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AFRICAN FISH EAGLES (Haliaeetus vocifer) and MARABOU STORKS (Leptoptilos crumeniferus) IN UGANDA: USE AS BIOMONITORS OF ENVIRONMENTAL CONTAMINATION

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Wilson K. Rumbeiha
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# AFRICAN FISH EAGLES (*Haliaeetus vocifer*) and MARABOU STORKS (*Leptoptilos crumeniferus*) IN UGANDA: USE AS BIOMONITORS OF ENVIRONMENTAL CONTAMINATION

**By**

**Simon Ralph Hollamby**

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## ABSTRACT

AFRICAN FISH EAGLES (*Haliaeetus vocifer*) and MARABOU STORKS (*Leptoptilos crumeniferus*) IN UGANDA: USE AS BIOMONITORS OF ENVIRONMENTAL CONTAMINATION

By

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A study was designed to evaluate concentrations of persistent organic pollutants and mercury in African fish eagles (*Haliaeetus vocifer*), marabou storks (*Leptoptilos crumeniferus*) and tilapia fish (*Oreochromis niloticus*) in Uganda. Total mercury concentration in breast feathers; plasma concentrations of a range of persistent organic pollutants; packed cell volume and plasma chemistry values were determined for adult and nestling African fish eagles at Lake Mburo (n = 18) and Lake Victoria near Entebbe (n =15), as well as marabou stork nestlings in Kampala (n = 21). Morphometric measurements were collected on adult fish eagles. A human and eagle food, *Oreochromis niloticus* were sampled for total body mercury and a range of persistent organic pollutants (n = 18). Feather mercury concentrations were significantly ($p \leq 0.05$) lower in fish eagles at Lake Mburo than fish eagles from Entebbe and marabou storks from Kampala. Five adult fish eagles and five *Oreochromis niloticus* from Entebbe had concentrations of 4,4'-DDE of less than 0.005 ppm wet weight in plasma and fish samples. The research establishes concentrations of these pollutants in these species and allows future trend analysis. African fish eagles and marabou storks meet many criteria of a suitable avian biomonitor of environmental pollution. With appropriate development, long-term research and integration with other monitoring initiatives, these species could become valuable tools to assess environmental change.

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# PREFACE

The chapters in this thesis are organized as independent pieces of work some of which are intended to be published in the scientific literature. As such, there may be some redundancy between chapters, especially in relation to the reporting materials and methods.

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| | |
|---|---|
| Aldrin | 1,2,3,4,10,10- hexachloro-1,4,4a,5,8,8a-exahydro-*endo*-1,4-*exo*-5,8-dimethanonapthalene |
| AST | aspartate transaminase |
| Chlordane | 1,4,5,6,7,8,8,-octochloro-3a,4,7,7a- tetrahydro-4,7- methanoindan |
| Chol | cholestrol |
| CK | creatine kinase |
| Dieldrin | 1,2,3,4,10,10- hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-*endo*-1.4-*exo*-5,8-dimethanonapthalene |
| DDD | 1,1,1 – dichloro- 2,2-bis (4-chlorophenyl) ethane |
| DDE | 1,1,1 – trichloro- 2,2-bis (4-chlorophenyl) ethylene |
| DDT | 1,1,1 – trichloro- 2,2-bis (4-chlorophenyl) ethane |
| DCPAH | Diagnostic Center for Population and Animal Health |
| Endrin | 1,2,3,4,10,10- hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro- 1.4- *endo, endo*- 5,8-dimethanonapthalene |
| Fish Eagle | African fish eagle (*Haliaeetus vocifer*) |
| Heptachlor | 1,4,5,6,7,8,8,-octochloro-3a,4,7,7a- tetrahydro-4,7- methanindane |
| HCH | 1,2,3,4,5,6- hexachlorocyclohexane |
| Lindane | gamma isomer of HCH |
| MSU | Michigan State University |
| Na/K | sodium/potassium ratio |
| ppm | parts per million |
| PCV | packed cell volume |

| | |
|---|---|
| P | phosphorous |
| POPs | persistent organic pollutants |
| PCBs | polychlorinated biphenyls |
| TT4 | thyroxine |
| TT3 | triiodothyronine |
| TPP refrac | total plasma protein measured by refractometry |
| TPP col | total plasma protein measured by the biuret method |
| TT4 RIA | thyroxine radioimmunoassay |
| TT4 ELISA | thyroxine enzyme linked immonoabsorbent assay |

# Chapter 1

# Project Summary, Literature Review, Hypothesis and Research Objectives

## Project Summary

With a history of social and political instability, there has been little monitoring or scientific evaluation of the health of Uganda's ecosystems (Koch RA 1996). The onset of social and political stability in the mid 1980s has led to an expanding economy, increased foreign investment and population urbanization centered in Kampala. The effects of this change on wildlife and the environment have not been adequately documented. The African fish eagle (*Haliaeetus vocifer*) is a tertiary avian predator in lake-based food chains throughout sub-Saharan Africa (Brown et al 1982). Piscivorous raptors have proved to be valuable biomonitors of ecosystem health and environmental pollution (Bowerman et al 2000 a). Much of the research on raptors as environmental monitors has come from countries with a developed database on species from temperate climates that show marked seasonality.

Marabou storks (*Leptoptilos crumeniferus*) are indigenous to tropical Africa where they are common to abundant in most parts of their range. In areas such as urban Kampala, they have a cosmopolitan diet, due to their efficient adaptation to a lifestyle as scavengers of human refuse (Hancock et al 1992). Their diet and exposure to pollutants in urban areas may reflect human exposure to the same pollutants making marabou storks potentially useful as biomonitors of toxic exposure in humans.

The hypothesis tested by this project is there is no significant difference in persistent organic pollutant and mercury concentrations in tissues of African fish eagles and *Oreochromis niloticus* from Lake Victoria near Entebbe compared to those from Lake Mburo.

The objectives of this project were to quantify the concentrations of persistent organic pollutants (POPs) in the blood and feathers of populations of African fish eagles at two study sites in Uganda. One site was an urbanized area of shoreline on Lake Victoria from Nfo Island (0°. 00' N, 32°. 26' E) to Kisubi Bay (0°. 05'N, 32°. 35' E) near Entebbe. The other site was Lake Mburo, a six km long freshwater lake within a 256 sq km national park situated in South Western Uganda (0°. 39' S, 30°. 57' E). Lake Mburo is 230 km and Entebbe 40 km from the capital, Kampala. The population of Kampala is rapidly increasing and virtually contiguous with that of Entebbe (Uganda Bureau of Statistics 2002). Concentrations of the same pollutants measured in fish eagles were quantified in whole body cross section samples of a representative eagle prey species, tilapia (*Oreochromis niloticus*). The whole body cross section samples were approximately 100g whole cuts including viscera, fat, skin and bone is taken from just cranial to the dorsal fin ventrally down to just caudal to the gill arch. *Oreochromis niloticus* are herbivorous fish occupying the littoral zone in African lakes. As fish can be the primary source of dietary protein for human populations in this region, sampling of fish may also give some indication of human exposure to these pollutants. Data on concentrations of these pollutants in the blood and feathers of nestling marabou storks from urban Kampala is presented for the purpose of comparison and contrast to the fish eagle data. Blood plasma was used for total polychlorinated biphenyls (PCB) and chlorinated pesticide analysis. The specific chlorinated pesticides examined were aldrin, DDT, α-HCH, dieldrin, endrin, heptachlor and their metabolites, β-HCH, 2,4'-DDD, 4,4'-DDE, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, heptachlor epoxide and lindane and

nonachlor. Breast feathers were used for total mercury analysis. Other parameters such as weight, packed cell volume (PCV), estimated age and plasma chemistry values were determined for marabou storks and African fish eagles. A survey of hematozoan parasites was performed for the marabou stork and African fish eagle populations sampled. Certain persistent pollutants, such as the coplanar PCBs that have an antiestrogenic effect. Some PCB congeners may disrupt thyroid gland homeostasis (Yamamoto et al 1996). Therefore, plasma total thyroxine (TT4), and plasma total triiodothyronine (TT3) were also determined. The sex of the African fish eagles and marabou storks was determined by analysis of erythrocyte DNA. Characterization of nest site habitat was made and population density was estimated but not quantified. Analysis of variance was conducted to assess the association between various factors and total feather mercury, plasma chemistry parameters, and morphological characteristics of African fish eagles and marabou storks (SAS PROC ANOVA for categorical risk factors, and SAS PROC GLM for continuous risk factors. SAS 8.2, 2001. SAS Inc. Cary, NC). These analyses were conducted both at the univariable (only one risk factor at a time) and multivariable level. Multivariable analyses were conducted to adjust the effect of selected risk factors simultaneously. The level of significance (type 1 [$\alpha$] error) was set at $p = 0.05$.

There has been no documentation of persistent organic pollutant or heavy metal concentrations in African fish eagles in Uganda. A study has examined metal concentrations (zinc, cadmium, lead, copper, iron, manganese, chromium and cobalt) in the feathers of adult marabou storks from Kampala city and surrounding areas (Nyangababo 2003). This study did not include mercury. Only a few studies have

reported plasma chemistry values for wild eagle species (Bowerman et al 2000 b; Garcia-Montijano et al 2002). Plasma chemistries have not been determined for wild populations of African fish eagles or marabou storks. Data generated from this project will document blood plasma concentrations of POPs, feather concentrations of total mercury and help establish parameters for plasma chemistry values of African fish eagles and marabou storks at selected sites in Uganda. The difference between the data gathered from Lake Mburo and the Lake Victoria region (that includes Entebbe and Kampala) may highlight the effects of greater anthropogenic environmental alteration at the latter site. The data will also increase knowledge of the biology of African fish eagles in Uganda. To this end, morphometric measurements were determined on adult African fish eagles and differences relative to sex were compared. The project results may also provide valuable comparisons to analogous work being conducted on tertiary avian predators in lake-based food chains in other areas of the world, such as bald eagles (*Haliaeetus leucocephalus*) in the Great Lakes region of North America (Bowerman et al 1993).

The project field methods are documented, as they may prove applicable to other raptor species or future research on African fish eagles and marabou storks. An assessment was made of the suitability of the development of the African fish eagles and marabou stork as biomonitors of environmental health. The conclusions are presented. Knowledge from this research should help improve the conservation management of African fish eagles and the Ugandan lake-based ecosystems they inhabit.

## Literature Review

### Mercury and Avian Species

Mercury can occur in a number of chemical forms in the environment. Microbial and biochemical reactions in soils and sediments can lead to the transformation of all chemical forms into methylmercury, the most toxic form (Heinz 1996). Sources of mercury can be natural or anthropogenic and include fluorescent lamps, batteries, thermometers, medicines, paints, metallurgical processes, fungicides in the paper industry, fossil fuel burning and natural release such as through volcanic eruptions (Heinz 1996). Two of Africa's most active volcanoes, Nyamuragira and Nyiragongo, situated in the Democratic Republic of Congo, bordering Uganda, erupted on July 26 2002 and January 16 2002. These eruptions were six months prior to this project's field periods and widespread atmospheric deposition from the eruptions occurred for months afterwards This may have contributed to the total mercury concentrations present within the lake-based ecosystems studied in this project.

Mercury is lipid soluble and bioaccumulative. It diffuses across the alveolar space into erythrocytes and the brain, accumulates in adipose tissue and can be passed into eggs. Methylmercury can have harmful effects on adult survival, reproduction, behaviour and cellular development (Burger 1994). The neurotoxic effects of methylmercury can alter nesting behavior and negatively impact reproductive success of avian species (Heinz 1996). Mercury can cause reduced egg production, lighter eggs and smaller clutches. Hatching success and chick survival were reduced in black ducks (*Anas rupripes*) and mallards (*Anas platyrhyncos*) fed diets containing methylmercury (Finley and Stendell

1978; Heinz 1979). The effects of mercury can be somewhat antagonized by exposure to other elements such as selenium and zinc. Animals have no physiological requirement for mercury (Eisler 1987). Anthropogenic activities that may contribute to environmental mercury concentrations include industrial pollution and use of fossil fuels that commonly occur in the industrial area along the shore of Lake Victoria near Kampala. The human population of Kampala increased from 774,241 in 1991 to a preliminary figure of 1,208,544 in 2001 and use of diesel fuel (inferior grades and quality) in Uganda increased from 125,621 in 1997 to 207,183 cubic metres in 2001 (Uganda Bureau of Statistics, 2002). Increased use of fossil fuels as an energy source may be associated with increased atmospheric mercury concentrations. Through cycling, increased atmospheric mercury may lead to an increase in mercury concentrations in aquatic organisms. No studies have been reported examining the potential impact of mercury pollution on wildlife in Uganda.

**Persistent Organic Pollutants (POPs) and Avian Species**

Over thirty organochlorinated compounds have been used as pesticides (Blus et al 1996). The effects of 1,1,1 - trichloro - 2,2-bis (4 - chlorophenyl) ethane (DDT) on avian species have been well documented (Lincer 1975; Peakall 1993; Ratcliffe 1967). 1, 1, 1 – trichloro - 2, 2 - bis (4-chlorophenyl) ethylene (DDE), a metabolite of DDT, is known to cause adverse effects on reproductive success and eggshell thinning in wild raptor populations (Ratcliffe 1967) and in experimental studies on raptors (Lincer 1975; Weimeyer and Porter 1970). Polychlorinated biphenyls (PCBs) have been used as insulating materials in transformers and capacitors, plasticizers in waxes, paper manufacturing, flame retardants and for a variety of other industrial applications (O'Hara

and Rice 1996). Various congeners of PCBs can produce teratogenic effects, such as bill defects or deformities (Gilbertson et al 1991). Some coplanar PCB congeners have been associated with dioxin like effects including wasting, thymic atrophy and endocrine and enzyme disruption in a number of species (Safe 1990). Experimentally, reproductive failure was produced with various concentrations of PCB congeners fed to chickens (Kubiak et al 1989). Nestling bill defects in Swedish white tailed sea eagles (*Haliaeetus albicilla*) were believed related to PCBs (Helander 1982). In studies on wild bald eagles, productivity was significantly and inversely correlated with concentrations of PCBs and p,p'-DDE in addled eggs (Bowerman 1993). The role of PCBs and their estrogenic/antiestrogenic reproductive effects compared to the eggshell thinning effects of DDE are still open to debate (Bowerman, 1995, 2000 a; O'Hara and Rice 1996).

Concentrations of organochlorinated pesticides, PCBs and mercury in bald eagles in Michigan have been documented using similar methods to those in this project (Bowerman, 1993). Organochlorinated pesticide concentrations were measured in eagle blood during 1988 to 2000, PCB and organochlorinated pesticide concentrations in eggs from 1986 to 1997 (175 eggs) and mercury in feathers from 1986 to 2000 (Bowerman et al 2000 a). Similar studies on *Haliaeetus* sp. have been conducted in Sweden, Siberia and the southeastern United States and are proposed for Norway (Bowerman, 2001).

Studies on POP contaminant concentrations in African fish eagles, marabou storks and other avian species in African countries are sparse. A survey of the African fish eagle population of Lake Kariba in Zimbabwe found all eggs collected contained

DDT or its metabolites (Douthwaite 1992). The study found high concentrations of mercury in adult birds and significant eggshell thinning in areas associated with high use of DDT. The author suggested that factors other than pesticide concentrations limit breeding success at the study site. An earlier study in Zimbabwe reported egg dry weight total DDT concentrations and found reduced eggshell thickness in African fish eagle eggs. (Thomson 1984). Lincer (1981) reported organochlorinated pesticide residues in a single fish eagle egg from Kenya's Rift Valley Lakes and Snelling et al (1984) reported values from a number of eggs from various sites in southern Africa. Ratcliffe's index of shell thickness (Ratcliffe 1967) was determined for 90 African fish eagle eggs collected within southern Africa. Eggshell thickness declined progressively in relation to the use of DDT (Davies and Randall, 1989). Interestingly, one multi year study of fish eagle productivity at Lake Chivero, a polluted dam in Zimbabwe, indicated that fish eagle numbers were increasing, despite years of eutrophication and heavy metal, sewage and pesticide effluent (Mundy & Couto 2000). The authors concluded, despite large decreases in eggshell thickness, that productivity was unaffected. The reason for the increases in productivity (in the largely piscivorous fish eagle) were thought to be an increased fish population caused by nutrient enrichment leading to proliferation of aquatic plant life. The authors concluded however, heavy metal concentrations may be a cause for future concern.

The lack of uniformity in relation to tissues and species sampled, differences in analytical methods and reporting of study results can complicate interpretation of POP data in avian species. We report all mercury and POP concentrations in part per million

(ppm). This is on a dry weight basis for feathers and a wet weight basis for whole body cross section samples of fish and avian plasma samples.

**African Fish Eagle Biology**

Much has been recorded on the biology of the African fish eagle (Brown, 1960, 1971, 1978, 1980; Brown & Hopcraft 1973; Erikson & Skarpe 1989; Ghiglieri 1983; Green 1964; Krueger 1997; Prout Jones & Milstein 1980, 1986; Stewart et al 1997; Sumba 1986, 1988, 1989; Virani 2001). Despite this information, there appears to be considerable debate over some key factors of fish eagle breeding and biology, including the proximate factors that stimulate breeding at tropical latitudes. Some of the information presented in these reports is anecdotal as opposed to scientifically designed studies. However, the majority of the studies provide valuable information based on observation but involved no capture or handling of fish eagles. One study examined nestling growth in African fish eagles (Sumba 1988) in Uganda while another measured growth in captive birds (Prout-Jones & Milstein 1986). One paper examined breeding seasonality of fish eagles in Queen Elizabeth National Park, Uganda (Sumba 1986) and concluded that breeding occurred year round with peak laying during the long wet season from August to November. No breeding records are available for Lake Mburo or Lake Victoria at Entebbe. However, population counts have been conducted usually twice yearly at Lake Mburo and Lutembe Bay, Lake Victoria (close to the Entebbe study site) (Nature Uganda, The East Africa Natural History Society, P.O.Box 27034, Kampala, Uganda). Our limited observations would suggest that egg laying occurs mid March to late August at Lake Mburo. Chicks of various ages from hatchlings to almost fledged as

well as eggs were observed in nests between late June and late August. Chicks at various stages of development were noted in nests in January, March and August at Lake Victoria near Entebbe. Eggs were observed in nests around Entebbe in August, December and January. To accurately determine the breeding season would require multi year analysis as the proximate factors that stimulate breeding are variable and not adequately documented (Virani 2001). However, it would be reasonable to assume these proximate factors could cause variation in the timing of breeding from year to year.

Four authors examined population structure and densities of fish eagles in various regions of Uganda (Brown 1970; Green 1964; Krueger 1997; Sumba 1988). African fish eagles usually build large, conspicuous nests in tall trees close to water. Breeding may not occur every year. The incubation period for fish eagle eggs is generally believed to be around 42 days. One to three (usually two) eggs are laid at two to three day intervals. Fledglings leave the nest at approximately 70 -75 days according to Brown (1980), while Sumba claimed the mean fledging time to be closer to 76 days (Sumba, 1988). Fish eagles attain adult plumage at five years of age. Brown (1980) recorded adult fish eagle weights as 1.98-2.49 kg for males (n = 4) and 3.00 to 3.63 kg for females (n = 3). The literature cited in this thesis, as well as our observations confirm fish eagles as one of the most territorial avian species, both to con-specifics and other avian species. Fish eagles are reported to be mainly piscivorous but birds, small mammals, reptiles, amphibians and other avian species can make up to 9 per cent by weight of the diet in some individuals (Stewart K.M. 1997). Our observations, and the communications of local fisherman, suggest that *Oreochromis niloticus*, usually below 25 cm in length and approximately

400g form the majority of the fish eagle's diet at Lake Mburo and Lake Victoria near Entebbe. However, diet can be variable and at Murchison Falls, the carnivorous tiger fish (*Hydrocynus forsaklii*) appears to be the main fish prey item.

**Marabou Stork Biology**

Unlike the fish eagle, the biology and reproductive cycle of the marabou stork in Uganda has been well described (Pomeroy 1973, 1975, 1977, 1978a, 1978b). Adult marabous weigh between 5 - 8 kg and diet of the birds in Kampala probably includes almost anything organic, such as garbage, fish remains, abattoir refuse and a large amount of vegetable matter (Brown, 1982). However, during the period of nestling growth, increased amounts of protein are taken in the form of fish, frogs and rodents. Marabous undertake short, mainly north south migrations in Uganda, coinciding with rainfall seasonality (Pomeroy, 1978a, 1978b). The Kampala population is greatest during the dry season around December and January. Marabous are colonial nesters with multiple nests being built in particular tree types, such as Mvule (*Chlorophora excelsa*) and ***Tabebuia pentaphylla***. The incubation period is 30.3 days and average clutch size is 2 - 3 eggs. Marabous have an extremely long period from hatching to fledging, being about 135 days with first flights out of the nest at 110 - 115 days. Marabous first breed at 6 - 7 years and may live up to 25 years. Breeding success is low but has reportedly increased in recent years in Kampala (Hancock et al, 1992). To the best of our knowledge, no studies have quantified POP concentrations or plasma chemistry values in wild marabou storks. A study has examined metal concentrations (zinc, cadmium, lead, copper, iron, manganese, chromium and cobalt) in the feathers of adult marabou storks

from Kampala city and surrounding areas (Nyangababo 2003). This study did not include mercury.

**Pesticide Usage In Uganda**

Accurate data on use of pesticides and monitoring of pesticide residues in Uganda are limited (Baliddawa 1991; Ejobi et al 1996 a,b; Kock 1996; Ogutu-Ohwayo 1997; Simonich and Hites 1997; Tukahirwa 1984, 1991; Wiktelius et al 1999). Ejobi et al (1996) quotes 80 tonnes of DDT per year used mainly for cotton growing and mosquito control. Dieldrin was used at the annual rate of 392 tonnes per year for banana weevils and termites while 30 tonnes was also used for Tsetse fly control. Lindane, aldrin, hexachlorobenzene, campheclor, chlordane and heptachlor were also used. Official figures can only be viewed as estimates of present day usage. It is clear from the literature that there is a regulatory need to evaluate and standardize methods of reporting and monitoring the use of potential chemical contaminants in Uganda. Ejobi et al (1996 a,b,) measured concentrations of DDT metabolites and other chlorinated hydrocarbons in human milk and cows milk in urban Kampala and a rural district. Concentrations of DDT in Ugandan mother's milk was higher than that in many developed countries such as Japan, Sweden and the USA, that had banned this pesticide. However, concentrations of DDT were lower than other developing countries such as Nigeria, India, Kenya and Ethiopia. No studies were found examining POPs or mercury in African fish eagles in Uganda.

## Hypotheses

The hypothesis to be tested was:

- There is no difference in persistent organic pollutant and mercury concentrations in tissues of African fish eagles (*Haliaeetus vocifer*) and tilapia (*Oreochromis niloticus*) from Lake Victoria near Entebbe compared to those from Lake Mburo.

Two important issues related to the hypothesis that will be examined in this thesis are:

- Whether concentrations of persistent organic pollutants and mercury are likely to be significant contributing factors to the population dynamics of the African fish eagle at Lake Victoria near Entebbe or Lake Mburo.

- Whether African fish eagles and marabou storks can be utilized as successful biomonitors of mercury and persistent organic pollutants.

## Research Objectives

The objectives of this study are to determine:

1. concentrations of organochlorinated pesticides and total PCBs from African fish eagle and marabou stork plasma from the stated study sites.

2. concentrations of total mercury in breast feathers from African fish eagles and nestling marabou storks from the stated study sites.

3. concentrations of organochloinated pesticides, total PCBs and mercury from whole body cross section samples of *Oreochromis niloticus* fish from Lake Mburo, Lake Victoria and Murchison Falls.

4. packed cell volumes (PCV) and selected plasma chemistry values from African fish eagles and marabou storks from the stated study sites.

5. plasma total thyroxine (TT4) and plasma total triiodothyronine (TT3) levels from African fish eagles and marabou storks at the stated study sites.

6. sex of all birds sampled by means of DNA analysis.

7. the presence and identity of blood hematazoa from fish eagle and marabou stork samples from the stated study sites.

8. morphometric measurements on adult fish eagles and band (ring) all adult eagles.

9. nest site habitat characteristics such as nesting tree species, tree height, nest height, diameter of tree at breast height (DBH), disturbance around nest, distance from the nest to water and canopy cover.

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**Figure 1.1. Uganda, East Africa with research sites highlighted (modified from worldatlas.com)**

# Chapter 2

## Methods and Equipment Used to Sample African Fish Eagles (*Haliaeetus vocifer*) and Marabou Storks (*Leptoptilos crumeniferus*) in Uganda

## Abstract

A study was designed to evaluate persistent organic pollutants and mercury levels in African fish eagles (*Haliaeetus vocifer*), marabou storks (*Leptoptilos crumeniferus*) and tilapia (*Oreochromis niloticus*) in Uganda. The objective of this paper is to describe the methods and equipment used in the study. Birds were sampled from the Kampala/Entebbe region and Lake Mburo in Uganda, in three field periods from December 2001 through January 2003. Adult eagles were captured on water using a fish "snare vest". The ratio of number of birds caught to eagle attempts to take the snared fish was 1: 6 at Lake Mburo and 1:10 at Lake Victoria near Entebbe. The ratio of the number of birds caught to the number of times the snared fish was placed in the water was 1:8 for Lake Mburo and 1:36 for Lake Victoria. One hundred and twenty capture attempts were made over ten days at Lake Mburo and 72 over nine days at Lake Victoria near Entebbe. Eighty-three percent of adult eagles were snared by the third digit. Nestling marabou storks and African fish eagles were captured using professional tree climbing methods and equipment. The sampling success rate, defined as trees climbed with at least one chick sampled was 100% for storks and 55% for eagles. The snare vesting technique described may be an effective method to catch adult African fish eagles with a success rate dependant on multi-factorial local site conditions. The climbing methods described were successful for the safe sampling of nestling marabou storks and African fish eagles. These capture methods may prove useful in the management and study of captive and wild populations of African fish eagles and marabou storks. Snare vesting, with modifications, knowledge of local site conditions and species biology could have applicability to the capture of other large piscivorous eagle species.

## Introduction

The African fish eagle (*Haliaeetus vocifer*) has a wide distribution along lakes and waterways throughout sub-Saharan Africa (Brown, 1980). A number of authors have described the biology of the species in various locations (Brown 1960, 1971, 1980; Brown and Hopcraft 1973; Green 1964; Krueger 1997; Sumba 1986, 1988, Prout Jones & Milstein 1986). These studies predominantly involved non-invasive observations. Studies examining the effects of persistent organic pollutants (principally DDT and its metabolites) on reproductive success in African fish eagles were conducted from the 1970s through the 1990s and mainly in southern Africa (Douthwaite 1992; Davies and Randall 1989; Lincer 1981; Snelling et al 1983; Thomson 1984). These studies examined eggs. However, the method used to obtain the samples are often only briefly described. The use of blood for pesticide analysis is advantageous as the effect on the population is less than the permanent removal of eggs for analysis. Methods have been described in certain species whereby total DDT residues in plasma can be adjusted to estimate the residues in the egg. (Henny and Meeker 1981). However, the use of blood as a sample tissue requires catching the bird. No known studies have described in detail the temporary capture of adult and nestling African fish eagles for scientific research.

The biology and breeding cycle of marabou storks (*Leptoptilos crumeniferus*) in Uganda has been well described (Pomeroy 1977, 1978). One report described the use of oral anesthetic agents for capture and sampling of adult marabou storks, but reported some degree of mortality associated with the method (Pomeroy and Woodford 1976). No known studies describe in detail the temporary capture of nestling marabou storks for

blood collection. With the increasing scrutiny all types of invasive wildlife field investigations are currently subjected to, and to facilitate future research, it behooves all researchers to adequately document and disseminate successful capture methods. It is also important to highlight unsuccessful capture methods so other researchers can assess, then modify, improve on, or discard the techniques. This is vital because as populations of many species continue to decline, proven and safe capture methods are required. This report describes methods used to capture adult and nestling African fish eagles and nestling marabou storks in the Kampala/Entebbe region and at Lake Mburo National Park in Uganda, East Africa during three field periods from December 2001 through January 2003. The captures formed part of a study to collect feathers for mercury and plasma for determination of organochlorinated pesticides, total PCBs, and plasma chemistry values in African fish eagles and marabou storks. In addition, morphometric data was collected.

## Materials and Methods

### Adult Eagle Capture

African fish eagles were sampled at Lake Mburo, a six $km^2$ freshwater lake in South Western Uganda (0°. 39' S, 30°. 57' E) situated in a 256 $km^2$ national park, and on Lake Victoria at Entebbe, from Nfo Island (0°. 00' N, 32°. 26' E) to Kisubi Bay (0°. 05' N, 32°. 35' E). Fish eagles were sampled in July, August and December 2002 at Lake Mburo and August 2002 and January 2003 at Entebbe. Forty five percent of birds were sampled between 0600 and 1200 hours and 55% between 1200 to 1800 hours.

Adult fish eagles were captured on the water using a "snare vest" technique. At Lake Mburo, the boat used was an inflatable 3 m x 1.46 m Seaeagle 8 with motormount (SeaEagle Boats, Port Jefferson, NY) crewed by three and powered by a five horsepower (hp) Yamaha outboard motor (Yamaha Motor Corporation, Kennesaw, GA). At Entebbe, a 6.1 m local fishing vessel crewed by four and powered by Yamaha outboards, ranging from 5 to 40 hp was used. Fish fitted with the snares were tilapia *(Oreochromis niloticus)* between 15-25 cm in length and approximately 300 - 400 g. A ventral midline incision from the anogenital opening to the lateral fins was made and the viscera removed. Foam and small pebbles were inserted in the coelomic cavity and mouth so the fish would float laterally on the water surface. The body cavity and mouth were sewn closed with a simple continuous suture pattern using 8 pound test (3.6 kg) 0.12" diameter monofilament fishing line (Stren Fishing Lines, Madison, NC). Multiple loops used to make the snares were constructed from 25 pound test (11.3 kg), clear or pale green, monofilament nylon, 4.8 mm (0.19") diameter fishing line (Pure Fishing, Spirit Lake, IA). To create the snares, a 60-80 cm piece of 25 pound test line was cut and a "figure of eight" knot tied loosely in one end (Figure 2.2 a,b). The end of the line used for the final knot was then placed through one circle of the eight to create a slipknot and excess line was pulled thru to make 5-6 cm diameter snares and a free end of line (Figure 2.2 c). Hemostats and tension were then applied to the line to loosely set the knot and excess line near the slipknot was cut. Eight to twelve snares were made per fish. The free end of the line was threaded through an eyed needle that was used to penetrate the body of the fish. This allowed the line to exit on the underside of the fish as it floated laterally in the water. Most often, snares were placed at equidistant intervals to cover the whole floating

surface of the fish. The free ends of the line were brought together and held in place by a lightly clamped hemostat. They were then tied on themselves with multiple square knots and the excess line cut. The completed snare vest (Figure 2.1) was then set aside until just before use. Sixty pound test (27 kg), 6.3 mm (0.25") diameter, monofilament fishing line (Danielson Company Inc., Auburn, WA) was used to attach the snare vest to a wooden reel, manually held by an operator after being attached to the boat. To prepare the snare vest for immediate use, the free ends of the 25 pound test line were attached to the 60 pound test line by a series of square knots. The snared fish was then placed in the water and allowed to gain distance from the boat by passively drifting or actively paddling away from the fish. Distance from the boat ranged from 6.1 m to 30 m. Once an eagle was entangled by a digit in a snare, the field crew paddled to it while maintaining tension on the line. Shoulder length, kevlar lined animal handling gloves (VetPro Warden gloves, Medical Service Associates, Newington, CN) were used to retrieve the eagle by securing both legs in the tibiotarsal region. A second operator then covered the head with a bag and supported the body until the bird could be taken to shore for sampling. Two pairs of gloves were used to ensure safe transfer of the bird from the operator in the boat to an operator on shore. Fish eagles swim well so there was little risk of drowning. Once all samples had been collected and measurements taken and recorded, the eagle was placed in a large cotton sack, weighed and released from land at the closest point possible to the capture location. Average time from capture to release was 32 minutes (range 20-45).

**Marabou Stork and African Fish Eagle Nestling Capture**

Marabou stork nestlings were sampled in January 2003 in the center of Kampala city, along Nile Avenue, one of the main thoroughfares. All nests were in the introduced white cedar tree (*Tabebuia pentaphylla*). Fish eagle nestlings were sampled at Lake Mburo and Entebbe locations as previously described for adults. Marabou Stork and fish eagle nestlings were temporarily captured for sample collection using professional tree climbing methods based on those described in the National Tree Climbing Field Guide (USDA Forest Service 1996), with modifications for tropical tree species and environmental conditions. The majority of the trees climbed were *Chlorophora excelsa*, *Antiarus toxocara* and *Acacia sieberiana*. Average tree height was 29.87 m at Entebbe (range 17.10 - 46.33 m) and 11.58 m at Lake Mburo (range 6.40 - 18.89 m) and average nest height was 22.55 m (range 12.80 - 34.74) at Entebbe and 8.84 m (range 3.96 - 16.46) at Lake Mburo. The main method of tree ascent was using tree climbers with 70 mm gaffs (Klein Tools Inc., Chicago, IL), a leather climbing saddle (Weaver Leather Inc., Mt. Hope, OH), locking carabiners (Petzl America, Clearfield, UT), tubular webbing and lanyards. Lanyard types used were 13 mm steel core 3.7 m (New England Ropes, Fall River, MA) combined with microcenders (Petzl America, Clearfield, UT) or a two in one Prusik lanyard. Rope type used for rappelling and occasional ascending was Kernmantle 46 m, 11mm diameter static line (New England Ropes, Fall River, MA).

When necessary fish eagle and stork nestlings were gently coaxed to the side of the nest using an "eagle hook". This was either a converted telescopic car aerial (Scosche, telescopic fender mount antennae, Oxnard, CA) twisted at the end to form a hook or an ice gaff (Mason Tackle Company, Otisville, MI), with the point of the hook covered with

protective plastic. The ice gaff was also used by the climber to fend off Marabou storks, who often were very protective of their young. Adult fish eagles did not interfere with sampling of nestlings. Fish eagle and stork nestlings were placed singly into a 40 cm diameter nylon bag specifically designed for similar work with bald eagle (*Haliaeetus leucocephalus*) nestlings (The Taku Tailor, Juneau, AK). The bag had a padded bottom, velcro tab fasteners and multiple ventilation holes. The bag was attached to a rope and lowered to the ground for sampling. Average time from capture to release for fish eagle nestlings was 32 minutes (range 10-60 minutes) and for marabou stork nestlings was 15 minutes (range 7-22 minutes).

All procedures utilizing birds in this study were carried out under approval of the Michigan State University All University Committee on Animal Use and Care. Research permits were granted by the Uganda National Council for Science and Technology and the Uganda Wildlife Authority.

## Results

Twelve adult eagles were captured (ten at Lake Mburo and two at Entebbe) using the snare vest method. The ratio of number of birds caught to attempts eagles made to take the snared fish was 1: 6 at Lake Mburo and 1:10 for Lake Victoria at Entebbe. The ratio of the number of birds caught to the number of times the snared fish was offered to a fish eagle, irrespective of whether an attempt was made to catch the snared fish was 1:8 for Lake Mburo and 1:36 for Lake Victoria. One hundred and twenty capture attempts were made over ten days at Lake Mburo and 72 attempts over nine days at Entebbe. Ten

eagles (83%) were trapped by a snare encircling the bird's third (middle) digit. There appeared to be no consistent area of the fish that was struck by the birds.

Eighteen fish eagle nestlings were sampled by the tree climbing methods described. Eight nestlings were sampled at Lake Mburo from five nest sites and ten from Lake Victoria at five nest sites. Nestlings often could not be visualized, even with binoculars before trees were climbed. At two nests (containing two nestlings each) the nestlings were at fledging age and flew off when the climber approached the nest. Two returned to the nest unaided while two swam to the lakeshore where they were retrieved, sampled and returned to the nest. Nests were viewed daily for an average period of three days after sampling and no problems such as abandonment or interrupted feeding were noted. However, visualization of the chicks was often impossible from the ground. Parents returned to their chicks either immediately after the retreat of the climber, or soon thereafter.

Twenty-one marabou stork nestlings were sampled from twelve nests, in six trees in one colony during an eleven-hour period. Parents either never left the nest (and vigorously defended the chicks) or remained close to the nest and returned upon retreat of the climber. The success rate, defined as trees climbed with at least one chick sampled (nestlings under 800g were deemed too small to sample safely) was 100% for storks and 55% for eagles.

## Discussion

The success of the snare vesting capture technique for African fish eagles was site dependant. The reasons that snare vesting was more successful at Lake Mburo than Lake Victoria were multi-factorial. Fish eagle population density is greater per 100 meters of shoreline at Lake Mburo than Entebbe. The density allowed the researcher's to exploit the extreme territoriality of fish eagles to aid the capture process. Laying a snared fish on the suspected borderline between two territories created competition for the food resource and led to more attempts to take the fish. Competition occasionally led to a rushed, less calculated approach to the snared fish by the eagle thus increasing capture success. Smaller territory size and closer placement of the snared fish to the eagle perching trees also appeared to increase the number of eagles caught at Lake Mburo. The smaller inflatable boat used at Lake Mburo was more maneuverable than the large wooden boat used on Lake Victoria. This facilitated quick and accurate placement of the snared fish at a desirable site. Quick placement of the snared fish was important as many eagle attempts to take the snared fish occurred five minutes or less after snare placement. Furthermore, the usually calmer waters at Lake Mburo helped with snare placement and thus capture success. Optimum time of day for capture at both sites was early morning (0700-0900 hr) and late afternoon (1600-1800 hr), corresponding to observed peak eagle fishing activity.

Color and size of fishing line was important, as eagles would avoid fluorescent green line and "abort" their approach to the snare at the last second. Clear line was the ideal color. Lines heavier than 25-pound test (11.3 kg) became too difficult to create small easily knotted nooses.

When releasing eagles, it was important to choose the release site carefully. The release site had to be within the eagle's territory or an area where the bird would not be attacked by con-specifics. The latter occasionally happened (with no untoward sequelae) to a bird snared in the water before retrieval could occur. A release site with a view of the lake, and far enough back from the shore so the eagle could orientate itself before attempting flight proved optimal. The only complication arising from this snare vesting technique was that on two occasions, an eagle broke free of the vest with a snare encircled around the third digit. One of these eagles was captured on a subsequent second attempt and the snare was no longer present. It is suspected that removal of the slipknots by the eagles could be accomplished with ease, however this was not proven.

The major variables with the snare vest technique that negatively impacted capture rates were snare number and size. Having small numbers (less than five) of larger snares (greater than 8 cm diameter) rarely proved successful. Eight to twelve snares per fish was optimal. However, the use of four snares proved successful for the capture of bald eagles (Bowerman 2001).

The high success rate for sampling marabou stork nestlings was largely due to easy nest accessibility, the high degree of visibility of chicks from the ground and the well defined seasonality of their breeding cycle (Pomeroy, 1977, 1978a). For eagle nestlings, the success rate was aided by predetermination of nest activity status, based on female eagle activity, nest size, state of nest repair and amount of droppings/prey remains

under the nest. The lower success rate with eagle nestlings was partially due to the lack of a defined and studied breeding pattern at both our sample sites and an inability to see into the nest from the ground.

We conclude that the climbing methods described in this paper are successful for the safe sampling of nestling marabou storks and African fish eagles. In addition, snare vesting, as described in this communication may be an effective method to catch adult African fish eagles with a success rate that depends on multi-factorial local site conditions. The technique, with modifications, knowledge of local site conditions and species biology could have applicability to the capture of other large piscivorous eagle species.

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**Figure 2.1:** Fish snare vest utilizing tilapia (*Oreochromis niloticus*). Note fish floats laterally due to Styrofoam in the coelomic cavity and small pebbles in the mouth. A total of eight to twelve snares were made per fish.

**Figure 2.2:** A modified "figure of eight" slipknot was used to tie nooses on the snare vest To create the snares, a 60-80 cm piece of 25 pound test line was cut and a "figure of eight" knot tied loosely in one end (**Figure 2.2 a,b top view**). The end of the line used for the final knot was then placed through one circle of the eight to create a slipknot and excess line was pulled thru to make 5-6 cm diameter snares and a free end of line (**Figure 2.2 c bottom view**) (drawings by A. R. Gandolf).

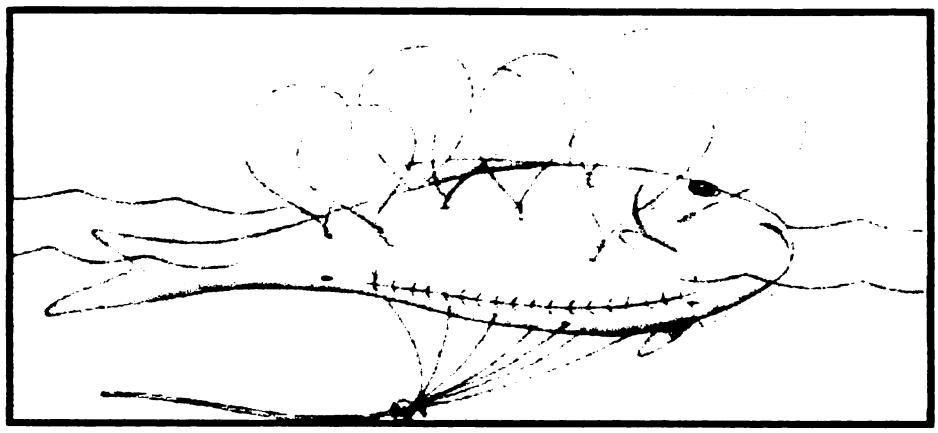

**Figure. 2.1: Fish snare vest**

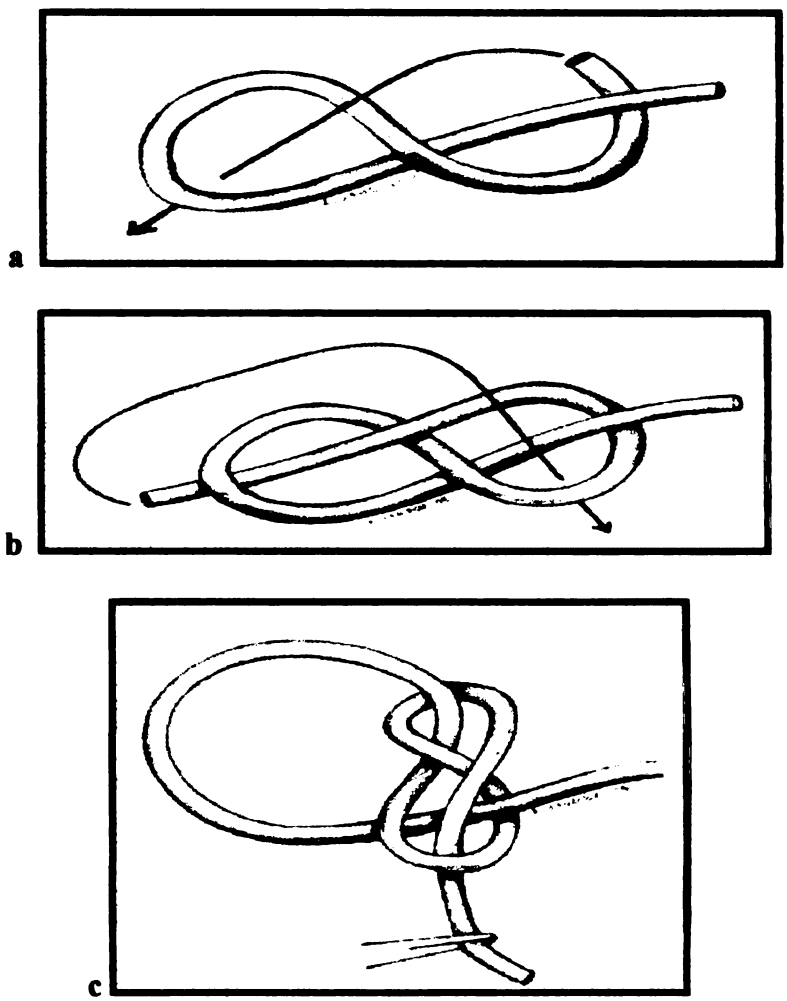


**Figure. 2.2: Modified "figure of eight" slipknot used to tie nooses on the snare vest (drawings by A. R. Gandolf)**

# Chapter 3

## Packed Cell Volume, Biochemical Values, Blood Parasites and Morphometric Measurements for African Fish Eagle (*Haliaeetus vocifer*) Nestlings and Adults at Two Sites in Uganda

## Abstract

Packed cell volumes (PCV) and plasma chemistry parameters were measured in 15 adult and 18 nestling African fish eagles (*Haliaeetus vocifer*) from June 2002 through January 2003. Morphometric measurements were taken on 15 adult eagles. All eagles were sampled for blood parasites and sexed by erythrocyte DNA extraction. Ten adults and eight nestlings were sampled from Lake Mburo and five adults and ten nestlings from Lake Victoria near Entebbe in Uganda. Analysis of variance was conducted to assess the association between site, age, gender and plasma chemistry parameters and the association between gender and morphological characteristics. Plasma chemistry values reported for nestling and adult African fish eagles are similar to those reported for other captive and free-ranging eagle species. Packed cell volumes for nestlings were markedly lower than values reported for similarly aged nestlings of other eagle species. There was no significant difference ($p \geq 0.05$) in PCV of nestling eagles of different body weights. There was variation in all measured plasma chemistry parameters between adults and nestlings, most significantly ($p \leq 0.05$) PCV, calcium, phosphorous, potassium, cholesterol and creatine kinase (CK), all of which were lower in adults, except aspartate transaminase (AST), which was higher. *Plasmodium cicumflexium* like parasites were present in the erythrocytes of three nestlings from Lake Mburo. Like other *Haliaeetus* sp. body weight, bill depth, culmen, toepad and hallux length as well as bill depth measurements were significantly ($p \leq 0.05$) greater for females than males. The data provides baseline biological and physiological information that may prove useful in the management and study of captive and wild populations of African fish eagles.

## Introduction

The African fish eagle (*Haliaeetus vocifer*) inhabits lakes and waterways throughout sub-Saharan Africa (Brown, 1980). It does not appear in the appendices of the Convention In Trade of Endangered Species (CITES). The African fish eagle fills a position of tertiary avian predator, its diet being predominantly fish (Stewart et al 1997). This is a similar niche to that filled by the bald eagle (*Haliaeetus leucocephalus*) in lake-based ecosystems in North America. The bald eagle has been proposed as an ecosystem monitor species of Great Lakes water quality, particularly in regard to the toxic effects of organochlorinated compounds on piscivorous wildlife (Bowerman, 2003). Given the widespread distribution of the African fish eagle and its relative abundance, it may also be a valuable indicator species of water quality in African lake-based ecosystems, such as Lake Victoria. However, there are many factors that determine what constitutes an effective biomonitor species, including a comprehensive knowledge of the basic biology and physiology of the species. Establishing baseline hematological, plasma chemistry and morphological parameters can help fulfill this requirement of an effective biomonitor species. With rapid population growth, urbanization and an expanding economy, Uganda needs effective methods to assess the quality of its environment. Development of suitable bioindicator species are part of a multifaceted approach to effectively assess environmental changes and to monitor the impact of such changes on wildlife.

In addition to providing baseline physiological data to create potential biomonitors, proactive data collection on raptor populations in their natural habitats is preferable to data collected on small numbers of captive specimens. The latter has

become necessary for some raptor species, such as the Spanish Imperial Eagle (*Aquila adalberti*), due to declines in the wild population (Garcia-Montijano et al 2002).

Much of the information on African fish eagle behavior, biology and physiology is anecdotal, dated, site specific and non-standardized. There are no known reports describing plasma chemistry or hematological parameters in adult or nestling African fish eagles. Morphometric data on adult African fish eagles, to the best of our knowledge, is not published in the scientific literature. This paper presents packed cell volumes (PCV), plasma chemistry values, blood parasite analysis, and morphometric data on nestling and adult African fish eagles of known sex. The sampling of African fish eagles for the parameters reported in this paper formed part of a larger study with objectives to determine concentrations of organochlorinated pesticides and total polychlorinated biphenyls (PCBs) from eagle plasma, total mercury content in feathers, characterize eagle nest site habitat and assess the species potential as a biomonitor. The objective of this paper is to provide physiological data that may prove useful in the development of this species as a biomonitor, as well as facilitate the conservation and management of captive and wild populations of African fish eagles.

## Materials and Methods

African fish eagles were sampled at Lake Mburo, a six km long freshwater lake in south western Uganda (0°. 39' S, 30°. 57' E) situated in a 256 $km^2$ national park, and on Lake Victoria near Entebbe, from Nfo Island (0°. 00' N, 32°. 26' E) to Kisubi Bay (0°. 05' N, 32°. 35' E). Fish eagles were sampled at Lake Mburo in July, August and December

2002 and at Lake Victoria in August 2002 and January 2003. Thirty-three eagles were sampled: ten adults and eight nestlings from Lake Mburo and five adults and ten nestlings from Lake Victoria. Forty five percent of birds were sampled between 0600 and 1200 hours and 55% between 1200 to 1800 hours.

Adult fish eagles were captured on water using a fish "snare vest" technique. Tilapia (*Oreochromis niloticus*) were fitted with fishing line snares (loops) and packed with foam so the fish floated laterally. A total of eight to twelve 5 - 6 cm diameter snares with a free end of line were made per fish. The free ends of line penetrated the body of the fish and were then tied on themselves and the excess line cut. The line was attached to a hand held reel and the fish placed in water. Once captured, the eagle was retrieved and secured by the legs in the tibiotarsal region. Fish eagles swim well so there was little risk of drowning. On shore, the eagles were placed in dorsal recumbency and the eyes covered. Ten ml of blood were collected from the brachialis vein via a 21 or 23 gauge x 1.9 cm (¾ inch) butterfly catheter (Surflo Winged Infusion Set, Elkton, MD) connected to a 10 ml syringe (Luer Lok Tip Syringe, Becton Dickinson and Company, Rutherford, NJ) flushed with sodium heparin (100 IU/ml). The blood was immediately transferred to a 10 ml lithium heparin vacutainer. An additional 4 ml of blood was drawn and placed in a 5 ml EDTA vacutainer (Becton Dickinson, Franklin Lakes, NJ). Three blood smears were made with fresh blood using the slide on slide technique (Campbell, 1988). Fresh whole blood was also used to determine blood glucose levels (Medisense 2® card glucometer utilizing precision plus sensors®, Medisense Inc, Bedford, MA). A drop of whole blood was placed on a commercially prepared paper sample card for molecular sex

determination based on total erythrocyte DNA (Avian Biotech International, Tallahassee, FL). Five whole breast feathers were hand plucked for determination of total mercury concentrations. A physical examination including scoring body condition (based on pectoral muscle mass and feather condition), whether the crop was empty or full and a visual description of any abnormalities were made. Body measurement methods used were the same as those described for the bald eagle (Bortolotti 1984 a,b). Length of the eighth primary feather and footpad were determined with a 60cm ruler (Figure 3.2) (Pickett brand Model ASE 24, Forestry Suppliers, Jackson, MI). Hallux and culmen length, as well as bill depth were measured using a dial caliper (Figure 3.3.) (model SPI 2000, Forestry Suppliers, Jackson, MI). Birds were banded with 18-22mm internal diameter metal rivet bands inscribed with a three letter sequential code and the word "MAKERERE" (Gey Band and Tag Company, Norristown, Pennsylvania, USA). The bands were colored either red or gold for Lake Mburo and black for Entebbe. Suspected female birds were banded on the left leg and suspected males on the right leg. Lastly, birds were placed in a cotton sack and body weight recorded by a spring balance with gradations of 100g (Homs model 20, Douglas Homs Corp., Belmont, CA). Eagles were then released from land at the closest point possible to the capture location. Average time from capture to release was 34 minutes (range 20-45 minutes). African fish eagles were classified as adult if they had attained full adult plumage color (i.e. were at least five years old).

African fish eagle nestlings were retrieved for sampling from the nest using professional tree climbing methods (USDA Forest Service, 1996). The main method of

tree ascent was using tree climbers (Klein Tools, Chicago, ILL). Eagle nestlings were gently coaxed to the side of the nest using an "eagle hook" modified from a car aerial or ice gaff. Eagle nestlings were placed singly into a ventilated nylon bag and lowered to the ground for sampling. Sampling of nestlings was as described for adults with the exception that the volume of blood collected varied from four to 14 ml depending on body weight. Eagles were aged to within +/- 3 days based on body weight and the calculations presented by Sumba (1988).

Samples were placed in a chilled cooler. Time of sampling to storage of plasma in liquid nitrogen was 3.5 hours (range 2-9 hours). Packed cell volume and total plasma protein (TPP refrac) were determined in the field. Microhematocrit capillary tubes (2) were centrifuged at 3000 rpm for 5 minutes (Vulcon Mobilespin PS126-6, Vulcon Technologies, Grandview, MO) and an average PCV reading recorded. Total plasma protein was determined using a temperature compensated refractometer (Leica Inc. Optical Products Division, Buffalo, NY). The remaining blood was centrifuged at 3000 rpm for ten minutes. Plasma was observed visually for hemolysis, icterus, and lipemia and these changes subjectively classified as slight, moderate or severe. Plasma was pipetted into five 2 ml cryovials (Cryogenic Vial, Corning Incorporated, Corning, NY) and deposited into a MVE Doble-20 Vapor Shipper/Liquid Nitrogen Tank (MVE Bio-Medical Systems, Burnsville, MN). Plasma samples were transported to the Diagnostic Center for Population and Animal Health (DCPAH) at Michigan State University Veterinary Medical Center (MSU) then transferred to a – 80°C freezer until analyzed. Analysis occurred five months after sampling for 14 of the samples and less than one

month for the remainder. Plasma chemistry analyses were performed at the clinical pathology and endocrinology laboratories of the DCPAH at MSU. The plasma chemistry analyses were performed on an Olympus AU640 chemistry analyzer, (Olympus America Inc., Irving, TX). The electrolyte analyses were performed with a sodium potassium crown ether membrane while the chloride analysis employed a molecular oriented polyvinylchloride membrane. Calcium (Ca), phosphorous (P), TPP col, albumin, aspartate transaminase (AST), creatine kinase (CK), cholesterol and uric acid were performed using olympus reagents. Sodium/potassium ratio, and globulin were calculated from the measured parameters. Total plasma thyroxine (TT4) was measured by two methods: a commercial radioimmunoassay (TT4 RIA) (Diasorin Inc., Stillwater, MN) and a colorometric ELISA (TT4 ELISA) specifically designed to assess thyroid function in a range of animal species, including birds (Oxford laboratories, Oxford, MI. Not commercially available at the time of publication). Total plasma triiodothyronine (TT3) was measured using an assay prepared in house at the endocrinology laboratory, DCPAH at MSU. Molecular sex differentiation used a polymerase chain reaction based on the first gene of the avian W chromosome (CHD) (Ellegren, 1996; Griffiths et al 1998).

Blood smears for blood parasite analysis were stored unstained in slide boxes. Prior to examination, they were fixed in absolute methanol and then stained with giemsa. Each slide was examined in its entirety (250X), then for ten minutes (500X) and for an additional ten minutes under oil immersion in two five minute sessions (1250X). Both red and white blood cells were examined. The degree of parasitemia was expressed as the percentage of erythrocytes infected per 1000 erythrocytes counted. Parasitological

analysis was conducted at the Michigan Department of Natural Resources Rose Lake Pathology Laboratory.

Analysis of variance was conducted to assess the association between the risk factors of site, age (nestling or adult), gender and plasma chemistry parameters. Analysis of variance was also used to assess the association between morphological characteristics and sex of adult fish eagles (SAS PROC ANOVA for categorical risk factors, and SAS PROC GLM for continuous risk factors. SAS 8.2, 2001. SAS Inc., Cary, NC). These analyses were conducted at the univariable (only one risk factor at a time) and multivariable level. Multivariable analyses were conducted to adjust the effect of selected risk factors simultaneously. The level of significance (type 1 [$\alpha$] error) was set at $p \leq 0.05$. Descriptive statistics were done using Excel (Microsoft Excel, Microsoft Corporation, Redmond, WA). An outlying value was defined as being 1.5 times greater or less than the interquartile range. Descriptive statistics are emphasized due to the small sample size. This emulates the methods of other studies examining wild avian hematological and plasma chemistry values where only small sample sizes could be obtained (Garcia- Montijano 2002; Lumsden 1998).

All procedures utilizing birds in this study were carried out under approval of the Michigan State University All University Committee on Animal Use and Care. The Uganda National Council for Science and Technology and the Uganda Wildlife Authority granted research permits for this project.

## Results

Plasma chemistry values and PCVs are presented for adult (Table 3.1.) and nestling (Table 3.2.) African fish eagles. Morphometric data are presented for adult African fish eagles divided by sex (Table 3.3.). Results of analysis of variance of the morphological data are presented in Table 3.4. Results of analysis of variance of the plasma chemistry data are presented in Table 3.5. Results of multivariable analysis of variance of PCV in African fish eagle nestlings is presented in Table 3.6. Two of the plasma samples, a nestling from Lake Victoria and an adult from Lake Mburo were slightly hemolyzed. Two plasma samples showed slight (nestlings from Lake Mburo), two moderate (nestlings from Lake Victoria) and one sample severe lipemia (nestling from Lake Mburo). Three nestlings from Lake Victoria had an empty crop and seven had full crops. Four nestlings from Lake Mburo had empty crops and four had full crops. Three adults from Lake Victoria had full crops and two had empty crops. Six adults from Lake Mburo had empty crops and four had full crops. All birds were in good body condition as assessed by pectoral muscle mass and general physical examination. There was a weak positive correlation between blood glucose levels and a full crop ($r^2 = 0.012$). The mean PCV for nestlings was 0.27 L/L. There was no significant difference ($p \geq 0.05$) in PCV of nestling fish eagles of different body weights. Plasma chemistry values that were significantly ($p \leq 0.05$) different between adults and nestlings were AST, phosphorous, potassium and CK, all of which were lower in adults, except AST. Aspartate transaminase values were significantly ($p \leq 0.05$) higher in adults than nestlings. There were significant differences ($p \leq 0.05$) in cholesterol, albumin, potassium, phosphorous, TT3 and TT4 RIA values in fish eagles between the study sites. There was a weak

positive correlation ($r^2$ = 0.032) for TT4 levels measured by radioimmunoassay and ELISA methods. The ratio of TT4 ELISA/TT3 was 4.74.

Total plasma protein values were consistently higher when measured using a temperature compensated refractometer in the field than with a colorimetric method in the laboratory. Mean values for nestlings were 9g/L higher and adult values 10 g/L higher for measurements taken with a refractometer. A strong positive correlation existed between values returned by each method (r = 0.76).

One female nestling (estimated age 52 ± 3 days) from Lake Mburo had moderate numbers of erythrocytes (6%) infected with a *Plasmodium circumflexium* like parasite. Two nestling siblings (estimated ages 42 and 44 ± 3 days), a male and female from Lake Mburo, had light burdens (2% erythrocytes infected) of the same parasite. The nestlings infected with blood parasites also had old, healing digital abrasions, showed the poorest body condition of all the birds examined and had moderate burdens of an unidentified species of lice and hippoboscid flies. One infected bird was sampled at 0900, the second at 1200 and the third (with the heaviest burden) at 1300.

Body weights, culmen, footpad, 8$^{th}$ primary feather length, and bill depth parameters were significantly ($p \leq 0.05$) greater in adult female than in adult male fish eagles. Female fish eagles were on average 20% heavier than male fish eagles. The ratio of male to female culmen, footpad, 8$^{th}$ primary feather length, and bill depths were 0.87, 0.89, 0.91 and 0.92 respectively.

## Discussion

Packed cell volumes for nestlings in this study were markedly lower than nestling values reported in other eagle species (Redig, 1993; Bowerman 2000; Hoefle et al 2000). The mean PCV of African fish eagle nestlings in this study was 0.27 L/L. Values reported for wild bald eagle nestlings are 0.34 L/L (Redig, 1993) and 0.32 L/L (Bowerman 2000). Values reported for wild Spanish imperial eagles *Aquila adalberti* were also 0.32 L/L (Hoefle et al., 2000). Despite African fish eagles having a similar fledging period as these two eagle species, birds of a similar age to those sampled in the above studies still had lower PCVs. Despite the small nestling sample size (n =18), there were no low outlying values (greater than 1.5 times lower than the 25$^{th}$ percentile of the sample) to confound the results. Only three of 18 nestlings had internal or external parasite burdens that may have contributed to the low PCV. Exclusion of nestlings with blood parasites from the sample size did not significantly raise the mean nestling PCV (27.44 to 28.13). However, the nestling with the greatest parasite burden had the lowest recorded PCV of all birds sampled (0.20 L/L). The two nestlings with light parasite burdens had PCVs equal to the 25$^{th}$ percentile of the total nestling sample size. All nestlings were considered to be well nourished by visual examination and no nestlings were considered in poor body condition. The finding that there was no significant association between PCV and body weight is surprising. Although there may be some variation due to factors such as nutrition, there should be a positive correlation between body weight and age over the period of fledging growth (Sumba 1988). Therefore, PCV should increase with age. Due to the small sample size, results may not be statistically

valid as PCV would be expected to increase with age, as reported in studies on captive white storks, *Ciconia ciconia* (Montesinos et al 1997) and psittacines (Clubb et al 1991). It may be that rapid increases in PCV only occur once nestlings have an increased oxygen demand at the time of fledging (Hawkey et al 1984). Few studies have examined PCV in relation to nestling age in wild raptors. The reason for the lack of studies may be the greater number of studies conducted on species from temperate regions. Raptors from temperate regions are likely to have well defined breeding seasons, hence nestlings sampled in a particular temporal and spatial period would be expected to be of a comparable age. Such is not the case with African fish eagles from tropical climates where populations may contain eagle pairs with eggs, just hatched young or nestlings about to fledge at the same time.

Sex based variation in AST levels has been recorded in some avian species (Gee et al 1981). The variation in potassium values between adults and nestlings and the absolute values are similar to those reported for bald eagles (Bowerman 2000; Redig 1993). Red blood cell lysis may precipitate elevated extra-cellular potassium levels (Fudge 1994). Variation in CK levels is most likely indicative of muscle damage or injection site trauma during sampling, although overtly, struggling was usually minimal. Due to the small sample size, the data presented may not be statistically significant thus caution should be exercised with interpretation.

Total plasma thyroxine and triiodothyronine values presented should be interpreted cautiously. Serum levels of thyroid hormones and the ratio of TT4 to TT3 can

vary greatly between genus, species and individuals (Rosskopf et al 1982). This is illustrated by the ratio of TT4 ELISA/TT3 being 1.79 for marabou stork nestlings and 4.74 for fish eagle nestlings in this study. The short half lives of avian TT3 and TT4 can lead to significant diurnal variation in plasma concentrations of these hormones. Hyperthermia and stress may also reduce serum TT4 concentrations (Rae 2000). Birds have relatively low concentrations of TT4 compared to humans, so human TT4 RIA may have low sensitivity in avian species. In addition, commercially available human radioimmunoassay kits that have not been standardized in a particular laboratory by using serum from euthyroid birds are difficult to compare between laboratories, different commercial kits and different species (Merryman and Buckles 1998).

The finding that TPP values were consistently higher when measured using a temperature compensated refractometer in the field than with a colorimetric method in the laboratory may support studies that demonstrated poor reproducibility of values using refractometry to determine avian plasma protein values (Lumiej and Maclean 1996). This may indicate that refractometric methods should be used only as an approximation of plasma protein levels.

Bennett et al (1977) recorded blood parasite prevalence in a variety of avian species (not including African fish eagles), over 5 years at Zika Forest (about 30 km from Kampala). They found an overall prevalence of 34.8 % with the vast majority of infections being *Haemoproteus* spp. *Plasmodium* spp. infection comprised only approximately two percent of all infections and there appeared to be a slight positive

correlation between parasite prevalence and rainfall. Our samples were taken during the dry seasons.

Little morphometric data exists on African fish eagles. Average body weights in this study are similar to those cited by Sumba (1988) for captive fish eagles at Entebbe of 2.250kg for males (n = 3) and 2.835 kg (n = 2) for females. Body weight ranges presented by Brown (1980), 3.000-3.600kg (n = 3) for females and 1.986-2.497 kg (n = 4) for males, fall within ranges found in this study. The morphometric data supports the conclusion that, like other *Haliaeetus* species, females have a larger body mass than males.

It is hoped that the information presented in this paper can be utilized as a foundation on which further research can construct a comprehensive biological and physiological database for the African fish eagle. Such a database may prove a valuable diagnostic tool for the conservation of individual species, and the management and monitoring of ecosystem health, through use of the species as a biomonitor. Such long-term conservation goals are necessary and warranted on the African continent.

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**Table 3.1:** Packed cell volumes and selected plasma chemistry values and morphometric measurements, from adult African fish eagles (*Haliaeetus vocifer*) sampled at Lake Mburo and Lake Victoria near Entebbe, Uganda (n =15).

[a] PCV = packed cell volume

[b] TPP refrac = total plasma protein measured by refractometry

[c] TPP Col = total plasma protein measured by a colorimetric method

[d] TT4 radioimmunoassay = thyroxine radioimmunoassay

[e] TT4 elisa = thyroxine enzyme linked immunoabsorbent assay

[f] TT3 = triiodothyronine

[g] AST = aspartate transaminase

[h] CK = creatine phosphokinase

[i] P = phosphorous

[j] Na/K = sodium/potassium ratio

[k] = determined on whole blood

**Table 3.1 Packed cell volume, plasma chemistry values and morphometric measurements from adult African fish eagles**

| | Mean | Median | SD | Q1 | Q3 | Min | Max |
|---|---|---|---|---|---|---|---|
| [a] **PCV** L/L | 0.45 | 0.45 | 0.0225 | 0.44 | 0.46 | 0.43 | 0.53 |
| [b] **TPP** Refrac g/L | 48 | 47 | 4.7 | 45 | 52 | 38 | 54 |
| [c] **TPP** Col g/L | 38 | 38 | 4 | 35 | 41 | 30 | 42 |
| [d] **TT4** nMol/l | 4.4 | 4.0 | 2.03 | 3.0 | 5.5 | 1.0 | 8.0 |
| [e] **TT4 Elisa** nMol/l | 14.56 | 12.50 | 8.61 | 9.25 | 16.20 | 6.60 | 41.60 |
| [f] **TT3** nMol/l | 1.6 | 1.5 | 0.41 | 1.2 | 1.7 | 1 | 2.4 |
| [k] **Glucose** mMol/L | 12.4 | 11.9 | 2.01 | 10.7 | 12.8 | 10.3 | 16.3 |
| [g] **AST** U/L | 194 | 152 | 117 | 139 | 199 | 121 | 590 |
| **Calcium** mMol/L | 2.4 | 2.4 | 0.13 | 2.34 | 2.50 | 2.12 | 2.57 |
| [h] **CK** U/L | 217 | 215 | 53 | 184 | 252 | 127 | 320 |
| [i] **P** mMol/L | 0.74 | 0.68 | 0.30 | 0.48 | 0.92 | 0.42 | 1.45 |
| **Uric Acid** mMol/L | 0.998 | 1.011 | 0.408 | 0.690 | 1.190 | 0.291 | 1.731 |
| **Albumin** g/L | 12 | 12 | 1.3 | 12 | 13 | 11 | 15 |
| **Globulin** g/L | 25 | 26 | 3.3 | 23 | 28 | 19 | 30 |
| **Sodium** mMol/L | 153 | 155 | 5.56 | 153 | 155 | 143 | 161 |
| **Potassium** mMol/L | 1.3 | 1.2 | 0.33 | 1.1 | 1.5 | 1.0 | 1.8 |
| [j] **Na/K ratio** | 123 | 127 | 31.67 | 95 | 144 | 87 | 161 |
| **Chloride** mMol/L | 115 | 116 | 5.21 | 112 | 117 | 105 | 124 |
| **Cholesterol** mMol/L | 4.69 | 4.56 | 0.644 | 4.22 | 5.05 | 3.86 | 6.16 |

**Table 3.2:** Packed cell volumes and selected plasma chemistry values from nestling African fish eagles (*Haliaeetus vocifer*) sampled at Lake Mburo and Lake Victoria near Entebbe, Uganda (n =18).

[a] PCV = packed cell volume

[b] TPP refrac = total plasma protein measured by refractometry

[c] TPP Col = total plasma protein measured by a colorimetric method

[d] TT4 RIA = thyroxine radioimmunoassay

[e] TT4 ELISA = thyroxine enzyme linked immonoabsorbent assay

[f] TT3 = triiodothyronine

[g] AST = aspartate transaminase

[h] CK = creatine kinase

[i] Na/K = sodium/potassium ratio

[j] P = phosphorous

[k] = determined on whole blood

**Table 3.2. PCV and plasma chemistry values from nestling African fish eagles**

| | Mean | Median | SD | Q1 | Q3 | Min | Max |
|---|---|---|---|---|---|---|---|
| **Age** days | 27 | 22 | 12 | 18 | 37 | 9 | 52 |
| **Weight** Kg | 1.45 | 1.33 | 0.63 | 0.91 | 1.94 | 0.50 | 2.60 |
| [a] **PCV** L/L | 0.27 | 0.28 | 0.03 | 0.26 | 0.30 | 0.20 | 0.33 |
| [b] **TPP** Refrac g/L | 45 | 45 | 4 | 44 | 48 | 36 | 52 |
| [c] **TTP** Col g/L | 36 | 37 | 4 | 32 | 39 | 27 | 42 |
| [d] **TT4 RIA** nMol/l | 8 | 7 | 4 | 5 | 11 | 2 | 15 |
| [e] **TT4 elisa** nMol/l | 11.44 | 10.50 | 2.48 | 9.80 | 13.20 | 8.10 | 16.00 |
| [f] **TT3** nMol/l | 2.28 | 2.30 | 0.43 | 2.10 | 2.60 | 1.40 | 2.80 |
| [k] **Glucose** mMol/L | 13.8 | 14.3 | 1.8 | 12.6 | 14.9 | 10.4 | 17.7 |
| [g] **AST** U/L | 123 | 120 | 34 | 95 | 143 | 75 | 185 |
| **Calcium** mMol/L | 2.62 | 2.65 | 0.1 | 2.60 | 2.70 | 2.40 | 2.80 |
| [h] **CK** U/L | 906 | 754 | 515 | 517 | 1201 | 178 | 1880 |
| [j] **P** mMol/L | 1.97 | 1.68 | 0.84 | 1.55 | 2.13 | 0.77 | 3.91 |
| **Uric Acid** mMol/L | 0.898 | 0.922 | 0.345 | 0.660 | 1.041 | 0.422 | 1.65 |
| **Albumin** g/L | 13.8 | 13.5 | 2.1 | 12.3 | 16.0 | 10.0 | 17.0 |
| **Globulin** g/L | 22.0 | 21.0 | 3.6 | 20.3 | 24.5 | 16.0 | 28.0 |
| **Sodium** mMol/L | 148 | 148 | 2 | 147 | 149 | 145 | 154 |
| **Potassium** mmol/L | 2.45 | 2.30 | 0.65 | 2.10 | 2.90 | 1.30 | 3.80 |
| [i] **Na/K ratio** | 65 | 65 | 19 | 51 | 71 | 38 | 112 |
| **Chloride** mMol/L | 110 | 111 | 5 | 107 | 113 | 101 | 118 |
| **Cholesterol** mMol/L | 5.49 | 5.36 | 1.11 | 4.76 | 6.24 | 3.29 | 7.41 |

**Table 3.3:** Morphometric measurements of adult male and female African fish eagles (*Haliaeetus vocifer*) (n = 15) from Lake Mburo and Lake Victoria near Entebbe.

**Table 3.3. Morphometric measurements of adult male and female African fish eagles**

| | Mean | Median | SD | Q1 | Q3 | Min | Max |
|---|---|---|---|---|---|---|---|
| **Adult Male Fish Eagles (n=9)** | | | | | | | |
| **Weight (Kg)** | 2.3 | 2.3 | 0.1 | 2.3 | 2.4 | 2.1 | 2.5 |
| **8th Primary (mm)** | 380 | 387 | 16 | 380 | 389 | 340 | 391 |
| **Footpad (mm)** | 109 | 110 | 5 | 106 | 112 | 101 | 116 |
| **Bill Depth (mm)** | 25.24 | 24.76 | 1.13 | 24.64 | 24.88 | 24.49 | 27.81 |
| **Culmen (mm)** | 39.97 | 39.54 | 0.94 | 39.50 | 40.85 | 38.35 | 41.28 |
| **Hallux (mm)** | 35.64 | 36.17 | 1.87 | 35.15 | 36.40 | 31.29 | 38.02 |
| **Adult Female Fish Eagles (n=6)** | | | | | | | |
| **Weight (Kg)** | 3.0 | 2.9 | 0.4 | 2.7 | 3.4 | 2.5 | 3.6 |
| **8th Primary (mm)** | 417 | 420 | 8 | 412 | 424 | 405 | 425 |
| **Footpad (mm)** | 123 | 124 | 5 | 120 | 125 | 115 | 128 |
| **Bill Depth (mm)** | 27.39 | 27.21 | 1.79 | 26.39 | 28.29 | 25.05 | 30.11 |
| **Culmen (mm)** | 45.71 | 44.31 | 5.14 | 43.35 | 44.96 | 41.55 | 55.89 |
| **Hallux (mm)** | 39.90 | 39.76 | 1.89 | 38.50 | 40.96 | 37.70 | 42.71 |

**Table 3.4. Univariable analysis of variance of morphological data by gender from African fish eagles from Lake Mburo and Lake Victoria near Entebbe (n=15)**

| Variable | Gender | n | Mean (sd) | F | p |
|---|---|---|---|---|---|
| Body weight (kg) | Female | 6 | 3.02 (.45) | 18.34 | .0009 |
| | Male | 9 | 2.36 (.12) | | |
| $8^{th}$ primary feather length (mm) | Female | 6 | 417.50 (8.22) | 26.91 | .0002 |
| | Male | 9 | 380.56 (15.95) | | |
| Footpad length (mm) | Female | 6 | 122.67 (4.76) | 28.30 | .0001 |
| | Male | 9 | 109.22 (4.82) | | |
| Bill depth (mm) | Female | 6 | 27.39 (1.79) | 8.30 | .0129 |
| | Male | 9 | 25.24 (1.13) | | |
| Culmen length (mm) | Female | 6 | 45.71 (5.14) | 11.07 | .0055 |
| | Male | 9 | 39.97 (.94) | | |
| Hallux length (mm) | Female | 6 | 39.90 (1.89) | 18.54 | .0009 |
| | Male | 9 | 35.64 (1.87) | | |

**Table 3.5:** Analysis of variance of plasma chemistry values in adult (n = 15) and nestling ( n = 18) African fish eagles (*Haliaeetus vocifer*) from Lake Mburo and Lake Victoria near Entebbe, Uganda.

[a] AST = aspartate transaminase

[b] Chol. = cholesterol

[c] CK = creatine kinase

[d] PCV = packed cell volume

[e] P = phosphorous

[f] TT4 ELISA = thyroxine enzyme linked immunoabsorbent assay

[g] TT4 RIA = thyroxine radioimmunoassay

[h] TT3 = triiodothyronine

[i] TPP (ref) = total plasma protein determined by refractometry

j TPP (col) = total plasma protein determined by a colorimetric method

[k] = determined on whole blood

**Table 3.5. Analysis of variance of plasma chemistry values in fish eagles (n = 33)**

| Plasma Chemistry | Variables | F | p | Plasma Chemistry | Variable | F | p |
|---|---|---|---|---|---|---|---|
| | Site | .82 | .3723 | | Site | 17.17 | .0003 |
| | Age | 5.84 | .0222 | | Age | 10.55 | .0029 |
| [a]AST | Gender | .12 | .7293 | Albumin | Gender | .66 | .4222 |
| | **Overall** | **2.26** | **.1024** | | **Overall** | **9.46** | **.0002** |
| | Site | .74 | .3980 | | Site | 9.03 | .0054 |
| | Age | 27.45 | < .0001 | | Age | 35.60 | < .0001 |
| [c]CK | Gender | 2.45 | .1282 | Calcium | Gender | 0.0 | .9724 |
| | **Overall** | **10.21** | **< .0001** | | **Overall** | **14.88** | **< .0001** |
| | Site | 9.84 | .0039 | | Site | .38 | .5405 |
| | Age | 7.82 | .0091 | | Age | 7.22 | .0118 |
| [b]Chol. | Gender | .05 | .8270 | Chloride | Gender | .32 | .5753 |
| | **Overall** | **5.90** | **.0028** | | **Overall** | **2.64** | **.0682** |
| | Site | 3.44 | .0739 | | Site | .73 | .4008 |
| | Age | 7.42 | .0108 | | Age | 4.62 | .0402 |
| Globulin | Gender | .74 | .3979 | [k]Glucose | Gender | .20 | .6591 |
| | **Overall** | **3.87** | **.0193** | | **Overall** | **1.85** | **.1608** |

**Table 3.5 (continued)**

| Plasma Chemistry | Variables | F | p | Plasma Chemistry | Variables | F | p |
|---|---|---|---|---|---|---|---|
| | Site | 6.51 | .0163 | | Site | .32 | .5744 |
| | Age | 50.21 | < .0001 | | Age | 7.61 | .0099 |
| **Potassium** | Gender | 0.0 | .9536 | **Sodium** | Gender | .55 | .4628 |
| | **Overall** | **18.91** | **< .0001** | | **Overall** | **2.83** | **.0559** |
| | Site | 10.40 | .0031 | | Site | .25 | .6225 |
| | Age | 39.30 | < .0001 | | Age | 275.30 | < .0001 |
| [c]**P** | Gender | .79 | .3824 | [d]**PCV** | Gender | 1.59 | .2181 |
| | **Overall** | **16.83** | **< .0001** | | **Overall** | **92.38** | **< .0001** |
| | Site | 17.76 | .0002 | | Site | 20.20 | .0001 |
| | Age | 35.56 | < .0001 | | Age | 16.55 | .0003 |
| [h]**TT3** | Gender | .72 | .4043 | [g]**TT4 RIA** | Gender | .36 | .5523 |
| | **Overall** | **18.01** | **< .0001** | | **Overall** | **12.37** | **< .0001** |
| | Site | .92 | .3451 | | Site | 3.71 | .0638 |
| | Age | 2.07 | .1608 | | Age | .65 | .4250 |
| [f]**TT4 ELISA** | Gender | 1.40 | .2474 | **Uric Acid** | Gender | 3.73 | .0633 |
| | **Overall** | **1.46** | **.2456** | | **Overall** | **2.70** | **.0641** |
| | Site | 2.36 | .1352 | | Site | .01 | .9347 |
| | Age | 2.99 | .0943 | [j]**TPP** | Age | 1.54 | .2249 |
| [i]**TPP (Ref)** | Gender | .03 | .8631 | **(Col)** | Gender | .62 | .4381 |
| | **Overall** | **1.79** | **.1703** | | **Overall** | **.72** | **.5477** |

**Table 3.6. Univariable and multivariable analysis of variance of packed cell volume in African fish eagle (n=18) nestlings.**

| Species | Variable | Level | n | Mean (sd) | F | p |
|---|---|---|---|---|---|---|
| Fish eagle nestlings | Site | Lake Victoria | 10 | 29.8 (1.99) | 22.29 | .0002 |
| | | Lake Mburo | 8 | 24.5 (2.78) | | |
| | Body weight | - | 18 | - | 2.35 | .1445 |
| | Gender | Female | 7 | 26.71 (4.57) | .47 | .5033 |
| | | Male | 11 | 27.91 (2.88) | | |
| | Multivariable ANOVA | Site | - | - | 21.39 | .0004 |
| | | Body Weight | - | - | .18 | .6759 |
| | | Gender | - | - | 1.17 | .2982 |
| | | Overall | - | - | 7.58 | .0030 |

**Figure 3.1:** Technique for measuring African fish eagle footpad length with a rigid ruler. Tendons must be stretched to obtain maximum extension of footpad for accurate reading. (Modified from Bortolotti 1984 a, b).

**Figure 3.2.** Technique for measuring African fish eagle culmen length (A), bill depth (C) and hallux length (D). (Modified from Bortolotti 1984 a, b).

**Figure 3.1. Technique for measuring African Fish Eagle footpad length (Modified from Bortolotti 1984 a, b)**

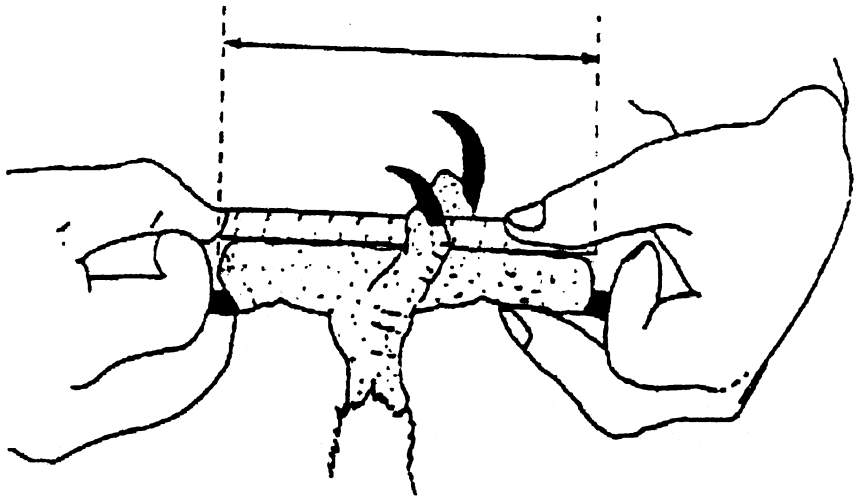
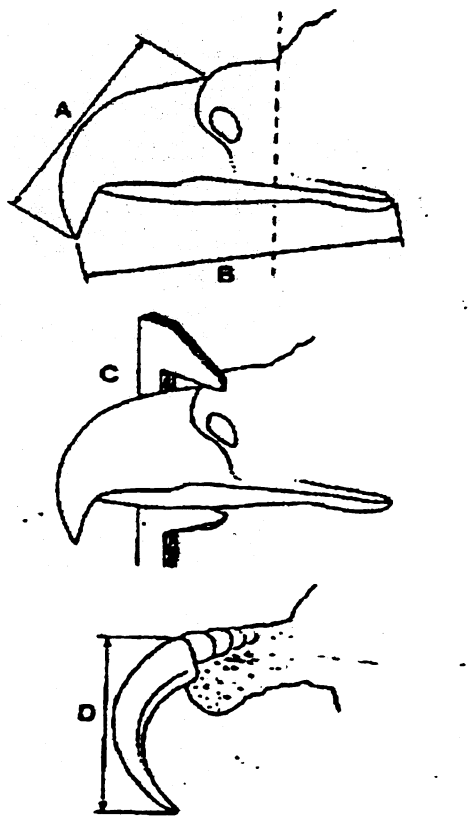


**Figure 3.2. Technique for measuring African fish eagle culmen length (A), bill depth (C) and hallux length (D). (Modified from Bortolotti 1984 a, b)**



# Chapter 4

# Packed Cell Volume, Plasma Chemistry Values and Survey for Blood Parasites in Nestling Marabou Storks (*Leptoptilos crumeniferus*) in Uganda

## Abstract

The purpose of this research was to establish baseline physiological parameters, such as plasma chemistry values in marabou storks and assess the feasibility of developing this species as a biomonitor of environmental change. Packed cell volumes (PCV) and plasma chemistry parameters were measured in twenty nestling marabou storks (*Leptoptilos crumeniferus*) in January 2003. Marabou nestlings were part of a colony located in the center of Kampala, Uganda. Nestlings were also surveyed for the presence of blood parasites. Sex was genetically determined using erythrocyte DNA. No blood parasites were found. There were no significant differences ($p \geq 0.05$) in plasma chemistry values or PCV between sexes. Total plasma protein (TPP), uric acid, phosphorous and creatine kinase were generally higher relative to published data on other avian species, including nestling white storks (*Ciconia ciconia*). Thyroxine levels measured by both ELISA and radioimmunoabsorbent assay were variable between nestlings. All other values were similar to those reported for a variety of avian species.

## Introduction

The marabou stork is indigenous to tropical Africa where it is common to abundant in most parts of its range. The marabou stork (*Leptoptilos crumeniferus*) has responded to increasing urbanization and centralization of human populations by adopting a scavenging lifestyle and cosmopolitan diet in urban areas. Populations have increased in Kampala and breeding colonies may be found in the city center (Hancock et al 1992). This research formed part of a study examining persistent organic pollutant concentrations in marabou storks and African fish eagles (*Haliaeetus vocifer*) and

assessing their potential as biomonitor species. To be an effective biomonitor, information is required on the biology and physiology of a species. The biology of the marabou stork in Uganda has been well described by Pomeroy (1977, 1978). Adult marabous weigh between 5 - 8 kg and the diet of the birds in Kampala probably includes almost anything organic, such as garbage, fish remains, abattoir refuse and a large amount of vegetable matter (Brown et al 1982). However, during the period of nestling growth increased amounts of protein are taken in the form of fish, frogs and rodents. Marabous undertake short, mainly north south migrations in Uganda, coinciding with rainfall seasonality (Pomeroy, 1978 a, 1978 b). Marabous are colonial nesters with multiple nests being built in particular tree types, such as Mvule (*Chlorophora excelsa*) and *Tabebuia pentaphylla*. The incubation period is 30.3 days and the average clutch size is 2 - 3 eggs. Marabous have an extremely long period from hatching to fledging, being about 135 days with first flights out of the nest at 110 - 115 days. Marabous first breed at 6 - 7 years and can live up to 25 years in captivity. Breeding success is low but has reportedly increased in recent years in Kampala (Hancock et al 1992). Despite its ubiquity, no plasma chemistry or hematological parameters have been reported for wild marabou storks. The objective of this study was to determine baseline plasma chemistry and packed cell volume (PCV) values for nestling marabou storks.

## Materials and Methods

Packed cell volumes, plasma chemistry parameters and body weights were recorded in twenty nestling marabou storks in January 2003. Eleven nestlings were male and nine were female as determined by molecular sexing based on total erythrocyte DNA

(Avian Biotech International, Tallahassee, FL). All nestlings were also surveyed for the presence of blood parasites. Marabou nestlings were part of a colony located along Nile Avenue, in central Kampala, Uganda (0° 19' N 35° 25' E). Kampala is the capital and largest city in Uganda with an estimated population of 1, 208, 544 in 2002 (Uganda Bureau of Statistics). Marabou stork nestlings were sampled from twelve nests, in six trees (*Tabebuia pentaphylla*), in one colony, on one day from 0700 till 1800. Marabou stork nestlings were temporarily captured for sample collection using professional tree climbing techniques (USDA Forest Service 1996), with modifications for tropical tree species and environmental conditions. The main method of tree ascent was using tree climbers or occasionally free climbing. When necessary, marabou stork nestlings were gently coaxed to the side of the nest using an ice gaff (Mason Tackle Company, Otisville, MI) with the point of the hook covered with protective plastic. Parents never left the nest (and vigorously defended the chicks) or remained close to the nest and returned upon retreat of the climber. Marabou stork nestlings were placed singly into a 40 cm diameter nylon bag (The Taku Tailor, Juneau, AK). The bag had a padded bottom, velcro tab fasteners and multiple ventilation holes. The bag was attached to a rope and lowered to the ground for sampling. Twelve ml of blood were collected from the medial metatarsal vein via a 23 gauge x 1.9 cm (¾ inch) butterfly catheter (Surflo Winged Infusion Set, Terumo, Elkton, MD) connected to a 3 ml syringe flushed with sodium heparin (100 IU/ml) to prevent coagulation. Eight ml of blood were immediately transferred to a 10 ml vacutainer tube containing lithum heparin anticoagulant and the remainder transferred to a 5 ml vacutainer tube containing calcium EDTA anticoagulant (Becton Dickinson, Franklin Lakes, NJ). Three blood smears were made from fresh blood using the slide on

slide technique (Campbell 1988). Fresh whole blood was also used to determine blood glucose levels with a hand held glucometer (Medisense 2® card glucometer utilizing precision plus sensors®, Medisense Inc. Bedford, MA). A drop of whole blood was placed on a commercially prepared paper sample card for molecular sex determination based on total erythrocyte DNA (Avian Biotech International, Tallahassee, FL). Five whole breast feathers were then hand plucked for determination of total mercury concentrations. A physical examination including scoring body condition (based on pectoral muscle mass and feather condition), whether the crop was empty or full and a visual description of any abnormalities was made. Bill depth, culmen length and bill circumference were measured. Lastly, birds were placed in a cotton sack and body weight recorded by a spring balance with gradations of 100g (Homs model 20, Douglas Homs Corp. Belmont, CA). Average time for removal from the nest until return was 14.5 minutes (range 7-22).

Samples were placed in a chilled cooler after collection. Separation of plasma occurred on the same day as sampling. Packed cell volume (PCV) and total plasma protein (TPP refrac) were determined in the field from blood in the EDTA vacutainer. Microhematocrit capillary tubes (2) were centrifuged at 3000 rpm for 5 minutes (Vulcon Mobilespin PS126-6, Vulcon Technologies, Grandview, MO) and an average PCV reading recorded. Total plasma protein was determined using a temperature compensated refractometer (Leica Inc. Optical Products Division, Buffalo, NY). The remaining blood in the EDTA and lithium heparin vacutainers was centrifuged at 3000 rpm for ten minutes. Plasma was observed visually for hemolysis, icterus, and lipemia and

subjectively classified as slight, moderate or severe. Plasma was removed and divided into five 2 ml cryovials (Cryogenic Vial, Corning Incorporated, Corning, NY) that were deposited into a MVE Doble-20 Vapor Shipper/Liquid Nitrogen Tank (MVE Bio-Medical Systems, Burnsville, MN). Plasma samples were transported to the Diagnostic Center for Population and Animal Health (DCPAH) at the Michigan State University (MSU) in the vapor shipper then transferred to a – 80 °C ultra-low freezer until analyzed. Analysis was done two weeks after sampling. Analyses of plasma chemistry values were performed at the clinical pathology laboratory of the DCPAH at the MSU. The plasma chemistry analyses were performed on an Olympus AU640 chemistry analyzer, (Olympus America, Inc. Irving TX). The electrolyte analyses were performed with a sodium potassium crown ether membrane while the chloride analysis employed a molecular oriented Polyvinylchloride membrane. Calcium, phosphorous, TPP col, albumin, aspartate amino-transaminase (AST), creatine kinase (CK), cholesterol and uric acid were performed using Olympus reagents. Sodium/potassium ratio, and globulins were calculated from the measured parameters.

Molecular sex differentiation was done using a polymerase chain reaction based on the first gene of the avian W chromosome (CHD) (Griffiths et al 1998).

Total plasma thyroxine (TT4) was measured by two methods: a commercial radioimmunoassay (TT4 RIA) (Diasorin Inc., Stillwater, MN) and a colorometric ELISA (TT4 ELISA) specifically designed to assess thyroid function in a range of animal species, including birds (Oxford laboratories, Oxford, MI. Not commercially available at

the time of publication). Triiodothyronine (TT3) was measured using a radioimmunoassay prepared in house at the endocrinology laboratory, DCPAH at MSU.

Blood smears for blood parasite analysis were stored unstained in plastic slide boxes. Prior to examination they were fixed in absolute methanol and then stained with giemsa. Each slide was examined in its entirety (250X), then for ten minutes (500X) and for an additional ten minutes under oil immersion (1250) in two five minute sessions. Both red and white blood cells were examined. The degree of parasitemia was expressed as the percentage of erythrocytes infected per 1000 erythrocytes counted. Parasitological analysis was conducted at the Michigan Department of Natural Resources Rose Lake Pathology Laboratory.

Analysis of variance (ANOVA) was conducted to assess the association between gender, body weight and plasma chemistry parameters of marabou stork nestlings (SAS PROC ANOVA for categorical risk factors, and SAS PROC GLM for continuous risk factors. SAS Inc., Cary, NC). These analyses were conducted both at the univariable (only one risk factor at a time) and multivariable level. Multivariable analyses were conducted to adjust the effect of selected risk factors simultaneously. The level of significance for type 1 ($\alpha$) error was set at a probability of 0.05. Descriptive statistics were done using Excel (Microsoft Excel, Microsoft Corporation, Redmond, WA). An outlying value was defined as being 1.5 times greater or less than the interquartile range. Outlying values occurred for four parameters and were removed from the results (Table 4.1.). This emulates the methodology of other studies examining wild avian

hematological and plasma chemistry values where only small sample sizes could be obtained (Garcia- Montijano 2002; Lumsden 1998).

All procedures utilizing birds in this study were carried out under approval of the Michigan State University All University Committee on Animal Use and Care. The Uganda National Council for Science and Technology and the Uganda Wildlife Authority granted research permits for this project.

## Results

All birds were in good body condition as assessed by pectoral muscle mass and no abnormalities were detected. The mean weight of the nestlings was 4.1 +/- 1.6 kg. The minimum and maximum nestling weights were 1.65 kg and 7.60 kg. Six birds had full and fourteen had empty crops. Eight of the samples showed slight, two moderate and five severe hemolysis.

Results of PCV, plasma chemistry parameters and body weight are presented in Table 4.1. Analysis of variance for plasma chemistry parameters in nestling marabou storks is presented in Table 4.2. No blood parasites were observed. There were no significant differences in plasma chemistry values or PCV between sexes except for globulin and TPP levels. Globulin levels also varied with bird body weight ($p \leq 0.05$). Female nestlings had significantly higher ($p \leq 0.05$) TTP levels as measured by refractometry and colorimetric methods. Weight of the nestling was not significant when assessed simultaneously with gender for its association with TPP. A strong positive

correlation existed between TPP values returned by using a temperature compensated refractometer in the field ($r^2$ = 0. 78) and the colorimetric method in the laboratory. Mean values for nestlings were 5 g/L higher when measured by refractometry. There was a positive correlation ($r^2$ = 0.576) between TT4 levels measured by radioimmunoassay to TT4 levels measured by the ELISA method. The ratio of the sum TT4 ELISA/TT3 was 1.79.

## Discussion

Most plasma chemistry parameters and PCVs reported in this study for marabou stork nestlings were similar to those recorded in wild and caged bird nestlings (Bowerman et al 2000; Redig 1993; Clubb et al 1993; Hoefle et al 2000). However, TPP, phosphorous, potassium and CK were higher and AST values lower compared to nestlings of other species recorded in the above studies. These same increases were apparent when compared to nestling white stork (*Ciconia ciconia*) plasma chemistry parameters (Montesinos et al 1997), with the exception of phosphorous and potassium, which were similar to values recorded for white storks. White storks were the only other Ciconiiformes in which plasma chemistry values were recorded from nestlings. Elevations in potassium and phosphorous values were most likely artifactual and caused by hemolysis. Elevated CK values were most likely a combination of sample hemolysis and struggling during handling. A possible explanation for elevated TPP levels in nestlings is that whole vertebrate prey, high in protein is favored by breeding adults during periods of nestling growth (Hancock et al 1992). The parents therefore provide an increased source of dietary protein to the nestlings and this may elevate plasma albumin

levels. Another explanation, especially in the colony sampled, is that an urban scavenging lifestyle may supply increased dietary protein for the entire year, irrespective of breeding status. Plasma chemistry samples from breeding, non-breeding and rural colonies would be necessary to validate these explanations.

The variation in TPP values measured by refractometry and the colorimetric method support studies that demonstrate poor reproducibility of values using refractometry to determine avian plasma protein (Lumiej & Maclean 1996). The correlation between both methods of measurement suggest refractometric measurements of TPP provide readings that are consistent but not precise. This may indicate that refractometry should be used only as an approximation, rather than an absolute indicator of TPP.

Differences in AST and Ca levels between sexes have been recorded in avian species (Gee et al 1981), but this has not been a consistent finding in all species studied and was not found in this study. Apart from a small sample size, no conclusions can be drawn from the higher globulin and TPP values returned for females

Total plasma TT4 and TT3 values presented should be interpreted cautiously. Serum levels of thyroid hormones and the ratio of TT4 to TT3 can vary greatly between genus, species and individuals (Rosskopf et al 1982). This is supported by the ratio of TT4 ELISA/TT3 for marabou stork nestlings found in this study. The short half- lives of avian TT3 and TT4 can lead to significant diurnal variation in measurable values.

Hyperthermia and stress may also reduce serum TT4 concentrations (Rae 2000). Birds have relatively low concentrations of TT4 compared with humans so human TT4 RIA may have low sensitivity in avian species. In addition, commercially available human RIA kits that have not been standardized in a particular laboratory by using serum from euthyroid birds are difficult to compare between laboratories, different commercial kits and different species (Merryman & Buckles 1998).

The lack of hematozoan parasites in blood smears from nestling marabou storks may be an artifact of a small sample size. The short temporal period over which infections could occur in the nestlings may also be a reason for the negative findings. Bennett et al (1977) recorded blood parasite prevalence in a variety of avian species (not including marabou storks), over 5 years at Zika Forest (about 30 km from Kampala). They found an overall prevalence of 34.8 % with the vast majority of infections being *Haemoproteus* spp. There appeared to be a slight positive correlation between parasite prevalence and rainfall. Our samples were taken at the conclusion of a prolonged wet season.

There is a paucity of physiological data on many common African avian species within their natural habitat. Species such as the marabou stork could possibly serve as useful monitors of ecosystem health, in both urban and rural environments. Plasma chemistry parameters will be very useful to help achieve this goal, and to establish a physiological database for the species.

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**Table 4.1:** Body weight, packed cell volume and plasma chemistry values from nestling marabou storks (*Leptoptilos crumeniferus*) in Kampala, Uganda (n =20).

[a] Q1 = the 25$^{th}$ percentile of the sample size

[b] Q3 = the 75$^{th}$ percentile of the sample size

[c] indicates outlying values (greater than 1.5 x the inter-quartile range) have been removed and the sample size adjusted accordingly.

[d] PCV = packed cell volume

[e] TPP Refrac = total plasma protein measured by refractometry

[f] TPP Col = total plasma protein measured by a colorimetric method

[g] AST = aspartate transaminase

[h] CK = creatine kinase

[i] Na/K = Sodium/potassium ratio

[j] TT4 radioimmunoassay = thyroxine measured by a radioimmonoassay

[k] TT4 elisa = thyroxine enzyme linked immunoabsorbent assay

[l] TT3 = triiodothyronine

[m] = determined from whole blood

**Table 4.1. Body weight, packed cell volume and plasma chemistry values from nestling marabou storks (n =20).**

| Parameter | Mean | SD | Median | Q1[a] | Q3[b] | Min | Max |
|---|---|---|---|---|---|---|---|
| **Weight** Kg | 4.1 | 1.6 | 3.8 | 3.1 | 4.9 | 1.6 | 7.6 |
| [d] **PCV** L/L | 0.34 | 0.04 | 0.33 | 0.31 | 0.37 | 0.28 | 0.40 |
| [e] **TPP** Refrac g/L | 46 | 4 | 47 | 43 | 49 | 40 | 52 |
| [f] **TPP** Col g/L | 41 | 5 | 42 | 39 | 45 | 30 | 50 |
| [m]**Glucose** (mMol/L) | 11.7 | 2.1 | 12.2 | 10.4 | 13.2 | 7.5 | 15.1 |
| [g] **AST** U/L | 135 | 28 | 143 | 115 | 155 | 84 | 178 |
| **Calcium** mMol/L (N=18)[c] | 2.70 | 0.17 | 2.74 | 2.68 | 2.82 | 2.35 | 2.95 |
| **Phosphorous** mMol/L | 2.84 | 0.87 | 2.53 | 2.15 | 3.53 | 1.74 | 4.49 |
| [h] **CK** U/L (N=16) | 1422 | 575 | 1320 | 988 | 1854 | 584 | 2705 |
| **Uric Acid** mMol/L | 0.873 | 0.355 | 0.806 | 0.643 | 1.100 | 0.357 | 1.422 |
| **Albumin** g/L | 17.1 | 2.3 | 17.0 | 16.0 | 19.0 | 12.0 | 20.0 |
| **Globulin** g/L | 24.2 | 3.5 | 25.0 | 22.0 | 26.0 | 18.0 | 30.0 |
| **Sodium** mMol/L (N=16)[c] | 149 | 2 | 149 | 148 | 151 | 143 | 153 |
| **Potassium** mMol/L | 4.2 | 1.3 | 3.9 | 2.9 | 5.3 | 2.7 | 6.5 |
| **Chloride** mMol/L (N=18)[c] | 107 | 5 | 107 | 105 | 111 | 96 | 115 |
| [i] **Na/K ratio** (N=16)[c] | 37 | 12 | 35 | 27 | 48 | 23 | 54 |
| **Cholesterol** mMol/L | 4.97 | 1.27 | 5.10 | 3.86 | 5.98 | 3.08 | 7.28 |
| [j] **TT4 RIA** nMol/l | 8.9 | 9.0 | 6.7 | 2.0 | 14.2 | 0.0 | 20.0 |
| [k] **TT4 elisa** nMol/l | 15.98 | 18.05 | 8.27 | 7.18 | 22.65 | 2.00 | 28.60 |
| [l] **TT3** nMol/l (N=18)[c] | 1.61 | 1.55 | 0.41 | 1.33 | 1.88 | 0.90 | 2.30 |

**Table 4.2:** Results of Analysis of variance of plasma chemistry values in nestling marabou storks (Leptoptilos crumeniferus) (n = 19) from Kampala, Uganda.

[a] AST = aspartate transaminase

[b] Chol. = cholesterol

[c] CK = creatine kinase

[d] TT4 ELISA = thyroxine enzyme linked immunoabsorbent assay

[e] TT4 RIA = thyroxine radioimmunoassay

[f] TT3 = triiodothyronine

[g] TPP (ref) = total plasma protein determined by refractometry

[h] TPP (col) = total plasma protein determined by the buiret method

[i] BW = body weight

**Table 4.2. Analysis of variance for plasma chemistry values in nestling marabou storks (n = 19)**

| Plasma Chemistry | Variables | F | p | Plasma Chemistry | Variables | F | p |
|---|---|---|---|---|---|---|---|
| [a]AST | BW | .12 | .7284 | **Albumin** | BW | 1.2 | .2892 |
| | Gender | 1.42 | .2504 | | Gender | 2.69 | .1205 |
| | **Overall** | **.77** | **.4779** | | **Overall** | **1.95** | **.1752** |
| [c]CK | BW | 10.45 | .0052 | **Calcium** | BW | .25 | .6250 |
| | Gender | .08 | .7870 | | Gender | .75 | .3982 |
| | **Overall** | **5.6** | **.0175** | | **Overall** | **.50** | **.6152** |
| [b]Chol. | BW | .22 | .6427 | **Chloride** | BW | .13 | .7251 |
| | Gender | 3.42 | .0831 | | Gender | 1.95 | .1820 |
| | **Overall** | **1.82** | **.1941** | | **Overall** | **1.04** | **.3769** |
| **Globulin** | BW | 6.47 | .0217 | **Glucose** | BW | 10.41 | .0050 |
| | Gender | 5.98 | .0264 | | Gender | .93 | .3496 |
| | **Overall** | **6.22** | **.0100** | | **Overall** | **5.67** | **.0130** |

**Table 4.2. (continued)**

| Plasma Chemistry | Variables | F | p | Plasma Chemistry | Variables | F | p |
|---|---|---|---|---|---|---|---|
| | BW | .06 | .8055 | | BW | 1.34 | .2645 |
| **Potassium** | Gender | .12 | .7358 | **Sodium** | Gender | 2.23 | .1551 |
| | **Overall** | **.09** | **.9141** | | **Overall** | **1.78** | **.2002** |
| | BW | .01 | .9267 | | BW | 2.16 | .1601 |
| **Phosphorous** | Gender | .16 | .6914 | **PCV** | Gender | 1.39 | .2539 |
| | **Overall** | **.09** | **.9180** | | **Overall** | **1.78** | **.1993** |
| | BW | 1.25 | .2806 | | BW | .28 | .6029 |
| **[f]TT3** | Gender | 1.08 | .3133 | **[e]TT4 RIA** | Gender | .01 | .9307 |
| | **Overall** | **1.17** | **.3369** | | **Overall** | **.14** | **.8664** |
| | BW | .09 | .7655 | | BW | .08 | .7789 |
| **[d]TT4 ELISA** | Gender | .02 | .8869 | **Uric Acid** | Gender | .01 | .9278 |
| | **Overall** | **.06** | **.9453** | | **Overall** | **.04** | **.9561** |
| | BW | 2.74 | .1161 | | BW | 3.99 | .0629 |
| **[g]TPP (Ref)** | Gender | 9.53 | .0067 | **[h]TPP (Col)** | Gender | 4.82 | .0432 |
| | **Overall** | **6.14** | **.0099** | | **Overall** | **4.41** | **.0298** |

# Chapter 5

# Persistent Organic Pollutant and Mercury Concentrations in African Fish Eagles (*Haliaeetus vocifer*), Marabou Storks (*Leptoptilos crumeniferus*) and Tilapia (*Oreochromis niloticus*) in Uganda

## Abstract

The purpose of this research was to determine whether there are significant differences in persistent organic pollutant (POP) and mercury concentrations in tissues of African fish eagles (*Haliaeetus vocifer*) from Lake Victoria near Entebbe and Lake Mburo. Secondly, we sought to determine POP and mercury concentrations in fish from these two lakes in addition to the Nile river delta near Wanseko (Murchison Falls). Thirdly, we sought to determine POP and mercury concentrations in marabou stork (*Leptoptilos crumeniferus*) nestlings from urban Kampala. Total mercury was measured in the breast feathers of 33 nestling and adult African fish eagles from Lake Mburo, and Lake Victoria near Entebbe and 20 nestling marabou storks from Kampala, Uganda from June 2002 through January 2003. Plasma concentrations of DDT, aldrin, hexachlorocyclohexane (α-HCH), dieldrin, endrin, heptachlor and their metabolites, β-HCH, 2,4'-DDD, 4,4'-DDD, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT, heptachlor epoxide, lindane, nonachlor and total polychlorinated biphenyl concentrations (PCBs) were also measured. Total body burdens of these chemicals and mercury were measured in 18 *Oreochromis niloticus*, a fish eagle prey item at Lake Mburo, Murchison Falls and Lake Victoria. Mercury concentrations were significantly higher in eagle adults and nestlings from Entebbe than adults and nestlings from Lake Mburo ($p \leq 0.05$). There were no significant differences ($p \leq 0.05$) in mercury concentrations between genders. Mercury concentrations for marabou stork nestlings in Kampala were slightly higher than concentrations in fish eagle nestlings from Entebbe. However, there was no significant difference ($p \leq 0.05$) in mercury concentrations between the entire fish eagle population sampled at Entebbe and marabou stork nestlings sampled at Kampala. Five adult eagles

and five *Oreochromis niloticus* fish samples from Entebbe had 4,4'-DDE in plasma in the range 0.001 – 0.005 ppm. Marabou stork samples did not contain 4,4'-DDE at 0.001 ppm. No samples contained PCBs at the 0.003 ppm detection level or other POPs (besides 4,4'-DDE) at the 0.001 ppm limit of detection.

## Introduction

The African fish eagle (*Haliaeetus vocifer*) is a widespread, often locally abundant tertiary avian predator in lake-based food chains throughout sub-Saharan Africa. The African Fish Eagle is very sensitive to the effects of persistent organic pollutants (POPs) (Douthwaite 1992). In this regard it is similar to other piscivorous tertiary avian predators such as the bald eagle (*Haliaeetus leucocephalus*) in North America (Bowerman 1993) and the white tailed sea eagle (*Haliaeetius albicella*) in Sweden (Helander et al 1982).

The marabou stork (*Leptoptilos crumeniferus*) is resident in tropical Africa where it is common to abundant in most parts of its range (Hancock et al 1992). The marabou stork has responded to increasing urbanization and centralization of human populations by adapting a scavenging lifestyle in urban areas (Pomeroy 1977, 1978). Populations have increased in Kampala and breeding colonies may be found in the city center. Although the marabou stork undertakes short seasonal migrations generally between the north and south of Uganda, a year round resident population in Kampala may be increasing (Hancock et al 1992). This and their geographical exposure in urban areas to greater industrial output may make these populations useful as indicators of exposure to a range of pollutants, including heavy metals such as mercury.

Studies on POP and metal contaminant concentrations in African fish eagles and marabou storks are limited and in the former species, were largely done in southern Africa (Davies and Randall 1989; Tannock et al 1983). South Africa and Zimbabwe had greater and more systemic use of POPs than Uganda during the latter decades of the twentieth century. However, the lack of accurate reporting procedures makes it difficult to determine the actual usage of many chemicals in Uganda. Ejobi et al (1996 a, b) quotes 80 tonnes of DDT per year being used mainly for cotton growing and mosquito control. Dieldrin was used at the rate of 392 tonnes per year for banana weevils and termites while 30 tonnes were also used for tsetse fly control. Lindane, aldrin, hexachlorobenzene, campheclor, chlordane and heptachlor were also used. In comparison importations to South Africa between 1974 and 1976 averaged approximately 950 tonnes per annum and in 1982 Zimbabwe imported approximately 1000 tonnes (Davies & Randall 1989). In 1985 DDT was banned for agricultural use in Zimbabwe. However usage was still reported to be up to 300 tonnes (Hartley and Douthwaite 1994) for Zimbabwe and 121 tonnes for South Africa in the mid 1980s (Davies & Randall 1989). No known studies on POPs in avian species have been conducted in Uganda.

Mercury pollution is a result of natural and anthropogenic activities. Natural degassing of the earth's crust is the major source of environmental mercury worldwide (Heinz 1996). Anthropogenic sources include industrial pollution and burning of fossil fuels which commonly occurs in the industrial area along the shore of Lake Victoria near Entebbe and Kampala. Methylmercury, the most toxic form, can have harmful effects on

adult and fledgling survival as well as reproduction, behavior and cellular development in avian species (Burger 1994). The neurotoxic effects of methylmercury can alter nesting behaviour and negatively impact the reproductive success of avian species. Methylmercury can also specifically impair hatching. Methylmercury pollution is of most concern because it is biomagnified along the food chain (Heinz 1996). A study has examined metal concentrations (zinc, cadmium, lead, copper, iron, manganese, chromium and cobalt) in the feathers of adult marabou storks from Kampala city and surrounding areas (Nyangababo 2003). This study did not include mercury. No studies have examined the potential impact of mercury pollution on wildlife in Uganda.

With increased political and economic stability, a rapidly expanding urbanized population, and industrial growth of 7 % (Central Intelligence Agency, 2002) baseline assessment and monitoring of pollution in Ugandan is needed. The bald eagle has been proposed as an ecosystem monitor species of North American Great Lakes water quality by the International Joint Commission (1985) and the 1998 State of the Lakes Ecosystem Conference (SOLEC 1998). It was proposed as an ecosystem monitor particularly in regard to the toxic effects of organochlorine compounds on piscivorous wildlife (Bowerman 2000). Given the African fish eagle's widespread distribution and its relative abundance, it may also be a valuable indicator species of water quality in the sub-Saharan lake-based ecosystems of Africa, such as Lake Victoria.

The hypothesis to be tested by this research is no difference exists in persistent organic pollutant and mercury concentrations in tissues of African fish eagles from Lake Victoria near Entebbe compared to those from Lake Mburo.

The objectives of this research were to establish baseline concentrations of POPs and total mercury in African fish eagle adults and nestlings at a site with significant anthropogenic disturbance (Lake Victoria near Entebbe) and at a relatively undisturbed site (Lake Mburo). Whole body concentrations of POPS and total mercury were determined for Tilapia fish (*Oreochromis niloticus*), an eagle prey item, to demonstrate bioaccumulation of these pollutants within the aquatic food chain. As fish can virtually be the primary source of dietary protein for human populations in this region, sampling of fish may also give some indication of human exposure to these pollutants. Marabou stork nestlings were sampled in Kampala as a contrast and comparison to the results from fish eagles, and to determine baseline concentrations of the pollutants in this common species.

A further objective of this study was to assess the feasibility of using fish eagles and marabou storks as biomonitors of environmental health. Multiple criteria including knowledge of species biology, breeding cycle, physiology, body size, diet and ease of sample collection determine species suitability for the role of biomonitor. To this end other baseline information on both avian species was collected including hematological, plasma chemistry values, body measurements and body weights. Adult fish eagles were banded and eagle nest site habitat preferences were also characterized.

The value of this study is in its establishment of baseline concentrations of contaminants in species that may fulfill the role of biomonitors of environmental health. In addition, the study also developed logistical and technical methodologies for sampling these species. To maximize benefits from this work, an assessment of the suitability of African fish eagles and marabou storks as biomonitors of environmental health in African lake-based ecosystems was made. However, also required is further long - term monitoring of contaminant trends with research on population dynamics and water quality, together with the application of developed techniques to monitoring other pollutants and indices of environmental change. Other indices of environmental change include species distribution, abundance, community structure and breeding. Apart from changes associated with specific pollutants these indices may change with natural climactic variation, global warming, ozone depletion, habitat change and fragmentation and changes in species composition within an ecosystem (Jarvis 1993). Whatever the potential sources of change, this study establishes an important database of protocols and values to help measure both the change and its impacts on both human and wildlife populations.

## Materials and Methods

African fish eagles were sampled at Lake Mburo, a 6 km long freshwater lake in South Western Uganda (0°. 39' S, 30°. 57' E) situated in a 256 $km^2$ national park, and on Lake Victoria at Entebbe, from Nfo Island (0°. 00' N, 32°. 26' E) to Kisubi Bay (0°. 05' N, 32°. 35' E). Lake Mburo is 230 km and Entebbe 40 km from the capital Kampala. Fish eagles were sampled at Lake Mburo in July, August and December 2002 and at Lake Victoria in August 2002 and January 2003. Thirty-three eagles were sampled: ten adults

and eight nestlings from Lake Mburo and five adults and ten nestlings from Lake Victoria.

Twenty marabou stork nestlings were sampled from a colony located along Nile Avenue, in central Kampala, Uganda (0°. 32' N, 32°. 58' E). Marabou stork nestlings were sampled from twelve nests, in six trees (*Tabebuia pentaphylla*), in one colony, on one day from 0700 till 1800, in January 2003.

*Oreochromis niloticus* were sampled at Lake Mburo and Lake Victoria at Entebbe in the same locations and times that fish eagles were sampled. In addition fish were sampled from the Nile river at Wanseko, close to Murchison Falls national park (2°. 15' N, 31°. 38' E), in January 2002.

Adult fish eagles were captured on water using a fish "snare vest" technique. *Oreochromis niloticus* were fitted with eight to twelve 5 - 6 cm diameter fishing line snares (loops). The free ends of the snares penetrated the body of the fish and were then tied on themselves and the excess line cut. The line was attached to a hand held wooden reel and the snared fish was then placed in the water. Once captured, the field crew paddled to the eagle and retrieved it by securing both legs. Fish eagles swim well so there was little risk of drowning. On shore, the eagles were placed in dorsal recumbency and the eyes covered by a baseball cap. Ten ml of blood were collected from the brachialis vein via a 21 or 23g x 1.90 cm (¾ inch) butterfly catheter (Surflo Winged Infusion Set, Terumo Medical Corporation, Elkton, MD) connected to a 10ml sterile syringe and were

immediately transferred to a 10 ml lithium heparin vacutainer (Becton Dickinson, Franklin Lakes, NJ). Four ml of additional blood were collected and transferred to a five ml calcium EDTA vacutainer. Three blood smears were made from fresh blood using the slide on slide technique (Campbell 1988). Fresh whole blood was used to determine blood glucose (Medisense 2® card glucometer, Medisense Inc. Bedford, MA). A drop of whole blood was placed on a commercially prepared paper sample card for molecular sex determination based on total erythrocyte DNA (Avian Biotech International, Tallahassee, FL). Five whole breast feathers were hand plucked for determination of total mercury concentrations. A physical examination and visual description of any abnormalities was made. Hallux, culmen, footpad and eighth primary length, as well as bill depth were measured (Figure 3.1 and Figure 3.2). Body measurements methods used were those described for the bald eagle (Bortolotti 1984 a, b) (Figure 3.1 and 3.2). Birds were banded with 18-22mm internal diameter metal rivet bands inscribed with a three letter sequential code and the word "MAKERERE" (Gey Band and Tag Company, Norristown, Pennsylvania, USA). The bands were colored either red or gold for Lake Mburo and black for Entebbe. Suspected female birds were banded on the left leg and suspected males on the right leg. Lastly, birds were placed in a cotton sack and weighed. (Homs model 20 spring balance, Douglas Homs Corp. Belmont, CA). Eagles were then released from land at the closest point possible to the capture location. Average time from capture to release was 34 minutes (range 20-45 minutes).

African fish eagle and marabou stork nestlings were retrieved from the nest using professional tree climbing techniques (USDA Forest Service 1996), with modifications

for tropical tree species and environmental conditions. The main method of tree ascent was using tree climbers (Klein Tools, Chicago ILL). Eagle and stork nestlings were placed singly into a ventilated bag and lowered to the ground for sampling. Sampling of nestlings was as described for adults except the volume of blood collected was between four and 14 ml. No more than one percent of body weight was collected and often considerably less. Venipuncture site on stork nestlings was the medial metatarsal vein. Eagle's ages were approximated (+/- 3 days) based on body weight by the calculations presented in Sumba (1988). Average time from removal of the nestling until return was 14.5 minutes (range 7-22) for marabou storks and 32 minutes (range 10-60) for fish eagle nestlings.

*Oreochromis niloticus* were sampled as they appeared to be the fish eagle's main prey item at Lake Mburo and Entebbe. Fish eagles feed on a variety of fish with cichlid species, (including *Oreochromis niloticus*, that are herbivorous) occupying the littoral zone comprising 80% of the fish taken (Stewart et al 1997). Fish samples were purchased from local fisherman as close as possible to where eagles were sampled. Fish less than 300-400 g and 300-450 mm in length were sampled as this closely corresponded to the limited information available on fish eagle prey selectivity (Stewart et al 1997). Fish were weighed and their body length recorded. Mean length and weight for fish from Lake Mburo were 225 mm and 343 g, Entebbe 267 mm and 569g and Murchison Falls 253 mm and 411g. A 100g cross trunk sample of fish including skin, muscle, bone and viscera was dissected just cranial to the dorsal fin and weighed. Samples were placed in freezer bags and transferred to a MVE Doble-20 dual-purpose vapor shipper/liquid nitrogen tank (MVE Bio-Medical Systems, Burnsville, MN).

Samples were placed in a chilled cooler after collection. Time of sampling to storage of plasma in liquid nitrogen was 3.5 hours (range 2-9 hours) for eagles and 8 hours (range 4-16 hours) for marabou storks. Samples were centrifuged for ten minutes at 3000 rpm (vulcon mobilespin PS126-6, vulcon technologies, Grandview, MO). Plasma was removed and divided into five 2 ml cryovials per bird (Cryogenic Vial, Corning Inc. Corning, NY) that were deposited into the vapor shipper/liquid nitrogen tank. Plasma samples were transported to the Diagnostic Center for Population and Animal Health (DCPAH) at Michigan State University's Veterinary Medical Center (MSU) then transferred to a - 80 °C ultra-low freezer until analyzed. Analysis occurred within two months of sampling in all cases.

All analysis was performed at the DCPAH at the MSU toxicology laboratory. Feathers for total mercury analysis were washed in reagent grade acetone, deionized water, then again in chromatography grade acetone (Burdick & Jackson, Muskegon, MI) to remove debris. Feathers were allowed to dry overnight in a fume hood and then weighed. Feathers were then placed in sealed 30 ml teflon vessels in a concentrated 2 ml nitric acid digest (Instra-analyzed grade, J.T. Baker Inc, Phillipsburg, NJ) at 95°C overnight. Ten ml of the digest was quantitatively transferred to a volumetric flask and mixed. A final acid concentration of 7 % HCl acid and 2 % $HNO_3$ to match the standard curve solutions 0, 25, 100, 500 ppt were made and diluted to volume. Aliquots were taken from the ten ml flask and diluted based on feather weight to achieve total feather dissolution and analyzed by cold vapor atomic absorption spectrophotometry at 253.7 nm. (LCD mercury monitor 3200, Thermo Separation Products, Riviera Beach, FL).

Commercial procedural blanks (2976 Mussell tissue with concentration 53.8 ppb to 68.2 ppb. National Institute of Standards and Technology Gaithersburg, MD) were used to monitor the accuracy of the analysis. Fish tissues were snap frozen for 60 seconds in liquid nitrogen then milled in an AIO analytical mill (Janke & Kunkel, IKA Labortechnik Germany). One gram of powdered fish was added to 2 ml of concentrated nitric acid. Further analysis followed the procedures outlined for feather sampling.

Tissues (serum, fish) for analysis of POPs were extracted and purified by the procedure described by Price et al (1986). Concentrations were determined by gas chromatography. Equipment used was a Varian 3400 gas chromatograph (capillary column method) with electron capture detector.

Analysis of variance (ANOVA) was conducted to assess the association between site, age (nestling or adult), gender and breast feather mercury concentrations in fish eagles and between body weight, gender and mercury concentrations in marabou storks (SAS PROC ANOVA for categorical risk factors, and SAS PROC GLM for continuous risk factors. SAS 8.2, 2001.SAS Inc., Cary, NC). Analysis of variance was also used to assess the associations between site, body weight and total body mercury burdens in *Oreochromis niloticus*. These analyses were conducted both at the univariable (only one risk factor at a time) and multivariable level. Multivariable analyses were conducted to adjust the effect of selected risk factors simultaneously. The level of significance for type 1 ($\alpha$) error was set at a probability of 0.05. Descriptive statistics were done using Excel (Microsoft Excel, Microsoft Office 2000 Professional. Microsoft Corporation, Redmond, WA). Descriptive statistics are emphasized due to the small sample size. This emulates

the methodology of other studies examining wild avian species where only small sample sizes could be obtained (Garcia- Montijano 2002; Lumsden 1998).

This study was conducted under approval of the Michigan State University All University Committee on Animal Use and Care. The Uganda National Council for Science and Technology and the Uganda Wildlife Authority granted research permits for this project.

## Results

Total mercury concentrations in breast feathers from adult and nestling African fish eagles, nestling marabou storks and in whole body section samples from *Oreochromis niloticus* are presented in Table 5.1. Feather mercury concentrations are presented on a dry weight basis and fish sample concentrations are presented on a wet weight basis. Analysis of variance for total mercury concentrations in African fish eagle and marabou stork breast feathers are presented in tables 5.2 and 5.4 respectively. Analysis of variance comparing mercury concentrations between fish eagles from Lake Victoria near Entebbe and marabou storks from Kampala are presented in table 5.3. Analysis of variance assessing the association between site, body weight and mercury concentrations in *Oreochromis niloticus* is presented in table 5.5.

Mercury concentrations in adult fish eagles from Entebbe were significantly higher than concentrations in adults from Lake Mburo ($p \leq 0.05$). Similarly, mercury concentrations in nestling fish eagles from Entebbe were significantly higher than

concentrations in nestlings from Lake Mburo ($p \leq 0.05$) (Figure 5.1). There were no significant differences ($p \geq 0.05$) in mercury concentrations in breast feathers of fish eagles based on gender or whether the bird was an adult or fledgling. Marabou stork nestlings from Kampala had slightly higher concentrations of mercury than fish eagle nestlings from Entebbe (Entebbe is 40 km from Kampala). However, there was no significant difference ($p \leq 0.05$) in mercury concentrations between the fish eagle populations from Entebbe and the nestling marabou stork population from Kampala. Two adult male fish eagles from Entebbe had much higher mercury concentrations (2.3 ppm and 1.4 ppm wet weight) than other fish eagles sampled.

There was a significant difference in mercury concentrations in whole body cross section samples of *Oreochromis niloticus* from the three study sites ($p \leq 0.05$). The highest concentration of mercury in fish came from the Murchison Falls site.

African fish eagle plasma, marabou stork plasma and whole body section samples from *Oreochromis niloticus* did not contain concentrations of the following chemicals at the 0.001 ppm limit of dedetection: aldrin, DDT, α-BHC, dieldrin, endrin, heptachlor and their metabolites, β-BHC, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, heptachlor epoxide and lindane and nonachlor. Total PCBs were not detected in African fish eagle plasma, marabou stork plasma or whole body section samples from *Oreochromis niloticus* at the detection limit of 0.003 ppm. Five adult eagles from Entebbe had 4,4'-DDE detectable in plasma, one at 0.005ppm (male), one at 0.003ppm (female), two at 0.002ppm (males) and one at 0.001ppm (female) wet weight. Samples were not

corrected for lipid content of plasma. Five *Oreochromis niloticus* samples from Entebbe contained 4,4'-DDE concentrations of 0.001, 0.001, 0.002, 0.003 and 0.003 ppm wet weight. Six eagle nestlings from Entebbe and one adult from Lake Mburo tested positive for 4,4'-DDE but at concentrations lower than 0.001ppm. At 0.001 ppm 4,4'-DDE was not detected in marabou stork plasma and whole body section samples of *Oreochromis niloticus.*

## Discussion

### Mercury

Scheuhammer and Bond (1991) suggest that mercury feather concentrations greater than 20 ppm may be associated with toxic effects in birds. Eisler (1987) states that concentrations above 5ppm fresh weight are thought to be associated with adverse affects in sensitive avian species. However, this study was not based on piscivorous birds but grain eating pheasants and mallards. Risk categories for mercury accumulation in common loons (*Gavia immer*) use 0-9 ppm wet weight as a low risk category that indicates background mercury concentrations in loons that have been minimally impacted by human activity (Schoch & Evers 2002). Jagoe et al (2002) found feather mercury concentrations in South Carolina bald eagle nestlings to be 3.06 ppm dry weight. They did not make any conclusions as to the affects of this level of exposure. Bowerman (1993) found concentrations in adult body feathers and nestling bald eagle feathers from the Great Lakes basin of North America of 21.4 ppm and 9.0 ppm respectively. He concluded that neither productivity (young per occupied nest) nor success was

significantly ($p \leq 0.05$) correlated with logarithmic concentrations of adult or nestling feather mercury. It may also be hard to determine the affects of mercury on reproduction when there are also tissue concentrations of chemicals such as DDE (Bowerman 1993). Mercury concentrations found in all age groups of African fish eagles and nestling marabou storks were well below all of the concentrations mentioned above.

Mercury accumulates over time with the two main routes of excretion being growing feathers and eggs (Heinz 1996). Therefore it would be expected that mercury concentrations would be greater in adult birds than nestlings and greater in male adults than female adults. Females would be expected to excrete mercury into the egg and also have a larger body weight than males and thus would be expected to have lower concentrations than males. The results of this study do not support these expectations, as there was no significant ($p \leq 0.05$) difference in mercury levels based on age (nestling or adult) or gender. When Burger (1994) summarized studies examining gender differences in mercury concentrations in feathers, she found only two out of eight studies that found significant differences. She also reported that most studies have failed to find any relationship between mercury levels and age. Although these studies may support our findings, the results may also be due to a small sample size. This reinforces the fact that due to the small sample size the results should be interpreted with caution. Sampling occurred at times of egg laying and at times of non breeding and it was impossible to determine the reproductive status of most adult female birds sampled. It was therefore impossible to determine whether egg laying, at some point in time before new feather growth, may have lowered the total body mercury concentrations through excretion of mercury into the egg. Another factor in this study was that a small percentage of the

samples from very small nestlings were breast down rather than breast feathers. Gariboldi et al (2001) found a significant ($p \leq 0.05$) correlation between mercury concentrations in blood, down and feathers of wood storks (*Mycteria Americana*) (n = 300) at four out of five sites in the southeast USA. There is some debate over whether the mercury in down originates mainly from the egg (Becker et al 1994) or dietary accumulation (Gariboldi 2001). This could be variable between species. The higher mercury concentrations in fish eagles from Entebbe may reflect greater bioaccumulation through dietary exposure than occurred with the fish eagles at Lake Mburo. *Oreochromis niloticus*, one of the main fish eagle prey items at both sites, had higher concentrations in fish from Entebbe than those from Lake Mburo. We do not believe the results for the two adult fish eagles with relatively high mercury concentrations compared to all other fish eagles sampled are spurious as they were captured in close proximity to each other, were nesting on the property of a large flower factory and both had 4,4'-DDE detectable at 0.002 ppm wet weight in plasma. Again, the results should be interpreted cautiously as the findings require more investigation given the small sample size.

Anthropogenic mercury emissions associated with fossil fuel burning are a possible explanation for the higher mercury concentrations in feathers returned from Entebbe and Kampala samples compared with samples from Lake Mburo. The fact the human population of Kampala increased from 774,241 in 1991 to a preliminary figure of 1,208,544 in 2001 and use of diesel fuel (inferior grades and quality) in Uganda increased from 125,621 in 1997 to 207,183 cubic metres in 2001 (Uganda Bureau of Statistics, 2002) lend support to this explanation. Another source of mercury may be point source emissions through the burning of garbage, which is a widespread practice in the

communities around the Lake Victoria shoreline. A factor that may have contributed to natural background mercury concentrations at all sites was the eruption of two volcanoes, Nyamuragira and Nyaragongo in the Democratic Republic of the Congo, near the border with Uganda, in July 2002. Volcanic eruptions are a natural source of atmospheric mercury, as is degassing of the earths crust (Heinz 1996).

Adult African fish eagles are extremely territorial (Brown 1980) thus concentrations of mercury in nestlings would be expected to reflect environmental concentrations in and around the nest site and confluent fishing territory. Therefore contaminant concentrations in nestlings and adults would be expected to reflect local concentrations of contamination. The significantly ($p \leq 0.05$) higher concentration of mercury in fish from Murchison Falls compared with Lake Mburo or Entebbe ($p = 0.02$) may reflect sources of contamination upstream from this sampling site or local conditions that favor the mobilization of mercury. Further investigation of the complex ecology of the area, the performance of water quality analysis and samples from fish eagles would be required to verify these assertions.

Feathers are an excellent, non-invasive tissue to sample for mercury analysis. Feathers contain higher concentrations of mercury than in other body organs (Westermark et al 1975) with approximately 70% of total body mercury found in feathers. (Honda et al 1986). Feather mercury concentrations can easily be measured. A positive correlation has also been shown between mercury content in feathers and internal tissues (Thompson et al 1991). Mercury concentrations are spread evenly throughout the feather, indicating endogenous incorporation methods, rather than atmospheric deposition

(Hahn et al 1993). Mercury concentrations of fish eagles and marabou storks can therefore be directly associated with dietary exposure. This dietary exposure may occur through bioaccumulation of mercury within the food chain. Feathers are a major excretory pathway for body mercury during molt as concentrations accumulate during periods of feather growth. Differing mercury concentrations are found depending on the order the sampled feather has in the sequence of the molt cycle. New feathers grown early in the cycle would be expected to have higher mercury levels than those grown later in the cycle. As the molt cycle continues and more mercury is excreted into growing feathers the total body pool of mercury is diluted. Thus the later in the molt cycle a new feather is grown the less mercury will be found in the feather. This has not been consistently shown to occur in all individuals within a population. Body contour feathers are the feather type that shows the least variation in mercury content (Furness et al 1986). This supports the methodology in this project of taking a pooled sample of breast feathers. Interpretation of total feather mercury concentrations should be made with caution. For example trace metals like selenium and zinc can lower the affects of high tissue residue concentrations of mercury. In addition, certain marine mammals and birds can demethylate methyl-mercury into inorganic mercury (Heinz 1996). It is suggested that similar mechanisms to tolerate high mercury concentrations may exist in piscivorous raptor species (Norheim & Froslic 1978). However, 95% of total mercury found in blood, feathers and eggs is in the methyl-mercury form (Thompson, 1996) and therefore total mercury concentrations may be a reasonable indicator of the potential for adverse affects in avian species.

Mercury concentrations in marabou stork nestling feathers were well below the concentrations reported to cause adverse reproductive and physiological affects in other avian species (Scheuhammer and Bond 1991; Eisler 1987; Scoch and Evers 2000). However, Gariboldi et al (2001) point out the need to relate tissue concentrations in piscivorous birds to sublethal effects at both the individual and population level. This would also apply to marabou storks and African fish eagles. The trend towards higher feather mercury concentrations in marabou stork nestlings compared to fish eagle nestlings from the same region (Entebbe and Kampala are 40 km from each other) may indicate species differences in accumulation or greater dietary exposure in marabou storks. The lack of any significant difference ($p \geq 0.05$) in mercury concentrations between the fish eagle population from Entebbe (including adults), and the nestling marabou stork population from Kampala may be caused by greater dietary exposure in marabou stork nestlings. This may be due to point source emissions such as the burning of garbage. Marabou storks also have a longer fledging period than fish eagles (135 days for marabou storks and 75 days for fish eagles) and therefore a longer period of feather growth and potential accumulation of mercury in the growing feather. The mercury concentrations found in marabou storks nestlings from Kampala may reinforce the conclusions in relation to concentrations of mercury found in fish eagles from the Entebbe region. That is, the concentrations of mercury found in biota at Entebbe/Kampala are higher than the concentrations found at Lake Mburo. In addition, this may reflect bioaccumulation from dietary sources. Marabou storks in Kampala have largely adapted to a cosmopolitan, broad ranging diet based on scavenging and it is stated that they will eat virtually anything organic (Hancock et al 1992). During nestling

growth, the parent marabou storks seek out dietary protein sources to cater for the extra growth requirements of nestlings (Hancock et al 1992). It is postulated that this may lead to seeking of food items that originate at higher trophic levels in the food chain, thus increasing susceptibility to persistent bioaccumulative endogenously derived toxicants. Concentrations of mercury in marabou stork nestlings in Kampala may be seen as a non-specific indication of mercury concentrations contained in human organic refuse in Kampala. However, caution should be exercised in attempting to ascribe exposure routes for mercury concentrations found in the marabou stork nestlings. Other exposure routes, in addition to diet may contribute to mercury concentrations in these birds. Atmospheric concentrations of mercury may also be higher in the center of a busy city and this may make a small contribution, through atmospheric deposition, and inhalation to the overall mercury concentrations found in the feathers of these marabou stork nestlings.

**Persistent organic pollutants**

Concentrations of 4,4'-DDE found in some adult fish eagle samples were below wet weight plasma concentrations linked to low productivity levels in bald eagle nestlings. Elliot & Norstrom (1998) calculated that 0.04 ppm wet weight DDE in plasma equals about 6 ppm DDE in eggs, and this level equates to a productivity of 0.52 young per occupied nest using the unweighted linear model of Wiemeyer et al (1993). Productivity of 0.7 young per occupied territory is necessary for population maintenance in the bald eagle and reproduction is considered impaired when productivity (young/occupied nest) is less than 1.0 (Bowerman 1993). The relationship between mean annual productivity and mean plasma 4,4'-DDE concentrations established for ten

subpopulations of bald eagles in the upper Midwest of the USA had productivity falling below 1.0 at approximately 0.011 ppm mean wet weight plasma 4,4'-DDE in nestlings (Bowerman 1993). Depending on the species, DDE concentrations in eggs of 2-20 ppm wet weight are associated with eggshell thinning, breakage and breeding failure sufficient to cause population declines (Hartley & Douthwaite 1994; Blus 1982; Henny et al 1984; Weimeyer et al 1984). Bald eagle productivity has been found to be near normal at breeding areas where DDE was 3 ppm or less wet weight in eggs (Weimeyer et al 1984). It must be remembered that all samples above the limits of detection in this study were adult birds and only trace levels below the 0.001ppm limit of detection were found in plasma from fish eagle nestlings. Unlike nestlings in the bald eagle studies, the adult birds in this study could not be accurately aged. Smith and Bouwman (2000) recorded a range of chlorinated hydrocarbon residues measured in blood plasma in four species of raptor from the North-West province of South Africa. Concentrations of DDE ranged between 0.0015 ppm to 0.0066 ppm (uncorrected wet weight) except for Lanner falcons (*Falco biarmicus*), an omiphagous species, had concentrations of 0.014 ppm. These levels were in adult birds and are similar to those recorded for the birds in this study. The authors of the South African study, which extrapolated egg DDE plasma concentrations from blood plasma, concluded that raptors are in no immediate danger of reproductive impairment on account of egg DDE residues. Evans and Bouwman (2000) also recorded blood plasma concentrations of DDE in pied kingfishers *Ceryle rudis* from various areas of northern KwaZulu-Natal, South Africa. Concentrations varied by location with the highest wet weight uncorrected concentration being 0.18 ppm. Pied kingfishers are common in Uganda and occur at a relatively high trophic level. It may be of interest to

sample these birds in the future. All the quantifiable concentrations of POPs in this study were in samples from the Entebbe area of Lake Victoria that also had higher mercury concentrations suggesting more effects of anthropogenic disturbance than at Lake Mburo. Concentrations of POPs were quantified in human and cows milk in the Kampala region in 1992/1993 (Ejobi et al 1996 a,b). Pesticide residues detected were dieldrin, 4,4'-DDD, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT, β-HCH, α-HCH and lindane. Mean 4,4'-DDE residues reported in human milk were 2.84 +/- 0.255 mg/kg milk fat and in cows milk 0.034 +/- 0.0004 mg/kg milk fat. These figures are not corrected for, percent recoveries. The mean percent extractable fat from cows milk was 2.9% and from human milk 4.3%. No PCBs were detected in these studies. Cows in this study were usually not grazed, but fed on crop residues that could have been contaminated with pesticides. Concentrations of pesticides were generally lower than concentrations reported in other developing countries. The widespread, systematic usage of persistent organochlorinated pesticides that occurred in African countries, such as Kenya, South Africa and Zimbabwe in the 1970s and 1980s surpassed usage in Uganda. This was probably due to the lesser development of plantation agriculture in Uganda during its time as a British protectorate in addition to the prolonged period of civil unrest that continued from independence in the early 1960s till the mid 1980s.

Raptor reproductive measures have been used to determine the effects of environmental contamination on the health of raptor populations, such as the bald eagle in North America. Bowerman (1993) in relation to bald eagles defined productivity as the number of young per occupied nest for each breeding area and success as the percent of occupied breeding areas fledging at least one young. Using these definitions and our

general observations, the results of the mercury/POP analyses would suggest healthy, self sustaining populations of fish eagles at Lake Mburo and Entebbe. Out of ten nests climbed that contained chicks only one had a single chick, seven had two chicks and two had three chicks. Five other nests contained eggs, three with two eggs and two with three eggs. Our general observations suggest that most of these nests were fledging two chicks. At Lake Mburo the average of three days of counting eagles from a boat led to a result of 74 adults and 15 immature fish eagles. The percentage of immature eagles was 20%. It is recognized that no scientific methodology was employed in this count and it is presented as observation only. Some authors suggest that a population of fish eagles with 20% immature birds can be considered a reproductively healthy population (Brown and Cade 1972). To determine the effects of environmental contamination on a population requires multiple sampling over many years as well as a thorough knowledge of the species biology and reproductive cycle at the study area. Again this was beyond the resources available for this study but given the residue concentrations reported here, and our general observations on productivity it could be inferred that environmental contamination by these chemicals does not play a significant role in determining the population dynamics of African fish eagles at either of the sites studied. However, this was not proven. We conclude that concentrations of mercury in African fish eagles is unlikely to be a major factor in determining population dynamics of fish eagles at either Lake Mburo or Lake Victoria at Entebbe. That there were no DDT residues found at the limits of detection for fish eagles, marabou storks or *Oreochromis niloticus* sampled in this study, may indicate that there is no ongoing exposure to this pesticide. It is worth noting, however the similar concentrations of plasma DDE in the Entebbe fish eagle

samples to those from South African raptors reported by Smith & Bouwman (2000). South Africa is generally thought to have had greater systematic usage of DDT than occurred may have occurred in Uganda. The concentrations of DDE in African fish eagles at Lake Victoria at Entebbe may warrant further investigation. This should include a larger sample size, analysis of water quality and multi year examination of fish eagle productivity. We also conclude that mercury concentrations in fish eagles are significantly higher at the more urbanized Entebbe site than at Lake Mburo. This was supported by similar mercury concentrations being found in nestling marabou storks from Kampala, which is almost contiguous with the Entebbe site. An expanding economy, population centralization, increasing use of fossil fuels and burning of garbage indicates that it would be reasonable to expect mercury concentrations to increase with time in wildlife living in the Entebbe/Kampala region.

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**Table 5.1** Total mercury concentrations (ppm dry weight) in breast feathers from adult and nestling African fish eagles (*Haliaeetus vocifer*) and nestling marabou storks (*Leptoptilos crumeniferus*) and in whole body section samples of tilapia (*Oreochromis niloticus)* (ppm wet weight) from Uganda.

[a] Q1 = 25$^{th}$ percentile of the sample size

[b] Q3 = 75$^{th}$ percentile of the sample size

[c] number indicates sample size

[d] LM= Lake Mburo

[e] Ebb= Lake Victoria near Entebbe

**Table 5.1 Mercury concentrations in marabou storks, African fish eagles and Tilapia *(Oreochromis niloticus)*.**

| | Mean | Median | SD | Q1[a] | Q3[b] | Min | Max |
|---|---|---|---|---|---|---|---|
| **FISH EAGLES** | **(feathers ppm dry weight)** | | | | | | |
| **Adult LM**[d] (10)[c] | 0.357 | 0.314 | 0.124 | 0.249 | 0.441 | 0.230 | 0.554 |
| **Adult Ebb**[e] (5) | 1.059 | 0.588 | 0.787 | 0.548 | 1.390 | 0.471 | 2.300 |
| **Adult Male** (9) | 0.730 | 0.450 | 0.680 | 0.320 | 0.590 | 0.230 | 2.300 |
| **Adult Female** (6) | 0.384 | 0.378 | 0.150 | 0.249 | 0.519 | 0.23 | 0.548 |
| **Adult Total** (15) | 0.591 | 0.450 | 0.552 | 0.299 | 0.551 | 0.230 | 2.300 |
| **Nestling LM** (8) | 0.185 | 0.171 | 0.100 | 0.110 | 0.210 | 0.096 | 0.398 |
| **Nestling Ebb** (10) | 0.666 | 0.669 | 0.230 | 0.489 | 0.840 | 0.309 | 1.03 |
| **Nestling Total** (18) | 0.452 | 0.424 | 0.305 | 0.205 | 0.702 | 0.096 | 1.03 |
| **Eagle Total** (33) | 0.515 | 0.450 | 0.433 | 0.233 | 0.588 | 0.096 | 2.300 |
| **STORKS** | **(feathers ppm dry weight)** | | | | | | |
| **Storks Male** (12) | 0.840 | 0.390 | 0.810 | 0.490 | 1.120 | 0.380 | 1.500 |
| **Storks Female** (9) | 0.720 | 0.600 | 0.470 | 0.370 | 0.800 | 0.230 | 2.000 |
| **Storks Total** (21) | 0.810 | 0.500 | 0.670 | 0.410 | 1.130 | 0.230 | 2.000 |
| **OREOCHROMIS** | **(whole body section samples ppm wet weight)** | | | | | | |
| **NILOTICUS** | | | | | | | |
| **Fish Ebb** (8) | 0.0055 | 0.0050 | 0.0025 | 0.0045 | 0.0070 | 0.0020 | 0.0100 |
| **Fish LM** (3) | 0.0030 | 0.0030 | 0.0000 | 0.0030 | 0.0030 | 0.0030 | 0.0030 |
| **Fish Murchison**(7) | 0.0077 | 0.0080 | 0.0022 | 0.0060 | 0.0090 | 0.0050 | 0.0110 |
| **Fish Total** (17) | 0.0059 | 0.0055 | 0.0027 | 0.0035 | 0.0078 | 0.0020 | 0.0110 |

**Table 5.2:** Analysis of variance of total mercury concentrations in breast feathers of African fish eagles (*Haliaeetus vocifer*) (n = 33) from Lake Mburo and Lake Victoria near Entebbe, Uganda (ppm dry weight).

**Table 5.2. Analysis of variance of mercury concentrations in fish eagle feathers (n = 33) (ppm dry weight)**

| Variable | Level | n | Mean (sd) | F | p |
|---|---|---|---|---|---|
| **Site** | Lake Mburo | 18 | .28 (.14) | 17.79 | .0002 |
| | Lake Victoria | 15 | .80 (.50) | | |
| **Age** | Adult | 15 | .59 (.55) | .84 | .3671 |
| | Nestling | 18 | .45 (.30) | | |
| **Gender** | Female | 13 | .43 (.22) | .82 | .3709 |
| | Male | 20 | .57 (.53) | | |
| **Gender (Adults)** | Female | 6 | .38 (.15) | 1.45 | .2505 |
| | Male | 9 | .73 (.68) | | |
| **Gender (Nestlings)** | Female | 7 | .47 (.28) | .03 | .8549 |
| | Male | 11 | .44 (.33) | | |
| **Multivariable ANOVA** | Site | - | - | 18.13 | .0002 |
| | Age | - | - | 1.31 | .2620 |
| | Gender | - | - | 1.29 | .2657 |
| | Overall | - | - | 6.91 | .0012 |

**Table 5.3:** Results of analysis of variance of breast feather mercury concentrations between Marabou storks (*Leptoptilos crumeniferus*) (n = 21) and African fish eagles (*Haliaeetus vocifer*) (n = 15) residing in the Entebbe/Kampala region of Uganda (ppm dry weight).

**Table 5.4:** Results of univariable analysis of variance of total mercury concentrations in breast feathers of marabou storks (*Leptoptilos crumeniferus*) (n = 21) from Kampala, Uganda (ppm dry weight)

**Table 5.5:** Results of univariable analysis of variance of mercury concentrations in Tilapia fish (*Oreochromis niloticus*) (n = 18) from three sites in Uganda (ppm wet weight).

**Table 5.3. Results of analysis of variance of feather mercury concentrations between avian species from Lake Victoria (ppm dry weight)**

| Variable | Level | n | Mean (sd) | F | p |
|---|---|---|---|---|---|
| **Species** | African fish eagles | 15 | .80 (.50) | 0.0 | .9510 |
| | Marabou storks | 21 | .81 (.49) | | |
| **Multivariable ANOVA** | Species | - | - | 5.17 | .0272 |
| | Gender | - | - | .30 | .5864 |
| | Overall | - | - | ***2.74*** | ***.0743*** |

**Table 5.4. Results of analysis of variance of feather mercury concentrations in marabou storks (ppm dry weight)**

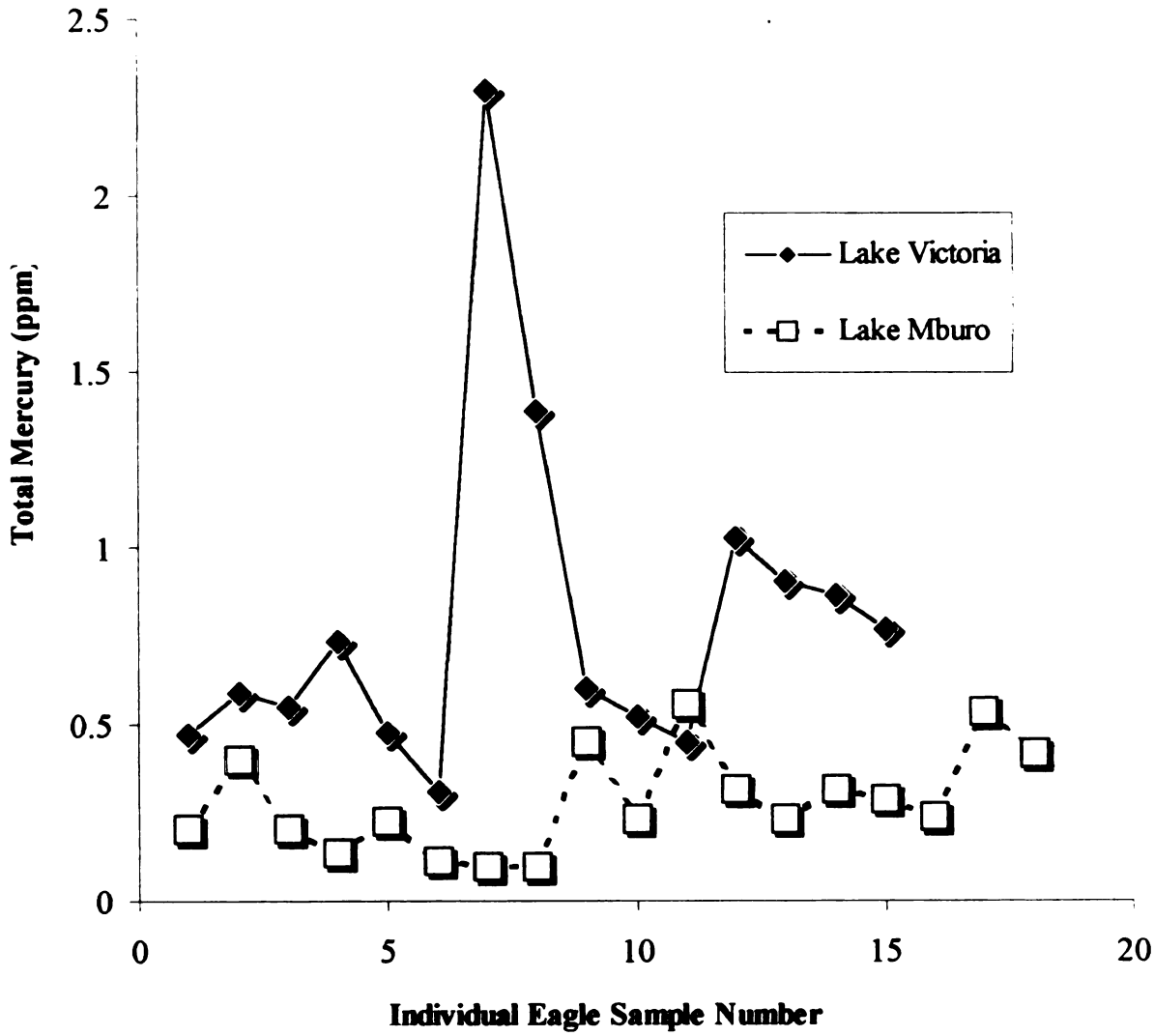
| Variable | Level | n | Mean (sd) | F | p |
|---|---|---|---|---|---|
| **Gender** | Female | 9 | .81 | 0.0 | .9549 |
| | Male | 12 | .80 | | |

**Table 5.5. Results of analysis of variance of total body mercury concentrations in *Oreochromis niloticus* fish (n = 18) from three sites in Uganda (ppm wet weight)**

| Variable | Level | n | Mean (sd) | F | p |
|---|---|---|---|---|---|
| **Site** | Lake Mburo | 3 | .0030 | 5.06 | .0209 |
| | Lake Victoria | 8 | .0055 | | |
| | Murchison Falls | 7 | .0077 | | |

**Figure 5.1:** Total mercury in breast feathers of adult and nestling African fish eagles (*Haliaeetus vocifer*) from Lake Mburo (n= 18) and Lake Victoria at Entebbe (n= 15), Uganda (ppm dry weight).

**Figure 5.1. Total mercury in breast feathers of adult and nestling African fish eagles (*Haliaeetus vocifer*) (n = 33) (ppm dry weight)**



# Chapter 6

## Nest Site Habitat Characterization of African Fish Eagles (*Haliaeetus vocifer*) from three sites in Uganda

## Abstract

The objective of this study was to document selected characteristics of the nest site habitat of African fish eagles (*Haliaeetus vocifer*) in Uganda. Nest tree species, nest tree height, nest height within the tree and nest tree diameter at breast height were recorded. Crown class, canopy cover, distance to water, whether the nest tree was living, decaying or dead and evidence of disturbance were recorded for each nest tree. Nest site habitat characteristics of breeding areas of African fish eagles (*Haliaeetus vocifer*) were examined at Lake Mburo (n =13), Murchison Falls National Park (n = 12) and Lake Victoria at Entebbe (n = 19). The most common tree species utilized for nesting were *Acacia sieberiana* (83%) at Lake Mburo; *Balanites aegyptiaca* (50%) and *Tamarindus indica* (33%) at Murchison Falls and a mixture of species including *Chlorophora excelsa* at Entebbe (26%). Average nesting tree height was 11.9 ± 3.6, 17.7 ± 6.7 and 29.6 ± 7.0 meters for Lake Mburo, Murchison Falls and Entebbe respectively. Average nest height was 9.7 ± 3.9, 13.1 ± 5.5, and 22.9 ± 5.8 meters for each site as above respectively. The average diameter at breast height of trees was 65 ± 14, 91 ± 37, and 145 ± 67 cm for each site as above respectively. Fish eagles preferred nesting in living trees. Most breeding areas contained significant alteration of natural vegetation features by animals or man. Characterization of nest site habitat may assist in future conservation planning for the African fish eagle in Uganda. Nest site characterization may also provide preliminary data necessary for development of the fish eagle as a biomonitor of environmental change.

## Introduction

The African fish eagle (*Haliaeetus vocifer*) has been called the "the voice of Africa" (Brown 1980). It has also been described as one of the best-known raptors throughout sub-Saharan Africa (Brown 1980). Despite this, much of the knowledge of African fish eagles is through observational records alone. Through observation, Brown (1980) characterized fish eagle breeding areas as usually having one to three nests, generally in tall trees and felt "..... quite certain that the fish eagle selects its nest tree, if there is a choice". The objective of this study was to measure selected nest site characteristics of African fish eagle nests at Lake Mburo, Murchison Falls and Lake Victoria near Entebbe in Uganda, East Africa. Data was collected as part of research determining concentrations of selected environmental pollutants in the tissues of fish eagles at the study sites. A second project objective was to assess the potential of fish eagles to be utilized as a biomonitor species of environmental change. Nest site habitat characterization may aid in this assessment by providing a quantitative description of selected nest site habitat requirements.

## Materials and Methods

Selected characteristics of the breeding area of African fish eagles (*Haliaeetus vocifer*) were examined at Lake Mburo (0°. 39' S, 30°. 57' E) (n =13), Murchison Falls National Park (2°. 15' N, 31°. 38' E) (n = 12) and Lake Victoria near Entebbe (0°. 04' N, 32°. 26' E) (n = 19). A breeding area was defined as approximately 100 square foot of land and water centered on a tree with a nest in it. To qualify, the nest had to have evidence of recent use such as eagles present in the tree, visible urates around the tree

base, nesting activity and presence of prey remains. Lake Mburo is a 6 km long freshwater lake situated in south western Uganda within a 256 sq. km national park. Areas of Entebbe studied included the Lake Victoria coastline from Nfo Island (0°. 00'N, 32°. 26'E) to Kisubi Bay (0°. 05'N, 32°. 35'E). Areas of Murchison Falls National Park sampled were the Nile river from the falls to Wanseko at the Lake Albert delta. Nest sites were sampled between December 2002 and January 2003.

Tree heights were determined using the percentage scale of a clinometer (Suunto Clinometer PM5 SPC, Carlsbad, CA). A fiberglass tape (Keson model OTR-18M-15, Forestry Suppliers, Inc. Jackson, MO) was used to measure 50 - 100 ft from the base of the nest tree. If vegetation was impenetrable, a rangefinder (TLR 75 Ranging Rangefinder, East Bloomfield, NY) was used for the same purpose. Tree height was calculated from the formula: *(percentage to top of tree + percentage to bottom of tree) x distance from tree (ft) = Height of Tree (ft)*. Nest height was calculated by the same method except the measurement was taken at the bottom of the nest. All results were converted to meters. Diameter of the tree at breast height (DBH) was measured with a DBH tape (Forestry Suppliers, Inc. Jackson, MO) at 1.37 meters ($4^1/_2$ ft) above ground on the uphill side of the tree. Distance to water was defined as the nearest straight line from the nest tree to the shore measured with a fiberglass tape, or a rangefinder, or if the distance was great, by visual estimation. Elevation and site location were recorded using a global positioning system (Eagle Expedition, Eagle Electronics, Catoosa, OK). Canopy cover was determined by use of a spherical crown densiometer (Spherical Densiometer Model C, Forest Densiometers, Bartlesville, OK). Readings were taken ten paces from the tree facing north, south, east and west. Vegetation had to be 7 m or more above the

ground to be included. The readings were averaged then multiplied by 1.04. This figure was then deducted to give the total area of the canopy occupied. Tree species were identified from the keys found in Eggeling (1951). Tree condition was assessed visually and recorded in the following categories living, 25% decay, 50% decay, 75% decay or dead. Crown class was defined as the nest tree height relative to the trees in a 100 ft radius immediately surrounding the nest tree. Trees were classified as dominant if the tree was taller than those immediately surrounding it, co-dominant if the tree canopy was the same height as the trees immediately surrounding it, intermediate if the nest tree canopy was lower than the trees immediately surrounding it and suppressed if the nest tree was completely dominated by the trees surrounding it. Age class of the nesting tree was classified as even if all of the trees within the breeding area were approximately the same size (signifying one canopy layer) or uneven, meaning the trees were of differing ages and sizes (signifying multiple canopy layers). Evidence of disturbance was classified as human or animal disturbance in an area of 100 ft radius from the base of the nesting tree.

Descriptive statistics were done using Excel (Microsoft Excel, Microsoft Office 2000 Professional. Microsoft Corporation, Redmond, WA). Direct correlation was used to assess the correlation between two variables.

## Results

Measurements of nest tree height, nest height, tree height above nest, DBH, elevation and canopy cover for African fish eagle nests at Lake Mburo, Murchison Falls and Lake Victoria near Entebbe are presented in table 6.1.

The most common tree species utilized for nesting were *Acacia sieberiana* (83%) at Lake Mburo; *Balanites aegyptiaca* (50%) and *Tamarindus indica* (33%) at Murchison Falls and *Chlorophora excelsa* at Entebbe (26%). Other trees utilized for nesting were *Euphorbia candelabra* and two unidentified hardwood species at Lake Mburo; *A. sieberiana* at Murchison Falls and *Antiarus africana moraceae*, *Newtonia buchanii*, *Antiarus toxocara*, *Canarium frankfurta*, an unidentified *Eucalyptus* species and an unidentified *Ficus* species at Lake Victoria near Entebbe.

Nest trees were highest and had a greater diameter at breast height at Lake Victoria near Entebbe. Lake Mburo had the smallest and thinnest nesting trees.

Crown class was dominant on 57%, 66% and 57% of the trees for Lake Mburo, Murchison Falls and Entebbe respectively. The remainder were classified as co-dominant except one tree at Entebbe that was classified as intermediate. No nesting trees were suppressed. The nesting areas were comprised of trees of uneven age classes at 100%, 25% and 84% of nest sites examined at Lake Mburo, Murchison Falls and Entebbe respectively.

At Lake Mburo and Murchison Falls 92% of nesting trees were living and at Entebbe 74% were classified as living. One tree at Lake Mburo was dead while 4 trees (21%) at Entebbe showed 25% decay and one tree 75% decay.

Evidence of human or animal disturbance was present at within the breeding area of all the nest sites. Human disturbance within the breeding area was seen at 89% of the Entebbe nest sites. Animal disturbance within breeding areas at Lake Mburo was largely due to hippopotamus and buffalo trails. Hippopotamus and buffalo trails were also numerous at Murchison Falls with the addition that many nests were located within crocodile breeding areas.

## Discussion

*Acacia sieberiana* at Lake Mburo and *Balanites aegyptiaca* and *Tamarindus indica* at Murchison Falls are numerically predominant tree types in the landscape at these sites. It can be inferred that the diversity of tree types chosen for nesting at Entebbe relects anthropogenic change to the landscape and fish eagle adaptation to these changes. Trees at Entebbe were generally taller than trees at the other two sites, but not as numerous. Eagle nests appeared to be more numerous at Entebbe where there were aggregations of tall trees (such as in the botanical gardens and animal breeding centre) and/or where there was restricted human activity (Nfo Island and smaller islands with difficult human access). The fewer trees available at Entebbe may have meant less available choice for nest site placement and thus adaptation to less than suitable habitat. Visual observation suggested that territories at Entebbe stretched a greater distance from shore than at the other sites. This greater territorial depth may have been associated with use of taller trees or differences in prey species density between sites. Visual observations (not scientific counts with methodology) suggest greater density and smaller territories of fish eagles at Lake Mburo than the other two sites. Determination of territory size may be

based on available resources, including suitable habitat, and this assertion may in part explain differing eagle densities between the study sites.

Data on the distance to water could be misleading as the distribution is highly skewed to the right suggesting a few nests were located at some distance from water with the majority closer. There was a weak positive correlation ($r^2 = 0.18$) between tree height and distance from water suggesting that higher trees maybe required or sought out the farther the nest was from the shore. This would be necessary for prey visualization. Uneven age class may signify anthropogenic disturbance precipitating variable vegetation growth rates or it could indicate the predominance of one or a few tree species. The high percentage of even age classes seen at Murchison Falls may be related to the topographical features of the area or the differences between nest sites located on a river with a strong current versus lake-based nest sites. Prey density could also affect nest site habitat preference.

The data presented can best be utilized to draw inferences into what may constitute suitable nesting site habitat for fish eagles. A more comprehensive statistical analysis, beyond the resources of this project is required to place many of the measurements into perspective. For example, the data would suggest fish eagles prefer to nest in living trees however this cannot be proved as the number of living: dead trees within each study site was not determined. Examination of the data in relation to the prevalence of the characteristic within a study site is also required (e.g. is there a predominance of even or uneven age classes of trees in general at Lake Mburo). The data

is therefore presented without analysis as baseline information that requires further, more intensive and wider ranging data collection before further conclusions can be made regarding fish eagle nest site habitat. However, in regards to nest tree species, nest tree height, nest height and canopy cover the data from each study site shows marked variation. One conclusion that may be drawn from this data is that fish eagles are an adaptable species able to nest under a fairly broad range of habitat parameters.

Further characterization of nest site habitat, utilizing this data may assist in future conservation planning for the African fish eagle in Uganda. Understanding, characterizing and quantifying the norm may assist in understanding, quantifying and interpreting deviations from the norm. In this way nest site characterization may also provide preliminary data necessary for development of the fish eagle as a biomonitor of environmental change.

## References

Brown L. 1980 The African Fish Eagle. Bailey Bros. & Swinfen Ltd. Folkestone, England.

Eggeling WJ. 1951. The Indigenous Trees of the Uganda Protectorate. The Government Printer, Entebbe, Uganda.

**Table 6.1:** Nest site characteristics for African fish eagle (*Haliaeetus vocifer*) nests at Lake Mburo (n = 13), Murchison Falls (n = 12) and Lake Victoria at Entebbe (n = 19), Uganda.

[a] LM = Lake Mburo

[b] MF = Murchison Falls National Park

[c] LV = Lake Victoria at Entebbe

## Table 6.1. Nest site characteristics for African fish eagle

| | LM (n = 13) [a] | MF (n = 12) [b] | LV (n = 19) [c] |
|---|---|---|---|
| **TREE HEIGHT (M)** | | | |
| Mean ± S.D. | 11.9 ± 3.6, | 17.7 ± 6.7 | 29.6 ± 7.0 |
| Median (range) | 11.9 (9.1-18.9) | 17.1 (12.8-30.2 | 29.9 (25-46.4) |
| **NEST HEIGHT (M)** | | | |
| Mean ± S.D. | 9.7 ± 3.9 | 13.1 ± 5.5 | 22.9 ± 5.8 |
| Median (range) | 8.8 (6.4-16.5) | 12.2 (8.8-24.4) | 23.8 (17.7-34.8) |
| **TREE HEIGHT ABOVE NEST (M)** | | | |
| Mean ± S.D. | 2.2 ± 1.5 | 4.6 ± 2.7 | 6.4 ± 1.5 |
| Median (range) | 2.4 (0.9-4.9) | 4.6 (2.4-9.8) | 5.8 (4.0-20.7) |
| **ELEVATION (M)** | | | |
| Mean ± S.D. | 1594 ± 144 | 959 ± 85 | 1448 ± 51 |
| Median (range) | 1582 (1570-1937) | 936 (922-1150) | 1456(1450-1512) |
| **DBH (CM)** | | | |
| Mean ± S.D. | 65 ± 14 | 91 ± 37 | 145 ± 67 |
| Median (range) | 67 (52-87) | 86 (60-166) | 118 (94-287) |
| **DISTANCE TO WATER (M)** | | | |
| Mean ± S.D. | 76.8 ± 166 | 48 ± 50.3 | 294± 355 |
| Median (range) | 12.2 (1.8-500) | 36.6 (20-176) | 73.4 (9.1-1000) |
| **CANOPY COVER (%)** | | | |
| Mean ± S.D. | 37 ± 32 | 61 ± 22 | 47 ± 30 |
| Median (range) | 42 (8-95) | 69 (55-88) | 43 (21-90) |

# Chapter 7

## Assessing the potential of African Fish Eagles (*Haliaeetus vocifer*) and Marabou storks (*Leptoptilos crumeniferus*) as biomonitors of environmental change.

## Abstract

The purpose of this study is to assess the suitability of using African fish eagles (*Haliaeetus vocifer*) and marabou storks (*Leptoptilos crumeniferus*) as biomonitors of environmental change in Uganda. A study was designed to evaluate concentrations of persistent organic pollutants and mercury in African fish eagles and marabou storks in Uganda. A series of criteria necessary for the successful development of a biomonitor species was established from the work of others and our own observations. With the results from this study and the existing body of literature, the suitability of marabou storks and fish eagles as biomonitor species was assessed against these criteria. African fish eagles and Marabou storks met most of the criteria of a suitable biomonitor species. However, both species failed to meet critical criteria. Chiefly, for fish eagles the reproductive cycle needs to be defined more clearly at the local level as does local variation in diet. Marabou storks require development of suitable capture methods for the adult portion of the population. Biomonitoring programs using marabou storks need to consider the wide ranging nature of the species diet, and its migratory behavior in its tropical range.

## Introduction

The use of mammals, birds and reptiles as monitors of the potential effects of chemicals in the environment is well documented (Burger & Peakall 1995; Jefree et al 2001). For a species to be a suitable biomonitor certain conditions must be met. These include characteristics that are defined at the class, order, genus and species levels for the animal being assessed. A characteristic at the class level may be that all birds molt

feathers and this uniquely avian tissue may be a useful indicator of whole body concentrations of certain heavy metals. A characteristic at the genus level might be the predominantly piscivorous diet and large body mass of members of the *Haliaeetus* genus. Documentation of the endpoint that is to be quantified for the chemical being examined, and the species in which it is to be examined are also important. An example would be eggshell thinning and its relationship to DDE concentrations in the parent birds. Establishing the relationship between an endpoint indicator effect and its hypothesized cause often requires laboratory toxicity testing on the species or a similar species. Selecting a tissue type in the correct quantity at a suitable body site, and establishing a correlation between tissue chemical concentrations and concentrations known to cause an endpoint effect are also critical. Finally, to utilize a species as a biomonitor, knowledge of ecological structure and function, and form and cycling of the chemical within the environment being examined are required. To meet these criteria, research teams with multidisciplinary expertise may be required. Apart from directly measuring residues of chemicals of concern in an animal species, information can be gained by use of other monitoring methods, such as stable radio-isotopic analysis, which can document changes in energy or mass flows through ecological communities (Harding & Stevens 2001). Chemical effects on an environment could be one of many reasons for changes in energy or mass flows that can be documented by radio-isotopic studies. Water quality analysis can help determine how a chemical may behave under different levels of anthropogenic alteration to aquatic ecosystems.

We sampled fifteen adult and eighteen nestling African fish eagles (*Haliaeetus vocifer*), twenty nestling marabou storks (*Leptoptilos crumeniferus*) and eleven tilapia fish *(Oreochromes niloticus*) from Lake Mburo and the Kampala/Entebbe region of Uganda for a range of persistent organic pollutants and mercury. The objectives of the research were to document baseline concentrations of these chemicals in marabou storks and African fish eagles, as well as collect other baseline data, such as plasma chemistry values, nest site habitat characteristics and body measurements. A further objective was to make an initial assessment of the utility of African fish eagles and marabou storks as biomonitors of environmental health. We wish to report our conclusions in relation to this objective.

## Materials and Methods

African fish eagles were sampled at Lake Mburo, a 6 km long freshwater lake in South Western Uganda (0°. 39' S, 30°. 57' E) situated in a 256 $km^2$ national park, and on Lake Victoria at Entebbe, from Nfo Island (0°. 00' N, 32°. 26' E) to Kisubi Bay (0°. 05' N, 32°. 35' E). Fish eagles were sampled at Lake Mburo in July, August and December 2002 and at Lake Victoria in August 2002 and January 2003. Thirty-three eagles were sampled: ten adults and eight nestlings from Lake Mburo and five adults and ten nestlings from Lake Victoria. Twenty marabou stork nestlings were sampled from a colony located along Nile Avenue, in central Kampala, Uganda (0°. 32' N, 32°. 58' E). Marabou stork nestlings were sampled from twelve nests, in six trees (*Tabebuia pentaphylla*), in one colony, on one day from 0700 till 1800, in January 2003.

*Oreochromis niloticus* were sampled at Lake Mburo and Lake Victoria at Entebbe in the same locations and times that fish eagles were sampled.

Adult fish eagles were captured on water using a fish "snare vest" technique. *Oreochromis niloticus* were fitted with eight to twelve 5 - 6 cm diameter fishing line snares (loops). The free ends of the snares penetrated the body of the fish and were then tied on themselves and the excess line cut. The line was attached to a hand held wooden reel in a boat. The snared fish was then placed in water. Once captured, the eagle was taken to shore and placed in dorsal recumbency. Ten ml of blood were collected from the brachialis vein via a 21 or 23 gauge x 1.9 cm (¾ inch) butterfly catheter (Surflo Winged Infusion Set, Elkton, MD) connected to a 10 ml syringe (Luer Lok Tip Syringe, Becton Dickinson and Company, Rutherford, NJ). The blood was immediately transferred to a 10 ml lithium heparin vacutainer. An additional 4 ml of blood was drawn and placed in a 5 ml EDTA vacutainer (Becton Dickinson, Franklin Lakes, NJ). A drop of whole blood was placed on a commercially prepared paper sample card for molecular sex determination (Avian Biotech International, Tallahassee, FL). Five whole breast feathers were then hand plucked for determination of total mercury concentrations. Body measurements and weights were recorded, blood smears made and the bird banded. Eagles were released from land at the closest point possible to the capture location. Average time from capture to release was 34 minutes (range 20-45 minutes).

African fish eagle and marabou stork nestlings were retrieved for sampling from the nest using professional tree climbing techniques (USDA Forest Service, 1996) with

modifications for tropical tree species and environmental conditions. The main method of tree ascent was using tree climbers (Klein Tools, Chicago, ILL). Eagle and stork nestlings were placed singly into a 40 cm diameter nylon bag (The Taku Tailor, Juneau, Alaska, USA) and lowered to the ground where sampling occurred. Sampling of nestlings was as described for adults with the exception that the volume of blood collected was between 4 to 14 ml depending on body weight. No more than one percent of body weight was collected and often considerably less. Venipuncture site on stork nestlings was the medial metatarsal vein. Average time from removal of the nestling until return was 14.5 minutes (range 7-22) for marabou storks and 32 minutes (range 10-60) for nestling fish eagles.

*Oreochromis niloticus* samples were obtained from local fisherman as close as possible to where eagles were sampled. Fish less than 300-400g and 300-450mm in length were selected. Fish were weighed and their body length recorded. A 100g whole body section sample of fish was dissected just cranial to the dorsal fin and weighed. The sample included skin, muscle, bone and viscera.

There are perhaps no species that would meet all the criteria of an ideal biomonitor. To test the suitability of developing the African fish eagle and marabou stork as biomonitors of environmental pollution the following criteria were established from the work of others (Gragnaniello et al 2001; Burger and Peakall, 1995, State of the Lakes Ecosystem Conference, SOLEC 1998; International Joint Commission. 1985) and our own observations. We believe the list constitutes the ideal for a biomonitoring program utilizing avian species. African fish eagles and marabou storks were subjectively assessed on their ability to meet the following requirements:

1. The population of the species must be large enough such that sampling will not adversely affect the population.
2. The species should be nonmigratory, at least for the part of their life cycle when sampling occurs, so as tissue concentrations of chemicals are an indication of local environmental contamination.
3. The biology of the species has been characterized and changes can be monitored.
4. The species should be large enough so that adequate samples can be obtained to meet analysis requirements.
5. Size, age and sex differences within the species are capable of being documented.
6. Size, age and sex variation in bioaccumulation of the chemical within the species can be documented.
7. The diet of the species can be determined for the environment under examination.
8. Species foraging range must be known, if local point source determinations are required.
9. The diet of the species must be relatively consistent within and between environments under study.
10. The species reproductive cycle must be known, and reproductive success and productivity able to be determined quantitatively.
11. Exposure routes for the chemical in the species concerned need to be documented.
12. An endpoint effect that is to be quantified for the chemical must be demonstrated in the species examined, or a similar species under experimental laboratory conditions.

13. Species must have a specimen that can be sampled, in the correct quantity at a suitable body site, in which there is a correlation between concentrations found and concentrations known to cause a specific endpoint.
14. The specimen chosen for sampling must be able to be stored from the time of sampling until analysis in a manner that will not affect the analysis or results.
15. The species can be sampled cost effectively and with relative ease, in the environment under examination.
16. If bioaccumulation of the chemical is to be studied, the species must occupy an upper trophic level in the food chain.
17. The species should be able to tolerate a range of concentrations of the chemical under examination.
18. Public and regulatory acceptance of the species as a biomonitor and the sampling methods utilized should be established.
19. Knowledge of the chemicals to be examined, their localized usage, their activity and reactions in the environment are required.
20. The species should have a long life span.
21. The species should be monitored over a number of seasons or biological cycles.

## Results

African fish eagles partially meet some of the criteria used to characterize an ideal biomonitor species. In summary, the population is large and secure at the study sites sampled, the species is nonmigratory as adults, the diet is known, the species is long lived, sex and size differences can be accounted for, sufficient volumes of blood and

feathers are available for analysis, the species occupies a high trophic level in the food chain and laboratory toxicity and field studies are available on similar species. In addition sampling could be cost effective once capital equipment is purchased and appropriate and willing collaborative partnerships are established. Criteria that have not been met are the biology of the African fish eagle is not well documented, nor is the reproductive cycle. In addition the diet of the fish eagle may not be consistent between locations.

Similarly to fish eagles, marabou storks meet some of the criteria of a suitable biomonitor. Marabou storks have large, relatively secure populations in Uganda, their biology and reproductive cycle has been recorded, sufficient sample volumes can be obtained, nestlings are relatively easy to sample, a portion of the population is nonmigratory (nestlings) and the species is long lived in the wild. However, adult marabous are mainly migratory, adult birds are very difficult to sample and the diet may not be consistent within and between sites.

## Discussion

### Fish eagle biology and biomonitoring

African fish eagles meet some of the criteria used to characterize an ideal biomonitor species. African fish eagles are tertiary avian predators in lake-based ecosystems and thus occupy a high trophic level in the foodchain (Stewart 1997). Local populations of fish eagles at both study sites appear adequate for sampling purposes. Population counts at both sites have been conducted approximately twice yearly (The East Africa Natural History Society, P.O. Box 27034, Kampala, Uganda) and suggest that

the population is stable. Fish eagles are thought to be nonmigratory as nestlings and as mature adults with an established territory (Brown 1980). Therefore, adults with established territories and nestlings should reflect local concentrations of contamination. One study related observations of productivity in fish eagles on a lake polluted with heavy metals and pesticides and made the observation that the fish eagle is considered to be a useful indicator of pollution in aquatic ecosystems (Mundy & Couto 2000). One of the key factors in making this assessment was the sedentary nature of adult fish eagles.

The biology of the fish eagle at the two sites examined has not been scientifically studied. Scientific studies at other sites are minimal, scattered and not comprehensive. Much of the knowledge of fish eagle biology has been through records of observations, rather than planned studies. Without a sound knowledge of what is normal it is difficult to recognize and assess variation that may be associated with environmental contamination.

**Fish eagle diet and biomonitoring**

Adult fish eagles in this study weighed 2.2-3.6 kg. Nestling fish eagles could be safely sampled at a body weight of 800 grams or above. The lower nestling weight gave enough plasma (3 ml) to complete all tests required. Avian sex in non-ratite species can easily be determined with almost 100% accuracy by examination of chromosomal DNA (Griffiths et al 1998). As DNA is present in all cells, commercial tests are available using blood and feather samples. Once adult plumage has been established at approximately 5 years, it is impossible to age fish eagles (Brown & Cade 1972). Although most studies with feathers have failed to find a relationship between mercury and bird age, Gochfeld et al (1996)

found that mercury concentrations in feathers of laughing gulls (*Larus atricilla*) decreased significantly with age. This highlights the importance of banding eagles so band returns may give some indication of longevity in the wild, which is unknown but calculated to be 19.8 years (Brown and Cade 1972). Thus fish eagles can be sexed, can be aged as nestlings, juveniles or adults and are large enough to obtain sufficient sample volumes for most types of analysis.

African fish eagles feed on a variety of fish with cichlid species such as tilapia (*Oreochromis niloticus*) that occupy the littoral zone comprising 80% of the fish taken (Stewart et al. 1997). *Oreochromis niloticus* were chosen for sampling as it appeared to be the fish eagles main prey item at Lake Mburo and Entebbe. Fish eagles are exposed to both mercury and persistent organic pollutants by dietary exposure, with bioaccummulation and biomagnification increasing contaminant concentration with increasing food chain trophic level. However our observations and anecdotal reports suggest that fish eagle diet may vary between sampling locations and even between individual birds. Our observations of prey remains at nests in Murchison Falls indicate tiger fish (*Hydrocynus forsaklii*), a carnivorous species often occupying surface waters forms a large percentage of the fish eagle diet in this area. In other areas, it is suggested some fish eagles are largely orniphagous (Brown, 1980). If carnivorous avian and fish species were the main prey items, this would mean another trophic level added to the food chain that may result in greater exposure of fish eagles to harmful chemicals through bioaccumulation. Such variation in diet between sites may complicate comparisons of chemical effects on indicator species between sites.

**Fish eagle reproduction and biomonitoring**

Details of the reproductive biology of the fish eagle have been reported and observed by Brown (1980) but not scientifically evaluated. Sumba (1986) examined breeding seasonality in fish eagles at Queen Elizabeth National Park. Sumba concluded that breeding in Queen Elizabeth National Park was non seasonal while Brown (1980) stated "...in some areas fish eagles breed at any time of the year; in others they are more strongly seasonal, though even the breeding season tends to be rather elastic." Our observations tend to agree with this statement. The breeding cycle of fish eagles at both sites in this study has not been characterized nor has reproductive success and productivity been measured over a number of years. Our observations at Lake Mburo suggest breeding at this site may be seasonal. No chicks were observed in nests during December or January. Chicks ranging from approximately one week old to almost fledged were found in nests during late June, July and August. Breeding at Lake Victoria near Entebbe may be less seasonal. Chicks ranging from approximately one week old to almost fledged were found in nests in July and August while chicks from one week to approximately 4 weeks old were found in nests in January and February. Lack of a defined seasonality to the breeding cycle indicates that any monitoring program ideally needs to have the logistical capacity to sample at a numerically low level over extended periods of time. A monitoring program needs to establish breeding patterns of the indicator species at the specific study site, rather than relying on data extrapolated from other areas. Population reproductive measures such as productivity (the number of young per occupied nest for each breeding area) and success (percent of occupied breeding areas

successfully fledging at least one young), as used in studies on bald eagles (Bowerman, 1993) provide useful information only if they are conducted as multiyear analyses.

**Laboratory and field toxicity studies: mercury**

Altered nesting behavior and reproductive failure have been documented in wild common loons (*Gavia immer*) with elevated mercury concentrations (Barr 1986). Laboratory feeding studies on Goshawks (*Accipiter gentiles*) and red-tailed hawks (*Buteo jamaicensis*) with mercury have shown acute death, neurotoxicity and altered nesting behavior (Borg et al 1970; Fimreite 1971). Scheuhammer and Bond (1991) suggest that mercury feather concentrations greater than 20 ppm may be associated with toxic effects. Eisler (1987) states that concentrations above 5ppm fresh weight in sensitive avian species are thought to be associated with adverse affects. However, toxic effects of mercury may vary among bird species (Gouter et al 1998).

**Feathers as a sample for biomonitoring mercury**

Feathers are an excellent, non- invasive tissue to sample for mercury analysis. Feathers contain higher concentrations of mercury than in other body organs (Westermark et al 1975) with approximately 70% of total body mercury found in feathers. (Honda et al 1986). A positive correlation has also been shown between mercury content in feathers and internal tissues (Thompson et al 1991). Mercury in feathers is strongly bonded to keratin disulphide bonds (Crewther 1965) and does not easily degrade therefore archived feathers can be used for analysis. Mercury concentrations are spread evenly throughout the feather, indicating endogenous incorporation methods, rather than atmospheric

deposition (Hahn et al 1993). Feathers are a major excretory pathway for body mercury during molt. Differing mercury concentrations are found depending on the order the sampled feather has in the molting sequence. This has not been consistently shown to occur in all individuals within a population. Body contour feathers are the feather type that shows the least variation in mercury content (Furness et al 1986). The methodology of feather sampling used in this study was based on these findings.

**Persistent organic pollutants, fish eagles and biomonitoring**

The effects of the pollutants tested for in this study have been well documented in laboratory toxicity studies and field reports on analogous raptor species, including the bald eagle (*Haliaeetus leucocephalus*) (Bowerman 1993; Lincer 1975). Eggshell thinning has been used as an indicator of toxic effects of DDE in avian species with 18 to 20% being related to declines in populations (Blus et al 1996). Egg concentrations of DDT have been positively correlated with blood concentrations. Sampling of blood can therefore give an indirect indication of egg concentrations of the contaminant (Henny and Meeker 1981). Determination of the lipid content of the plasma is important in that a study by Elliot et al (1998) found a positive correlation between mean plasma lipid in nestlings and productivity (Elliot et al 1998). Plasma concentrations of POPs need to be analyzed in relation to population reproductive success and productivity to assess the effect of the chemical. This was not possible within the limited temporal period of this project.

**Fish eagle sampling methodology and biomonitoring**

Fish eagle nestlings are difficult to sample. Nests are located in tree species that are challenging to climb and require professional tree climbing equipment. Nests are frequently located on thin branches that require ingenuity and resourcefulness on the part of the climber to reach. Occasionally, other complications such as wasps, ants and the tree being too tall for the equipment were encountered. The majority of trees at both sites were accessible only by water. Climbing the nesting tree was the only accurate way to determine whether a nest contained chicks. However, the nests were generally very visible and within a reasonable distance of each other, thus facilitating logistics. The use of a fish snare vest may be an effective method to catch adult African fish eagles but the success rate is very site dependent due to a host of multi-factorial local site conditions.

**Marabou storks as biomonitors**

Marabou storks provide an interesting comparison to fish eagles when assessing the species potential as biomonitors. In contrast to the fish eagle, the biology and reproductive cycles of the marabou stork in the study area have been well-documented (Pomeroy 1977, 1978). Marabou storks have a well-defined breeding season and are communal nesters. Multiple nests can occur in a single tree. Marabou storks have an extremely long fledging period, with first flights out of the nest occurring at 110-115 days (Hancock et al 1992). Marabou stork nestlings, when they are unable to fly, are relatively easy to sample. Nesting trees generally present less of an access challenge than fish eagle nests. Marabou parents can vigorously defend their young but, like fish eagles, seem minimally affected by the sampling process. Once the location of a colony is

known, multiple nests in a single tree can facilitate rapid sampling of individuals. Nesting trees are often used for many years making estimations of population reproductive parameters easier to assess. Marabou storks first breed at 6-7 years and may live up to 25 years. Marabou storks are large, conspicuous birds, with average adult weights recorded being 5.66 kg for females and 7.06 kg for males (Pomeroy 1977). All these factors make marabou storks an eminently suitable biomonitor species. However, marabous have adapted to human activities by adopting a scavenging lifestyle. The diet of the birds in Kampala probably includes almost anything organic, such as garbage, fish remains, abattoir refuse and a large amount of vegetable matter (Brown, et al 1982). However, during the period of nestling growth increased amounts of protein are taken in the form of fish, frogs and rodents. The cosmopolitan nature of the urban marabou's diet makes assessing sources of dietary exposure and establishing bioaccumulation in the food chain difficult. In addition, marabous undertake seasonal, mainly north south migrations in Uganda, coinciding with rainfall seasonality (Pomeroy 1977). Although marabou storks are present year round in Kampala, the majority of the population is migratory. Leg bands to identify non-migratory birds would be difficult due to the bands being obscured by urate wastes coating the legs of this species. Other methods of permanent adult identification have proved difficult and challenging (Pomeroy 1975). Concentrations of chemical residues in adult marabou stork tissues may not be wholly due to local exposure. This problem is partially overcome by sampling nestlings. In addition, adult marabou storks are very difficult to capture. Cannon nets, drop nets (baited for many months to allow habituation) and use of oral anesthetics may be the only methods to achieve multiple captures. All these methods have their disadvantages. Oral

anesthetics have been tried previously with success but also caused a number of mortalities (Pomeroy and Woodford 1977). Our study unsuccessfully attempted capture with drop nets and hand held nets. Interestingly, Nyangababo (2003) was able to capture greater than 30 birds by using a piece of meat or fish as bait then hand catch the bird. We tried and failed with this method at many sites as marabous are extremely cautious and quickly learn to differentiate between a human bypasser and one attempting to capture them. Marabou storks also have the disadvantage as a biomonitor that laboratory based avian heavy metal toxicity trials have mostly been conducted in raptors and domestic avian species, with no known laboratory studies on stork species. With rapid increases in motor vehicle numbers and usage and poor quality grades of fuels being used in Uganda (Uganda Bureau of Statistics, 2002) the marabou stork could be utilized in well-designed and reported field research to monitor pollutants such as lead, cadmium and copper. The marabou stork could also be used to establish a pathology database, as many injured birds have to be euthanased each year. A number of studies in other avian species have assessed the use of feathers as biomonitors of atmospheric pollutants that have a component derived from exogenous deposition (rather than dietary exposure alone), such as atmospheric lead (Dauwe et al 2002; Wren et al 1994; Nyagababo 2003).

## Conclusion

We conclude that African fish eagles could be valuable monitors of selected environmental pollutants if the following preconditions are met:

1. Any study should be a multiyear analysis that requires a minimum low-grade constant surveillance component.

2. Any study should initially address the issue of more completely characterizing the biology of the fish eagle, with particular emphasis on reproduction.

3. Preliminary research should address what contaminants are of most concern in Uganda and can the fish eagle serve as an appropriate monitor specifically for these pollutants.

4. Research programs should be designed to collect as much peripheral information as possible (e.g. parasite loads and species, serological exposure to various avian pathogens) and establish linkages with other investigators that can utilize this information. This not only increases the physiological database for the species but maximizes the use of field resources and project budgets.

5. Adult capture techniques should be refined so they work consistently at multiple study sites.

6. Prey item selectivity for fish eagles and marabou storks needs further characterization by prey remain studies at multiple study sites.

7. Appropriate collaborative partnerships with individuals, institutions and regulatory authorities within Uganda should be developed. Resource allocation to provide for local personnel development and training should be established with the goal of devolution of management responsibilities to local personnel.

One way to achieve some of these goals may be integration with other field research projects such that resources and personnel are shared.

Marabou storks could be developed as a biomonitor species with the precondition that development of any research program recognize the advantages and disadvantages discussed above and that the research is designed accordingly.

An area of research complimentary to residue analysis would be examination of temporal and spatial dietary shifts in marabou storks or fish eagles utilizing stable radio-isotopic analysis (Harding & Stevens 2001). The shifts could then be correlated with changes in many parameters, such as population density, chemical use or habitat degradation. Water quality analysis in aquatic biomonitoring programs would also be useful. This would enhance knowledge gained from residue analysis alone.

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# Chapter 8

# Project Conclusions and Recommendations for Future Research

## Conclusions

In the research project we demonstrated that:

- There is no significant difference in persistent organic pollutant and mercury concentrations in tissues of African Fish Eagles (*Haliaeetus vocifer*) and tilapia (*Oreochromis niloticus*) from Lake Victoria near Entebbe compared to those from Lake Mburo.

We found a significant ($p \leq 0.05$) difference between concentrations of mercury in African fish eagle feathers as well as whole body cross section samples of *Oreochromis niloticus* collected from Lake Mburo and Lake Victoria near Entebbe. The mercury concentrations in African fish eagle feathers and whole body section samples of *Oreochromis niloticus* from Lake Victoria were consistently higher than those from Lake Mburo. Feather mercury concentrations in fish eagles from Lake Victoria were still significantly higher ($p = 0.0012$) than concentrations from Lake Mburo when multivariable analysis was performed to account for gender and age (nestlings or adults). Further to this we found no significant ($p \leq 0.05$) difference in feather concentrations of mercury in marabou stork nestlings from Kampala and those from African fish eagles from Lake Victoria near Entebbe. We therefore find the alternative hypothesis proved (at the 0.05 level of significance) and reject the null hypothesis in relation to mercury concentrations. The higher mercury levels at Entebbe and Kampala are most likely the result of anthropogenic emissions, particularly the use of fossil fuels as well as the burning of garbage. African fish eagle plasma, marabou stork plasma and whole body section samples from *Oreochromis niloticus* did not contain the following chemicals at

the 0.001 ppm limit of detection: aldrin, DDT, α-HCH, dieldrin, endrin, heptachlor and their metabolites, β-HCH, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, heptachlor epoxide and lindane and nonachlor. Total PCBs were not detected in African fish eagle plasma, marabou stork plasma or whole body section samples from *Oreochromis niloticus* at the 0.003 ppm limit of detection. Five adult eagles from Entebbe had 4,4'-DDE detectable in plasma, one at 0.005ppm, one at 0.003ppm, two at 0.002ppm and one at 0.001ppm wet weight. Five *Oreochromis niloticus* samples from Entebbe contained 4,4'-DDE concentrations of 0.001, 0.001, 0.002, 0.003 and 0.003 ppm wet weight. At the 0.001 ppm limit of detection 4,4'-DDE was not detected in nestling marabou stork plasma samples from Kampala. We therefore find the alternative hypothesis proved and reject the null hypothesis (at the 0.05 level of significance) in relation to 4,4'-DDE but accept the null hypothesis in relation to all other sampled pesticides and PCBs.

Important issues related to the hypothesis that were addressed by this research were:

- Whether concentrations of persistent organic pollutants and mercury are likely to be major contributing factors to the population dynamics of the African fish eagle (*Haliaeetus vocifer*) at Lake Victoria near Entebbe or at Lake Mburo.

Reproductive parameters were not quantified for the African fish eagle populations as this would require multiyear analysis. However the concentrations of mercury and 4,4'-DDE found in African fish eagle feathers and plasma respectively are below concentrations known to cause toxic and reproductive change in a variety of avian

species under field and laboratory conditions. Our observations of African fish eagle nest productivity, breeding success and population structure lend support to this statement. However with an adult sample size of five birds from Entebbe, the statement should be interpreted cautiously in relation to our results. To definitively investigate the relationship between chlorinated hydrocarbons and productivity in African fish eagles a larger adult sample size, continued nestling sampling and long-term population reproductive analysis is required. Also required is greater knowledge of the clinical and sub clinical effects of mercury on piscivorous avian raptorial species, such as the African fish eagle and Ciconiini, such as the marabou stork.

- Whether African fish eagles (*Haliaeetus vocifer*) and marabou storks (*Leptoptilos crumeniferus*) can be utilized as successful biomonitors of mercury and persistent organic pollutants.

We found that African fish eagles and marabou storks met most of the criteria of a suitable biomonitor species as stated in this thesis. However, both species failed to meet critical criteria: the biology of the African fish eagle is poorly understood, the reproductive cycle has not been characterized, the reproductive cycle and diet may show site variation, nestlings are difficult to sample and the success of methods utilized to catch adults appears dependant on multi-factorial local site conditions. The marabou stork is migratory, has a varied diet often based on scavenging off human waste and the adult birds are extremely difficult to capture for sampling. With development and research most of these impediments to meeting the stated criteria could be met. We

conclude that both species show promise as biomonitors of chemical and heavy metal contaminants but require more development. In the case of the fish eagle, further research investigating species biology and reproduction is required. For the marabou stork, research projects require a design and purpose that can achieve project objectives within the limitations of species-specific characteristics (e.g. the species is migratory, varied diet).

## Recommendations

Relative political and economic stability in Uganda for the last 16 years has led to increased foreign aid and investment, industrialization, urbanization and centralization of government. The fact the human population of Kampala increased from 774,241 in 1991 to a preliminary figure of 1,208,544 in 2001 and use of diesel fuel (inferior grades and quality) in Uganda increased from 125,621 in 1997 to 207,183 cubic metres in 2001 (Uganda Bureau of Statistics, 2002) lend support to these assertions. There are a number of regulatory authorities charged with the management of Uganda's environment. To be proactive in their respective environmental mandates these authorities require baseline data to establish and monitor trends in environmental indices. The use of mammals, birds and reptiles as biomonitors can form part of this process. With this in mind the following recommendations are made in relation to this research project:

In relation to the concentrations of mercury and persistent organic pollutants found in African fish eagles, marabou storks and *Oreochromis niloticus* fish, no remedial management actions are advised.

In relation to the development and use of African eagles and marabou storks as biomonitors the following is advised:

1. Develop a multiyear research program to address the following relating to fish eagle biology-
    - Characterization of the reproductive cycle of fish eagles at multiple sites in Uganda.
    - Characterization of productivity and seasonality of fish eagle reproduction at multiple sites in Uganda.
    - Characterization of the diet of fish eagles at multiple sites in Uganda.
    - Determine what happens to juvenile fish eagles between fledging and establishing an adult territory. Continue banding all eagles caught for scientific research.
    - Quantify water quality indices at fish eagle study sites and assess the associations between water quality and fish eagle reproductive parameters.

2. Assess the ability of fish eagles to act as biomonitors of other pollutants, such as lead.

3. Develop banding techniques to assess whether a significant proportion of the Kampala marabou stork population is resident or migratory.

4. Resample the previously sampled avian and fish populations for mercury in five to seven years or more often if possible as per the sampling and analytical protocols of this research project. Particularly investigate the levels of 4,4'-DDE and mercury present in the Entebbe region of Lake Victoria.

5. Investigate the use of analytical techniques complementary to residue analysis such as stable radio-isotopic assays. Utilize this technique to answer specific questions relating to fish eagle and marabou stork biology e.g. examine trophic levels of prey of fish eagles at different sites to determine differences in diet composition.

6. Examine marabou stork nestlings in urban Kampala and a number of rural nests for differences in concentrations of heavy metals from exogenous and endogenous exposure routes.

7. Investigate alternative fish eagle capture techniques and refine existing ones. Techniques that exploit the fish eagle's extreme territoriality require investigation.

8. Develop techniques for the capture of adult marabou storks.

9. Collect pathology specimens from dead birds for toxicological analysis

10. Establish appropriate collaborative links between ex-situ researchers and in-situ individuals, private and government organizations.

11. Train appropriate Ugandan nationals in all sampling techniques and in management of fieldwork logistics and administration. Ensure all field programs are developed with realistic timeframes and adequate mandates for field personnel to achieve project objectives.

12. All projects developed should be multiyear research programs with a continuous low-level in-situ presence by the research team.

13. Develop flexible and realistic monitoring programs that can evolve with the changing needs of Uganda, its people and environment. Ensure research empowers Ugandans to make realistic and knowledgeable decisions regarding their future.

Serious consideration should be given to developing a battery of biomonitor species for assessing environmental change in aquatic ecosystems in Uganda. Recommended species are:

The African fish eagle (*Haliaeetus vocifer*)

The spot necked otter (*Lutra maculicollis*)

The Nile crocodile (*Crocodylus niloticus*)

Crocodiles have been used to assess the effects of persistent organic pollutants and heavy metals in Australia as have alligators (*Alligator mississpiensis*) in Florida lakes (Jefree et al 2001). Crocodiles however, are limited to certain waterways within Uganda. River otters (*Lontra canadiensis*) have been studied in relation to environmental PCB concentrations in North America (Wren 1991). The spot necked otter is common throughout most large waterways in Uganda. Both species meet many of the criteria of suitable biomonitors as stated in this thesis. The advantages of constructing multi- species biomonitoring programs in countries such as Uganda are many and varied and include:

1. maximization of economic, logistical, management and field resources.

2. reduction of the effects of variation and nonpredictability between years in relation to the timing of biological events within a species. This is achieved by allowing sampling of at least one species to occur in all seasons.

3. increased accuracy of assessments by reducing the impact of confounding effects that may occur in a single species monitoring program.

4. establishes a bank of indicator species which may be called upon to assess different aspects of environmental change.

5. establishes a database and tissue bank specific for African species.

As with any biomonitoring program, utilization of a multi species approach should occur in two phases: development of the biomonitor then utilization of the biomonitor.

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