water source (borehole) are; Hardness = 110 mg/L; Alkalinity = 128 mg/L; Chloride = 0.1 mg/L; Fluorides = 0.09 mg/L; conductivity = 271 µS/cm.

4.0.0 Results and Discussion

4.1.0 Diseases

Pathogens isolated from African lungfish under experimental conditions and from its natural environment include monogenetic trematodes (*Dactylogyrus sp*), digenetic trematodes (*Heterorchis sp.)*, cestodes, protozoans (*Trichodina* sp. and *Tetrahymena sp*.), fungus (*Fusarium* spp. and *Aspergillus* sp. and bacteria (*Flexibacter columnaris*, *Aeromonas sp, Pseudomonas sp*.). There was a high occurrence of fungal (60%) infection in experimental fishes with moderate infections of monogenetic and digenetic helminthes (34%) and bacteria (6%) shown in (Fig. 4.1).

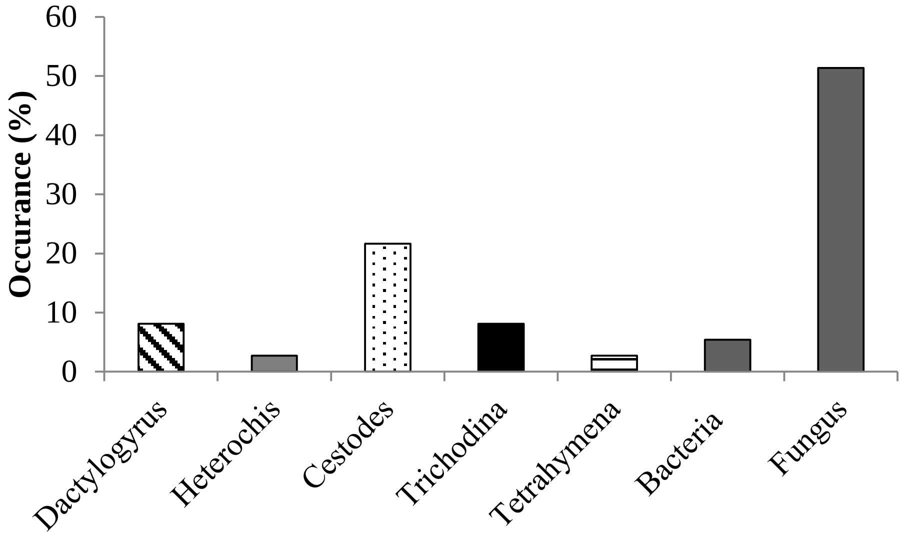


Figure 4. 1 . *Pathogens isolated from African lungfish prior, during and after the experimental period.*

Fungal and water mold infections mostly occurred at; i) dented caudal fin along the diphycercal tail, ii) posterior part of the cranial and, iii) lateral side of the medial line and the abdominal region of the fish (Fig. 4.2). Skin ulceration associated with petechial hemorrhage and ecchymosis mostly manifested on moribund fish. Internal bloody ascites were present in some heavily infested fish.

Figure 4. 2 . *Fungal and water mold infections of wild caught juveniles with skin ulcerations along the abdominal region and the caudal fin (a & b).*

Preliminary identification of pathogens collected from skin scraps indicated prevalence of fungal and water mold infections of *Fusarium solani* (42.4%), *Fusarium moniliforme* (1.5%), *Fusarium equisitte* (6.1%), *Saprolegnia sp.* (1.5%) and *Aspergillus sp*. (1.5%). There was a low prevalence (< 5.4%) found on freshly wild-caught fish. Other pathogens observed from skin include protozoans *Trichodina sp.* (3%) and *Tetrahymena sp.*(1.5%), all isolated from freshly collected wild fish.

A high prevalence (80%) of pathogen community (*Tetrahymena sp*, and *Fusarium solani*) is found in fish size ranging 0 ─ 49 mm. Prevalence of size clusters 100 – 149, 150 – 199 and > 200 mm were 27.2, 42.3 and 37.5%, respectively (Fig 4.3). A high prevalence of parasite infection in class-length 100 ─149 and 150 ─199 mm; most mortality affected this population. Class size 50 ─ 99 mm was not collected for the feed trials.

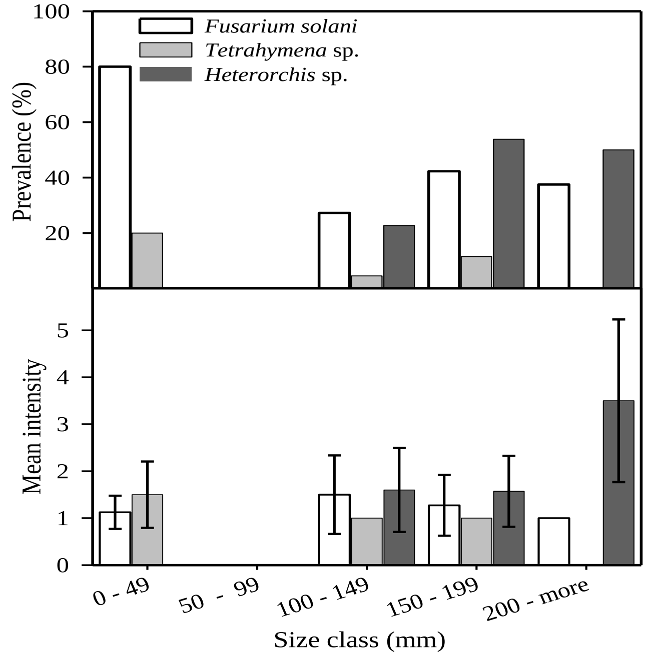


Figure 4. 3. *Prevalence and mean intensity of main pathogens infecting lungfish of different size classes.*

4.1.1 Bacteriology

Bacteriological tests revealed that all colonies isolated were gram negative bacteria (*Aeromonas sp. F. columnaris* and *Pseudomonas sp*). Colonies also collected from lesions grew on Hsu-Shotts and TYES agar; indicative of *F. columnaris*. This implies that cannibalism of African lungfish juveniles made the injured fish to be susceptible to bacterial infections. This led to mortalities among fish populations that fed commercial diets containing low crude proteins (<30%).

Bacteria are ubiquitous in aquatic environments and most organisms are opportunistic. Pathogens *Aeromonas sp. F. columnaris* and *Pseudomonas sp*, are ubiquitous, opportunistic bacteria with facultative and obligate forms that infect all finfish (Woo and Bruno 1999; Austin and Austin 2007). Losses in eggs, embryos and hatchlings of Australian lungfish kept in laboratory conditions were reported to be caused by Aeromonas (*A. hydrophila*) and pseudomonads (*Pseudomonas sp*) infections (Kemp 1994). Infections can spread rapidly in fish under high stock densities when water quality conditions are poor (Boyd 1979). Heavy ectoparasitemia is usually associated with systemic bacterial infections that enter through damaged parts of the skin (Noga 2010). Opportunistic facultative pathogens will cause diseases in compromised hosts living stressed environments (Plumb 1999). Bacterial infections could have been the primary cause of mortalities in experimental African lungfish juveniles kept in tanks.

4.1.2 Mycology

Presumptive identification of fungus based on morphology revealed presence of *Fusarium spp, Aspergilus sp and Saprolegnia sp.* Majority of the fungus species were *Fusarium spp* isolated from moribund fish. Fusarium species identified include *Fusarium solani* (56%), *F. equiseti* (33%) and *F. moniliforme* (11%). Fungal infestations were characterized by chronic mortalities in tanks, lesions and emaciation of lungfish. Kemp (1994) isolated *Saprolegnia sp* from eggs and embryos of Australian lungfish (*Neoceratodus forsteri*). Hence, lungfish is susceptible to fungal infections which may have enhanced mortalities in laboratory conditions.

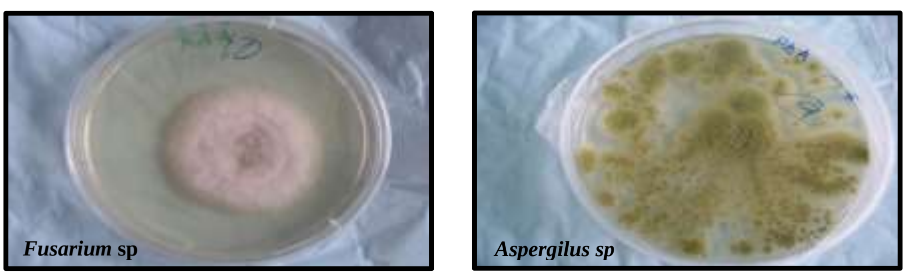


Figure 4. 4 . *Surface Colony of Fusarium sp and Aspergillus sp.*

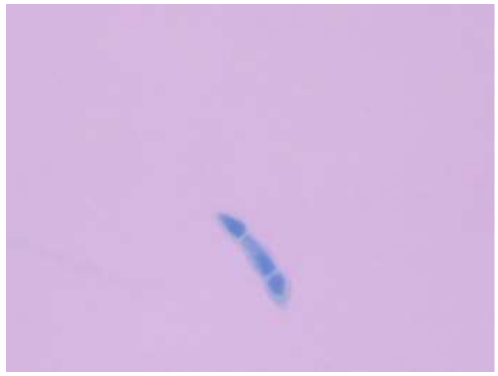


Figure 4. 5 . *Macroconidium of Fusarium solani visible using Lacto Phenol Cotton Blue stain*

Fungus is usually a secondary opportunistic pathogen that thrives in necrotic tissues or lesions of injured fish. Mortalities observed in the feed are mainly attributed to fungal infections (*Fusarium sp.*) Disease outbreaks caused by *F. solani* include cultured lobsters (*Homarus vulgaris*) and scalloped hammerhead sharks (*Sphyrna lewini*) (Alderman 1981; Crow et al. 1995; Hatai 2012). Fusarium is known to produce mycotoxins which affect the health of cultured finfish and shellfish. Fungus, *F. moniliforme* is associated with contaminated cereal grains (e.g. rice and corn); a key component of fish feeds (Bacon and Williamson 1992). Mycotoxins produced by *F. moniliforme* include fumonisins, moniliformin, gibberelins and fusaric acid (Rabie et al. 1982; Nelson et al. 1993). Fumonisins are toxic to fish and are reported to decrease weight gain and increase mortality in cultured channel catfish (Goel et al. 1994). Presumptive identification indicated a low prevalence of *F. moniliforme* suggesting experimental feed were contaminated.

4.1.4 Effects of Salt and formalin on African lungfish Juveniles

Mortality data of African lungfish exposed to different salt and formalin concentrations is presented in table 8. Mortalities did not occur in control for salt experiment but occurred in formalin experiment. Salt dips of 0.5 to 1.0 g/L are used for osmoregulatory purposes and preventing shock while rates of 3 g/L are recommended to control parasites (Noga 2010; (www.fda.gov/animalveterinary). The approved rate for finfish in tanks when using formalin is 250 mg/L for 1 hour (Noga 2010; www.fda.gov/animalveterinary). In this study, juveniles of African lungfish can tolerate concentrations up to 1.6 g/L of salt for 8 h and 480 mg/L of formalin for 4 h (Table 8). The estimated 96h LC 25, 50 and75 for salt is 0.3, 1.84 and 5.6 g/L, respectively. The LC50 is very low compared to other freshwater fish. The 24 h LC50 for lungfish exposed to acute levels of salt is 2.59 g/L (Fig. 4.7).

Table 10. *African lungfish juveniles in salt and formalin toxicity tests showing pooled data of mortalities from various treatments (duplicates) and LD50 (lethal dose of 50% fish in a given group) at 95 %CI.*

Concentration	Total number of fish (*N*)	Number of fish dead at								LC_{50} (95% CI)
		2 h	4 h	6 h	8 h	24 h	48 h	72 h	96 h	
Salt (g/L)										
0.00	18	0	0	0	0	0	0	1	1	1.84 (0.6 – 4.2)
1.00	18	0	0	0	0	0	0	2	2	
1.60	18	0	0	0	0	1	5	8	10	
2.50	18	1	4	8	9	15	15	18	18	
4.00	18	11	16	18	18	18	18	18	18	
6.30	18	9	18	18	18	18	18	18	18	
7.90	18	15	18	18	18	18	18	18	18	
10.00	18	14	18	18	18	18	18	18	18	
Formalin (mg/L)										
0	18	0	0	0	0	0	0	0	1	193.8 (125.0 - 262.5)
7.5	18	0	0	0	0	0	0	0	0	
15	18	0	0	0	0	0	0	0	0	
30	18	0	0	0	0	0	0	0	0	
60	18	0	0	0	0	0	0	0	0	
120	18	0	0	0	0	0	6	6	6	
240	18	0	0	0	0	18	18	18	18	
480	18	0	0	2	13	18	18	18	18	
960	18	1	2	18	18	18	18	18	18	

Linear models that fit well for salt and formalin doses are shown below;

i) Mortality (%) = 22.2169 + 10.1881*Salt (g/L); (R^2 = 0.72156, P= 0.0076)

ii) Mortality (%) = 3.12263 + 0.24207 *Formalin (mg/L); (R^2 = 0.844741, P = 0.0012).

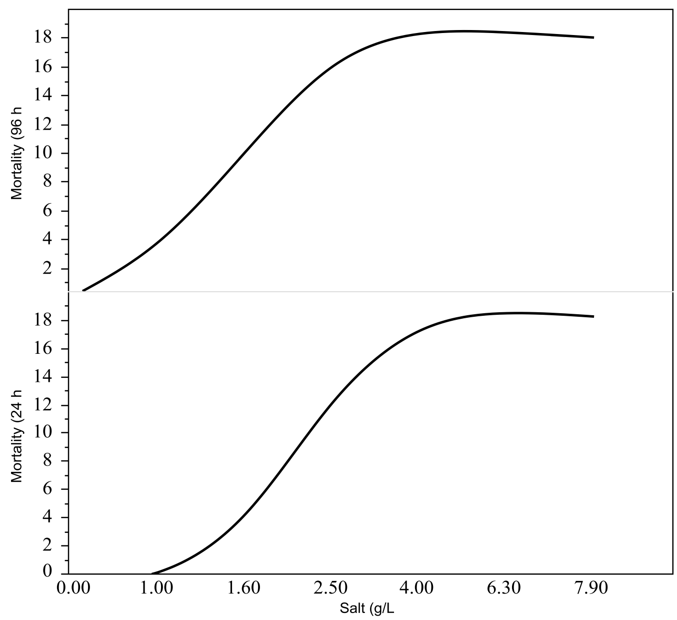


Figure .4.6 *Mortality of African lungfish (Protopterus sp.) exposed to lethal concentrations of formalin for 24 and 96 h.*

Dipnoans are classified as primary freshwater fishes restricted to freshwater containing total dissolved salt concentration less than 0.5 g/L and will not survive in protracted exposures (Myers 1949; Val and Randall 2005). Other studies show the 96h LC50 for salt in freshwaters fish; silver perch (*Bidyanus bidyanus*), freshwater catfish (*Tandanus tandanus*), common carp (*Cyprinus carpio*), mosquito fish (*Gambusia holbrooki*) and other ostariophysians is 13.7, 13.6, 7.3, 19.5 and 15 ─ 17 g/L, respectively (Alderman et al. 1976; Jackson and Piece 1992; Bianco and Nordlie 2008). Therefore, lungfish may be sensitive to salinity conditions and low concentrations of salt (about 1 g/L) should be administered.

Fish behaved normally in low salt concentrations (0 ─ 1.6 g/L) and fish exhibited equilibrium loss after 24 h. Mucus lining was visible on fish exposed to 1.6 g/L salt for 72 h. When concentrations reached 4.0 g/L, fish became erratic, unstable, lethargic and eventually died within 4 h. Mortality occurred within 4 h when fish were exposed to salt doses more 4 g/L. Copious mucus secretions along the skin integument were evident with fish exposed to higher salt doses (> 4.0 g/L).

Blood salt concentration in most freshwater fish ranges 7 to 13 g/L (Bacher and Garnham 1992; James et al. 2003), and threshold for juveniles to pre-harden eggs, permit growth and survivorship ranges from 2 ─ 5 g/L (James et al. 2003). Plasma of primitive fish (e.g. African lungfish) is hyperosmotic to its environment hence strategies to maintain this physiological status by eliminating large quantities of hypotonic urine while actively taking up ions through the branchial epithelia (Wright 2007). Chloride cells are responsible for salt exchange across membranes of gills and skin in freshwater fish (Sturla et al. 2001). Unfortunately, the osmoregulatory mechanism in African lungfish is not well understood because of its air-breathing behavior (thus reduced gill area); however, the ventral skin may be used for this purpose (Wright 2007).

When salt concentration increase, sodium ions are absorbed into the fish. This triggers a feed-back mechanism and regulatory processes occur to maintain the salt balance in the body (Maetz 1971). At lethal levels of salt concentration, fishes probably fail to maintain their osmotic balance with vital processes (e.g. respiration) are adversely affected. When exposures in high salt concentrations are prolonged the fish loses excess water to the environment until death occurs.

The estimated 96h LC 20, 50 and 80 for formalin in this study is 60, 193.8 and 312.5 mg/L. In other studies the 96h LC50 of formalin for African catfish (*C. gariepinus*) juveniles, snakehead fish (*C. striatus*), silver barb (*Puntius gonionotus)* and common carp (*C. carpio*) were 112.2, 166, 80, and 128 mg/L, respectively (FAO-NACA ;Ayuba et al. 2013). Conversely, *C. gariepinus* fry have an LC50 of 130 mg/L (Mbaru et al. 2010). Formalin is used to treat parasite and fungal infections with doses of 150 ─ 250 mg/L in tank condition. This study revealed lungfish juveniles can tolerate doses up to 480 mg/L for 4 h. The 24 h-LC50 for lungfish exposed to acute toxicity concentrations of formalin was 220.8 mg/L(Fig 4.8).

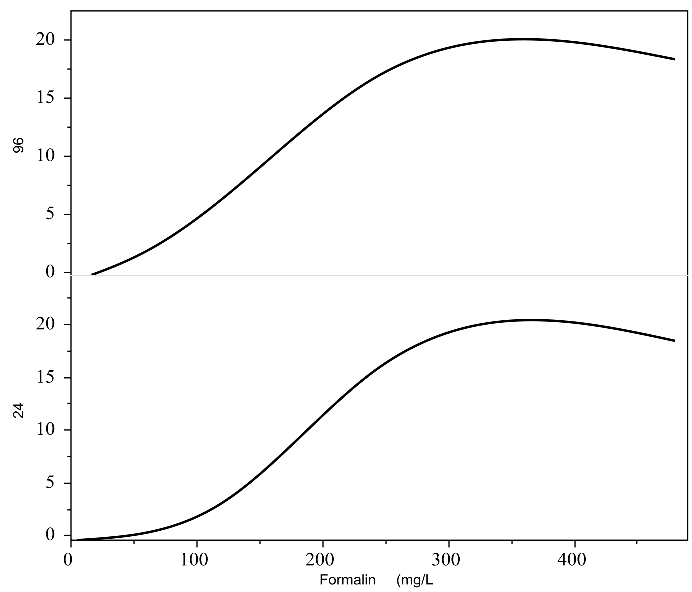


Figure 4.7 *Mortality of African lungfish (Protopterus sp.) exposed to lethal concentrations of formalin for 24 and 96h*

Lungfish juveniles are well adapted to acute formalin levels with a highest predicted mortality number of 5 fish. Fish dehydrates when exposed for long hours in formalin and effect is faster in small sized fish (Rothen et al. 2002). High temperatures above 20 C increase toxicity of formalin (Noga 2010). Formalin also affects oxygen levels in water because it is a reducing agent (Post 1985). Therefore, regular monitoring of water temperature and oxygen is important when formalin is used. In this study, DO levels of 4.77 to 5.93 mg/L and temperatures of 23.57 to 25.43 C were maintained with continuous aeration. However, lungfish exposed to doses of 60 mg/L lost equilibrium. Stress related behaviors of African lungfish juveniles occurred during the 6 ─ 8 h exposures of 240 ─ 480 mg/L, formalin with lungfish producing copious mucous along the body surface. Secretion of mucous along the skin integument is assumed to be the defense mechanism against formalin intrusion. Other fish like the rainbow trout develop necrotic tissues in the gills and become lethargic when formalin levels reach 200 mg/L.

African lungfish juveniles are susceptible to fungal, bacteria and parasite infections when reared in tank conditions. All pathogens (except Fusarium and *F. columnare*) in this study have been isolated from lungfish, before. Lungfish juveniles appear to be sensitive to salt but can tolerate high concentrations of formalin. This information is important for the development of management strategies against diseases affecting lungfish. It will enhance our knowledge on fish diseases affecting fish farms in Uganda and the eastern African region.

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Appendix A Survey Instrument

FISH FARMER'S QUESTIONNAIRE

Date of interview: ……………………………………………………………………

Name of interviewer: …………………………………………………………….

	Fish Farmer identification	
1	District	
2	Sub-county	
3	Parish	
4	Village	
	House hold ID	
1	Name of respondent (optional)	
2	Type of household	Female headed Male headed Others (specify)
3	Gender of respondent	

4	Ownership of the enterprise	Owner Family members Others (specify)
	Questions	
	Type of Fish farmed	
	Ever stocked lungfish?	
	Do you have future plans to culture lungfish?	
	Major Reasons	
	Culture system and size	Number of fish stocked
	Nutrition	
	Type of feeds given. Amount?	

	Regime?	
	Source of fish feeds	
	Harvesting and handling techniques	
	Frequency	
	Gears	
	Size	
	Survival	
	Ever caught a lungfish in culture system?	
	Challenges	
	Markets	
	Location	
	Size	
	Price/kg	

	Volume	
	Challenges	
	RECORD KEEPING	
	Do you keep records?	
	If yes, what type of records?	

CONSUMER'S QUESTIONNAIRE

Date of interview ………………………………………………………………

Name of interviewer……………………………………………………………

Respondent's details

Name …………………………………………………………………………. ***(Optional)***

Sex of the consumer…………………………………………………

1. How often do you eat lungfish?	1.Daily 2.Once a week 3.Once a month 4. Others (*specify*)………………………………………………
2. Where do you normally buy your fish?	1.Vendors 2.Whole sellers 3.Retailers in markets 4.Supermarkets
3. Give reason as to why you like that particular fish species.	
4. How much money did you spend on	

the fish?	
8. What product form do you usually buy?	

FISH TRADERS/RETAILERS

Date of interview ……………………………………………………………

Name of interviewer………………………………………………………….

1.Name of trader (optional)	
2.Name of market	
3.Location of market	
4.Sex of trader	1.Male 2.Female
5. Type of fish sold	
6.Cost of fish (kgs)	
7. Which is your major source of fish?	
8. In which form do the consumers prefer buying your product?	1.Live 2.Fresh 3.Dipfried 4.Smoked 5.Salted 6.Filleted

	7.Vacuum ceiled on disposal plates
9. Which markets do you sell your fish to?	1.Local markets 2.Supermarkets 3.Local restaurants 4.Export markets 5.Contract buyers
10. What are the major problems you face during the marketing of lungfish?	

Appendix B Survey Results

Lungfish Aquaculture: FISH FARMERS

		Numbers (%)
	Household type of lungfish farmers (n=36)	
	Male headed	6(17)
	Female headed	Nil
	Ownership;	
	i) Single	4(67)
	ii) Family	1(16.5)
	iii) Group	1(16.5)
	Household type willing to rear lungfish (n=30)	
	Male headed	17(57)
	Female headed	Nil
	Main reason for rearing lungfish (n=23)	
	Markets (availability and lucrative)	14(61)
	Taste	5(22)
	Large size	4(17)
	Main reason for not rearing lungfish (n=13)	
	Culture prohibits	3(23)

	Predates on other stocked fish (tilapia & catfish)	3(23)
	Burrows and escapes from pond	2(15)
	No technology (seed & feed)	2(15)
	Lack of capital	1(8)
	Dangerous to handle	1(8)
	Not interested	1(8)
	Farmed fish (n=36)	
	Lungfish	3(8)
	Tilapia + Lungfish	3(8)
	Tilapia + Catfish	30(84)
	Seed source for lungfish culture (n=6)	
	Wild-caught fingerlings from Lakes	5 (83)
	Wild-caught fingerlings from Swamps	1(17)
	No. Stocked/m^2/year (n=6)	
	1 fish	2(33)
	2-3 fish	4(67)

	FISH FARMERS (CONT.)	
	Culture system for rearing lungfish (n=6)	
	Pond	4(67)
	Ditch	1(16.5)
	Tank	1(16.5)
	Survival (%) in culture system	
	Pond	0 – 40
	Ditch	< 10
	Tank	100
	Feeding lungfish (n=6)	
	Farmers who feed	4(67)
	Farmers who do not feed	2(33)
	Feed type (lungfish culture)-ranking	
	Kitchen leftover	1
	Meat by-products	2
	Fish meal	3
	Snails	4
	Insects	5

	Maize bran	6
	Blood meal	7
	Ponds where lungfish is usually harvested but not stocked (n=12)	
	Eastern Region	10(83)
	Central Region	2(17)
	Harvesting method (n=18)	
	Gear/instrument;	
	i) Hooks	9(50)
	ii) Spears + hoes	3(17)
	iii) traps (basket)	1(6)
	iv) Seine net	2(10)
	v) Draining pond	3(17)
	Challenges during harvesting (n=18)	
	Handling a fish which can harm a person	4(22)
	Complete drainage of pond to harvest all fish	9(50)
	Effective harvesting gear	5(28)

FISH FARMERS (CONT.)

	Harvests were for: (n=18)	
	Home consumption only	10(56)
	Home consumption + Market	4(22)
	Markets only	4(22)
	Markets (n= 8)	
	Harvested lungfish is sold at;	
	i) Pond site	5(63)
	ii) Local market	3(37)
	Estimated market size kg (n=8)	
	0.5 – 1	5(63)
	>1	3(37)
	Respective Price range (US $/kg) {n=8}	
	2.50 – 3.00	
	3.00 –4.00	
	Challenges- ranking	
	Limited supply while demand is increasing	1

	Technologies/information to enhance market	2
	Record keeping (n=6)	
	Lungfish farmers keeping records	Nil
	Lungfish farmers who do not keeping records	6

LUNGFISH CONSUMERS

	Respondent's gender (n=100)	
	Male	36
	Female	64
	Lungfish consumers (n=73)*	
	Male	32(56)
	Female	41(44)
	Frequency	
	Once per week	17(23)
	Once per month	24(33)
	Twice per month	28(10)
	Once per year	25(34)
	Source of fish (n=73)	
	Vendors	21(29)
	Whole sellers	8(11)
	Retailers in markets	44(60)
	Supermarkets	0
	Why lungfish preference ?	

	Less fat in the tissue	8(11)
	Cheaper	18(25)
	Adequate for average family	21(29)
	Less bones	6(8)
	Less smell	5(7)
	Good taste	12(16)
	'Medicinal'	3(4)
	Lungfish products	
	Fresh/Fried pieces	43(59)
	Soup	7(10)
	Whole fresh fish (gutted/un-gutted)	9(12)
	Smoked pieces	4(5)
	Whole smoked	10(14)
	Retail Price range (US $/kg)	
	3 – 5	

*Based on respondents

TRADERS/RETAILERS

	Gender of trader	
	i. Male	24(35)
	ii. Female	44(65)
	Fish sold	
	Tilapia	36(52)
	Catfish	6(9)
	Lungfish	15(21)
	Nile perch	7(10)
	Silver fish	1(2)
	Synodontis	1(2)
	Bagrus docmac	1(2)
	Barbus sp	1(2)
	Price range of fish (US $/kg)	
	Tilapia	3.5 – 4.6
	Catfish	1.6 – 2.3
	Lungfish	0.6 – 2.5
	Nile perch	3.1 – 4.3
	Silver fish (dried)	0.9 – 2.1
	Synodontis	0.6 – 1.2

	Bagrus docmac	2.9 – 4.1
	Barbus sp	2.5 – 4.3
	Major source of fish (lungfish)	
	Fishermen	4(6)
	Wholesalers	11(94)
	Major consumers' preference (lungfish)- ranked	
	Fresh	1
	Dip fried	2
	Smoked	3
	Destiny markets (lungfish)	
	Local markets	7(47)
	Supermarkets	0
	Local restaurants/bar	5(33)
	Export markets	1(7)
	Contract buyers	2(13)
	Major problems- ranked	
	Limited supplies	1
	Quality	2