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Source: Journal of Wildlife Diseases, 40(3): 501-514

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-40.3.501

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## MERCURY AND PERSISTENT ORGANIC POLLUTANT CONCENTRATIONS IN AFRICAN FISH EAGLES, MARABOU STORKS, AND NILE TILAPIA IN UGANDA

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ABSTRACT: The purpose of this research was to evaluate persistent organic pollutant (POP) and mercury concentrations in tissues of African fish eagles (Haliaeetus vocifer) and Nile tilapia (Oreochromis niloticus) from Lake Victoria near Entebbe and Lake Mburo, Uganda. Marabou stork (Leptoptilos crumeniferus) nestlings from urban Kampala (40 km from Entebbe) also were sampled for POPs and mercury. Total mercury was measured in the breast feathers of eight nestling and 10 adult African fish eagles from Lake Mburo, 10 nestling and five adult African fish eagles from Lake Victoria near Entebbe, and 20 nestling marabou storks from Kampala from June 2002 through January 2003. Mercury concentrations in all samples were below levels associated with adverse effects in similar species. Mercury concentrations were significantly higher in eagle adults and nestlings from Entebbe than in adults and nestlings from Lake Mburo ( $P \leq 0.05$ ). No significant differences ( $P \ge 0.05$ ) were found in mercury concentrations between sexes or between the entire fish eagle population sampled at Entebbe and marabou stork nestlings sampled at nearby Kampala. Plasma samples from the same birds were analyzed for 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, aldrin, hexachlorocyclohexane ( $\alpha$ -HCH), dieldrin, endrin, heptachlor and their metabolites, as well as total polychlorinated biphenyls (PCBs). Nile tilapia whole-body cross sections collected from Lake Mburo (n=3) and Lake Victoria near Entebbe (n=8) also were analyzed for these POPs and mercury. No samples contained POPs or PCBs at the limits of detection except for 4,4'-1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene in five adult eagle plasma samples  $(0.0026\pm0.0015 \text{ ppm wet weight})$  and five Nile tilapia samples  $(0.002\pm0.001 \text{ ppm wet weight})$ from Entebbe.

Key words: African fish eagle, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, Haliaeetus vocifer, Leptoptilos crumeniferus, marabou stork, mercury, Nile tilapia, Oreochromis niloticus, Uganda.

#### INTRODUCTION

The African fish eagle (*Haliaeetus voci-fer*) is a widespread, often locally abundant, highly territorial tertiary avian predator in lake-based food chains throughout sub-Saharan Africa (Brown, 1980). Seasonality of breeding appears to vary greatly in tropical parts of the fish eagle's range (Brown, 1980). In western Uganda, breeding occurs year-round with a peak from August to November (Sumba, 1986). Eggs were observed in nests at Lake Mburo in July and August and at Entebbe in August,

December, and January. In dense populations, breeding may not occur each year and timing of breeding may vary between years. Fish eagles fledge at approximately 72–76 days (Brown, 1980; Sumba, 1988). First feathers can be seen through down at 24 days and the chick is completely feathered with no down visible by day 50 (Steyn, 1959; Brown, 1980). The fish eagle appears to molt continuously and sequentially and attains adult plumage by 5 yr of age (Brown, 1980). The African fish eagle is very sensitive to the effects of persistent organic pollutants (POPs) (Douthwaite, 1992). This characteristic is shared by other piscivorous tertiary avian predators such as the bald eagle (*Haliaeetus leucocephalus*) in North America (Bowerman et al., 2003) and the white-tailed sea eagle (*Haliaeetius albicella*) in Sweden (Helander et al., 1982).

The marabou stork (Leptoptilos crumeniferus) is resident in tropical Africa and is common to abundant over much of its range (Hancock et al., 1992). Most of Uganda has two dry seasons, usually from December to March and from July through September (Pomeroy, 1978). Most eggs at Kampala are laid in November or December, with incubation lasting a month and chicks often remaining in the nest for 4 mo (Pomeroy, 1973). Marabou storks molt their feathers sequentially, continuously, and slowly, except when gaining their first set of feathers, which takes 3 mo, with these feathers being retained for a further 8 mo after fledging. The duration of molt varies with type of feather, and is 1 yr for primaries and 12-15 mo for secondaries (Pomeroy, 1977). The marabou stork has responded to increasing urbanization of human populations by adopting an omnivorous, scavenging lifestyle in urban areas (Pomeroy, 1977). 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 tendency to populate urban areas with resulting exposure to potentially greater levels of pollutants may make these populations useful as bioindicators of a range of substances, including heavy metals such as mercury. The African fish eagle and the marabou stork have not been explored as environmental sentinels 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 and garbage, which commonly occurs in the catchment area along the shore of Lake Victoria near Entebbe and Kampala (National Environment Management Authority, 2000; Matagi, 2002). Mercury released through these activities is converted to methylmercury in water sediments, which are taken up by fish and eventually piscivorous birds. Methylmercury pollution is of most concern because it is biomagnified along the food chain (Heinz, 1996). 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 (Finley and Stendell, 1978; Burger, 1993). Apart from fish species, few studies have examined mercury concentrations in wildlife in Uganda.

The effects of 1,1,1-trichloro-2,2-bis(4chlorophenyl)ethane (DDT) on avian species have been well documented (Lincer, 1975). The DDT metabolite, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (DDE), causes eggshell thinning and decreased reproductive success in free-ranging raptors (Wiemeyer et al., 1984) and in laboratory studies on captive raptors (Lincer, 1975). Studies on POPs 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; Douthwaite, 1992). Of 1,829 records of organochlorine residues in African fauna reviewed by Wiktelius and Edwards (1997), birds comprised 60% of all records, with DDT and its metabolites being the most commonly reported residues. By far the greatest number of reports are from Zimbabwe (Douthwaite, 1992; Tannock et al., 1983; Hartley and Douthwaite, 1994), Kenya (Lincer et al., 1981; Kairu, 1994), and South Africa (Davies and Randall, 1989) between 1975 and 1985. Wiktelius and Edwards (1997) stated that the temporal and regional distribution of reports may have been due to increased organochlorine usage in these countries at the time, or alternatively, better research, reporting, and infrastructural capacity than in other African countries where usage might also have been significant. Ejobi et al. (1996a,b) quoted 80 tonnes of DDT per year being used mainly for cotton growing and indoor mosquito control in Uganda. Dieldrin was used at the rate of 392 tonnes per year for banana weevils and termites, whereas 30 tonnes also were used for tsetse fly control. Lindane, aldrin, hexachlorobenzene, campheclor, chlordane, and heptachlor were also used. However, the lack of accurate data reporting makes it difficult to determine the actual usage of POPs in many African countries, including Uganda (Wiktelius and Edwards, 1997).

The human population of Kampala increased from 774,241 in 1991 to approximately 1,208,544 in 2001. Some associated environmental problems in the Kampala-Entebbe region include inadequate solid waste collection, increasing traffic and industrial pollution, wetland drainage, biomass burning, soil erosion, deforestation, and unsustainable urban agricultural practices (National Environment Management Authority, 2000; Matagi, 2002). With increased political and economic stability and a rapidly expanding urbanized population, monitoring of pollution in Uganda is needed. The piscivorous bald eagle has been used to monitor organochlorine compounds in North American Great Lakes water (International Joint Commission, 1985; State of the Lakes Ecosystem Conference, 1998). Given the African fish eagle's widespread distribution and relative abundance, it may also be a valuable indicator species of heavy metals and POPs in the sub-Saharan lake-based ecosystems of Africa, such as Lake Victoria.

The objectives of the research were to determine concentrations of POPs and total mercury in African fish eagles at a site with observed anthropogenic disturbance

(Lake Victoria near Entebbe) and at a site with minimal observed human impact (Lake Mburo). Persistent organic pollutants and total mercury were determined in whole-body cross sections of Nile tilapia (Oreochromis niloticus), a fish eagle prey item. Fish eagles feed on a variety of fish with cichlid species (including the mostly detritivorous Nile tilapia) that occupy the littoral zone comprising 80% of the fish taken (Stewart et al., 1997; Campbell et al., 2003). Because fish are a primary source protein for human populations in this region; sampling of fish also may indicate potential human exposure to these pollutants. Marabou stork nestlings were sampled in Kampala to determine concentrations of these pollutants in this common avian species.

#### MATERIALS AND METHODS

The study sites and techniques for collection of samples have been described elsewhere (Hollamby et al., 2004). Thirty-three eagles were sampled, including 10 adults and eight nestlings from Lake Mburo and five adults and 10 nestlings from Lake Victoria. Five whole breast feathers were hand plucked for determination of total mercury concentrations.

The most common tree species utilized for nesting were Acacia sieberiana (83%) at Lake Mburo (n=13) and a mixture of species including mvule (Melicia excelsa) (26%) at Lake Victoria near Entebbe (n=19). Average nesting tree heights were 11.9±3.6 m and 29.6±7.0 m and average nest heights were 9.7±3.9 m and 22.9±5.8 m for Lake Mburo and Lake Victoria near Entebbe, respectively. The average diameters at breast height of trees were 65±14 cm and 145±67 cm for each site as above, respectively. Nile tilapia were sampled at Lake Mburo (n=3) and Lake Victoria at Entebbe (n=8) in the same locations and times that fish eagles were sampled.

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 12 nests in six white cedar trees (*Tabebuia pentaphylla*), in one colony, on 1 day from 7:00 AM until 6:00 PM in January 2003. The trees averaged 5 m in height with nests usually located in the uppermost branches.

Average time from removal of the nestling until return was 14.5 min (range 7–22 min) for marabou storks and 32 min (range 10–60 min) for fish eagle nestlings. Fish eagle nestling weights were 0.50–2.45 kg and marabou stork nestling weights were 1.65–7.60 kg.

Blood samples were placed in a chilled cooler after collection. Time of sampling to separation of red blood cells from plasma and subsequent storage in liquid nitrogen was 3.5 hr (range 2–9 hr) for eagles and 8 hr (range 4–16 hr) for marabou storks. Samples were centrifuged for 10 min (Vulcon Mobilespin PS126-6, Vulcon Technologies, Grandview, Missouri, USA) and plasma was removed and frozen as described previously (Hollamby et al., 2004). Plasma samples were transported to the Diagnostic Center for Population and Animal Health (DCPAH) at Michigan State University's Veterinary Medical Center (East Lansing, Michigan, USA) and stored at -80 C until analyzed within 2 mo of sampling for all avian samples.

Nile tilapia were purchased from local fisherman as close as possible to where eagles were sampled. Fish were weighed and their body lengths were recorded. Mean lengths and weights for fish from Lake Mburo and Lake Victoria were 205 g and 217 mm and 307 g and 569 mm, respectively. A 100-g whole-body cross-section sample of fish including skin, muscle, bone, and viscera was dissected 1 cm cranial to the dorsal fin and weighed. Samples were placed in freezer bags and transferred to a vapor shipper–liquid nitrogen tank for transport to DCPAH.

All analyses were performed at the DCPAH toxicology laboratory. Feathers for total mercury analysis were washed three times in acetone (chromatography grade, Burdick & Jackson, Muskegon, Michigan), and three times in ultrapure water (four-bowl MilliQ system, Millipore, Bedford, Massachusetts, USA), and one additional time in acetone. Feathers were dried overnight in a fume hood and then weighed. Feathers were cut into several small pieces, and the calamus was discarded. Feather pieces were then placed in sealed 30-ml Teflon® vessels (Savillex, Minnetonka, Minnesota, USA) in 2 ml of concentrated nitric acid (Instra-analyzed grade, J.T. Baker Inc., Phillipsburg, New Jersey, USA) and digested at 95 C overnight. The samples were transferred to a 10-ml volumetric flask, mixed with 100 µg of yttrium (JMC Specpure ICP/DCP Analytical Standards, Johnson-Matthey/Aesar, Ward Hill, Massachusetts) as internal standard, and diluted to volume (10 ml). An aliquot of the sample was then taken from the 10-ml volumetric flask and diluted to fit the standard curve of 25-500 parts per trillion (ppt). Samples were analyzed by cold vapor atomic absorption spectrophotometry at 253.7 nm (LCD mercury monitor 3200,

Thermo Separation Products, Riviera Beach, Florida, USA). Accuracy was monitored by concurrent analysis of procedural blanks (in triplicate) and mussell tissue (SRM 2976, National Institute of Standards and Technology, Gaithersburg, Maryland, USA) with mercury certified at  $\overline{61}$  µg/kg dry mass basis. Fish samples were cut into small pieces then snap frozen for 60 sec in liquid nitrogen and milled in an AIO analytical mill (Janke & Kunkel, IKA Labortechnik, Germany). One gram of powdered fish was added to 2 ml of 2% nitric acid for digestion. The digest was then brought to volume (100 ml) with 7% hydrochloric acid. Further analysis followed the procedure outlined above for feathers.

Tissues (serum and fish) analyzed for chlorinated pesticides (DDT, aldrin, hexachlorocyclohexane [a-HCH], dieldrin, endrin, heptachlor, and their metabolites, β-HCH, 2,4'-dichlorodiphenyldichloroethane [2,4'-DDD], 4,4'-DDD, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT, heptachlor epoxide, lindane, and nonachlor) and polychlorinated biphenyls (PCBs) were extracted and purified by the procedure described by Price et al. (1986). Briefly, fish samples were cut into small pieces then snap frozen for 60 sec in liquid nitrogen and milled in an AIO analytical mill. Ten grams of ground fish sample was then mixed and ground with 75–100 g of sodium sulfate. The ground sample was transferred into a 400-ml beaker, to which 100 ml of mixed solvent (ethyl ether:petroleum ether, 1:1) was added. The beaker was covered loosely with foil and set in a warm-water bath at 60 C for 5 min. The ether solution was then decanted through Na<sub>2</sub>SO<sub>4</sub> into a weighed 250ml beaker. The process of ether extraction was repeated two more times, yielding a total extraction volume of 300 ml. The combined extract was set over a warm-water bath to dry. The dry extract was subsequently dissolved in hexane and 1 ml of this solution was subjected to a Silica Gel 60 column (5 g of Silica Gel 60, 9-mm column inner diameter [Sigma-Aldrich Corporation, St. Louis, Missouri]) clean-up. A blank tissue (10 g of liver) and a spiked sample (10 g of liver, spiked at 0.1 parts per million (ppm), for chlorinated pesticides and 0.2 ppm for Arochlor 1260) also were extracted with each set of extracts.

For serum or plasma, 2 ml of serum was added to a  $16 \times 125$ -mm screw capped tube and 6 ml of hexane:acetone (9:1) was added and vortexed for 30 sec. The sample was then centrifuged at 2,500 rpm for 10 min. The organic layer (upper layer) was transferred into a 50-ml conical test tube. This process of extraction was repeated twice. The combined organic extract was dried under nitrogen. The pellet was reconstituted with 1 ml of hexane and subsequently subjected to a Silica Gel 60 column (5 g of Silica Gel 60, 9-mm column inner diameter) clean-up. A blank serum and a serum spike (PCBs at 1.0 ppm and chlorinated pesticides at 0.1 ppm) also were extracted with each set of extracts. The following reagents were used to prepare the standard curve: Organochlorine Pesticides Mix containing 20 analytes at 2,000 µg/ml each in hexane:toluene (50:50; 47426-U, Supelco, Bellefonte, Pennsylvania, USA), cisnonachlor and trans-nonachlor at 100 µg/ml in methanol for each (7130 and 7851, respectively, Crescent Chemical Company, Islandia, New York, USA), and Arochlor PCBs (Arochlor 1221, 1232, 1242, 1248, 1254, 1260, and 1262) at 1,000 µg/ml each in isooctane (44809, Supelco, Bellefonte, Pennsylvania, USA). Standard solutions were prepared by progressive dilution of the above standard solutions. Linear standard curves at 0.002-0.5 ppm for pesticides and 0.05-2.0 ppm for PCB (Arochlor 1260) were used for quantification. Concentrations were determined by gas chromatography (GC) with electron capture detection (Varian 3400 gas chromatograph, Varian Instruments, Walnut Creek, California, USA). The quantification limits for serum were 0.002 ppm for pesticides and 0.05 ppm for PCBs (Arochlor 1260). The quantification limits for fish samples were 0.001 ppm for pesticides and 0.02 ppm for PCBs (Arochlor 1260). The recovery rates were 70-100% for pesticides and 85-90% for PCBs (Arochlor 1260). For quality control, solutions of known concentrations of pesticides were injected at the beginning and at the end of each set of GC runs. In addition, a blank control sample and a spiked sample were run with each set of extracts.

Analysis of variance (ANOVA) was performed on the data to assess the association between site, age (nestling or adult), sex, and feather mercury concentrations in fish eagles and between sex and mercury concentrations in marabou storks (SAS PROC ANOVA for categorical risk factors and SAS PROC GLM for continuous risk factors, SAS 8.2, SAS Inc., Cary, North Carolina, USA). Analysis of variance also was used to assess the associations between site, body weight, and total body mercury burdens in Nile tilapia. These analyses were conducted at the univariate level (only one risk factor at a time) and, where appropriate, at the multivariate level. Multivariate analyses were conducted to adjust the effect of selected risk factors simultaneously. The level of significance was set at P=0.05. Statistical analysis was not performed on the POP results because of the small number of positive samples. Descriptive statistics were done by using Excel

(Microsoft Excel, Microsoft Office 2000 Professional, Microsoft Corporation, Redmond, Washington, USA). Descriptive statistics are emphasized because of 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 et al., 2002).

#### RESULTS

Mercury concentrations in fish eagle feathers from Entebbe were significantly higher than concentrations in eagle feathers from Lake Mburo ( $P \leq 0.05$ ). No significant differences ( $P \ge 0.05$ ) were found in mercury concentrations in breast feathers of fish eagles based on sex or age (Table 1). No significant difference ( $P \ge 0.05$ ) was found in mercury concentrations between the fish eagle populations from Entebbe and the nestling marabou stork population from Kampala (Table 1). Mean whole-body cross-section mercury concentrations in Nile tilapia were  $0.0055 \pm 0.0025$  ppm wet weight (n=8) from Entebbe and 0.003±0.00 ppm (n=3) from Lake Mburo (Table 1). Positive correlations were found between body weight and feather mercury concentrations for fish eagle nestlings at Entebbe (Fig. 1) and marabou stork nestlings at Kampala (Fig. 2).

African fish eagle plasma, marabou stork plasma, and whole-body cross-section samples from Nile tilapia were negative for the following chemicals at the 0.001 ppm limit of detection: aldrin, DDT,  $\alpha$ -HCH, dieldrin, endrin, heptachlor and their metabolites, β-HCH, 2,4'-DDD, 4,4'-DDD, 2,4'-DDT, 4,4'-DDT, heptachlor epoxide, lindane, and nonachlor. African fish eagle plasma, marabou stork plasma, or wholebody cross-section samples from Nile tilapia were negative for total PCBs at the detection limit of 0.003 ppm. Five adult eagles from Entebbe had 4,4'-DDE detectable in plasma, one at 0.005 ppm (male), one at 0.003 ppm (female), two at 0.002 ppm (males), and one at 0.001ppm (female) wet weight. These data were not corrected for plasma lipid content. Five

	Mean	Median	$SD^{a}$	Minimum	Maximum
Fish eagles					
Adult LM <sup>b</sup> (10)	0.36	0.31	0.12	0.23	0.55
Adult LV <sup>b</sup> (5)	1.06	0.56	0.79	0.47	2.30
Adult male LM (6)	0.37	0.36	0.12	0.31	0.23
Adult female LM (4)	0.30	0.26	0.14	0.23	0.54
Adult male LV (3)	0.10	1.39	0.10	0.31	2.30
Adult female LV (2)	0.51	0.51	0.05	0.47	0.55
Adult total (15)	0.60	0.45	0.55	0.23	2.30
Nestling LM (8)	0.19	0.17	0.10	0.10	0.40
Nestling LV (10)	0.67	0.67	0.23	0.31	1.03
Nestling total $(18)$	0.45	0.42	0.31	0.10	1.03
Total (33)	0.52	0.45	0.43	0.10	2.30
Marabou storks					
Male (12)	0.84	0.39	0.81	0.38	1.50
Female (9)	0.72	0.60	0.47	0.24	2.00
Total (21)	0.81	0.50	0.67	0.24	2.00
Nile tilapia					
Fish LV (8)	0.01	0.01	0.01	0.01	0.01
Fish LM (3)	0.01	0.01	0.01	0.01	0.01

TABLE 1. Total mercury concentrations (parts per million [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 cross-section samples of Nile tilapia (*Oreochromis niloticus*) (ppm wet weight) from Uganda. Sample sizes are given in parentheses.<sup>a</sup>

 $^{a}$  SD = standard deviation.

<sup>b</sup> LM = Lake Mburo; LV = Lake Victoria near Entebbe.

Nile tilapia samples from Entebbe contained 4,4'-DDE concentrations with a mean concentration of  $0.002\pm0.001$  ppm wet weight. All nestling marabou stork plasma samples were negative for 4,4'-DDE at 0.001 ppm limit of detection.

#### DISCUSSION

#### Mercury

Feather mercury levels in this study were lower than a reported mean of 5.6 ppm in 180 avian studies (Burger, 1993). Eisler (1987) stated that feather mercury concentrations above 5 ppm fresh weight are thought to be associated with adverse affects in sensitive avian species. However, his study was not based on piscivorous birds but rather on grain-eating pheasants and mallards. Gariboldi et al. (2001) pointed out the need to relate tissue concentrations in piscivorous birds to sublethal effects at both the individual and population level. Bowerman et al. (1994) found geometric mean concentrations in adult and nestling bald eagle body 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 (percent of occupied breeding areas fledging at least one young) was significantly ( $P \le 0.05$ ) correlated with logarithmic concentrations of adult or nestling feather mercury. It also

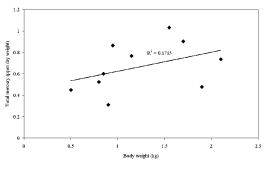


FIGURE 1. Total mercury in breast feathers of nestling African fish eagles (*Haliaeetus vocifer*) (n=10) from Lake Victoria near Entebbe, Uganda, as a function of body weight.

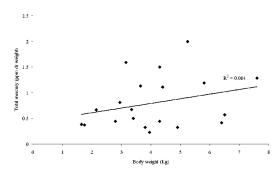


FIGURE 2. Total mercury in breast feathers of nestling marabou storks (*Leptoptilos crumeniferus*) (n=20) from Kampala, Uganda, as a function of body weight.

may be difficult to determine the affects of mercury on avian reproduction separately because of the presence of other chemicals such as DDE or other elements such as selenium (Bowerman et al., 1994). Mercury concentrations found in all age groups of African fish eagles and nestling marabou storks were below the concentrations cited above. Mercury studies in other African avifauna are few and comparisons are difficult because of variation in sampling and analytical techniques, species examined, and environmental conditions as well as the temporal period over which the study occurred. Mercury levels in livers of four African fish eagles analyzed in 1986-87 from the man-made Lake Kariba in Zimbabwe ranged from 66 to 395 ppm dry weight, whereas the liver levels in reed cormorants (*Phalacrocorax africanus*) (n=10) from the same lake were 0.9–13.6 ppm dry weight (Douthwaite, 1992). A study of feather mercury levels in birds from coastal Namibia revealed mean levels of 0.251, 1.310, 0.924, and 0.077 ppm dry weight (reported as parts per billion) in Cape comorants (*Phalorcrocorax capensis*) (n=6), Hartlaub's gulls (*Larus hartlaubii*) (n=10), kelp gulls (*Larus dominicanus*) (n=21), and lesser flamingos (*Phoenicon*aias minor) (n=8), respectively (Burger and Gochfeld, 2001).

The feathers is an excellent tissue to sample noninvasively for mercury analysis. Feathers contain higher concentrations of

mercury than found in other body organs (Westermark et al., 1975), with approximately 70% of total body mercury found in feathers, thus indicating that the majority of mercury in a bird's body is excreted by molting (Honda et al., 1986). Feather mercury concentrations can be measured easily and a positive correlation has been shown between mercury content in feathers and that in internal tissues (Thompson et al., 1991). The mercury concentration in feathers remains constant once the feather is mature until the feather is molted and indicates blood levels at the time of feather maturity (Gochfeld et al., 1996). Mercury concentrations are evenly distributed throughout the feather, indicating mainly endogenous incorporation methods, rather than atmospheric deposition (Hahn et al., 1993). Therefore, feather mercury concentrations of fish eagles and marabou storks can be associated with dietary exposure. This dietary exposure may occur through bioaccumulation of mercury within the food chain. However, 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 because as the molt cycle continues more mercury is excreted into growing feathers and the total body pool of mercury is diluted (Furness et al., 1986). 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 in some species (Furness et al., 1986). Body contour feathers are the feather type that shows the least variation in mercury content (Furness et al., 1986). Mercury accumulates over time, with the two main excretion routes in birds being deposition in growing feathers and in eggs (Heinz, 1996). Therefore, mercury concentrations would be expected to be greater in adult birds than in nestlings and greater in male adults than in female

adults. Because female fish eagles excrete mercury into the egg and typically have a larger body weight than males, they would be expected to have lower feather mercury concentrations than males. The results of this study do not support these expectations, because no significant difference  $(P \le 0.05)$  was found in mercury levels based on age (nestling or adult) or sex in African fish eagles. A summary of studies examining sex differences in mercury concentrations in feathers (Burger, 1993) identified only two of eight studies that showed significant differences. That summary also reported that most studies failed to find any relationship between mercury levels in feathers and age. Although these studies are consistent with our findings, results of our study should be interpreted cautiously because of the small sample size. Sampling in Uganda occurred at times of egg laying and at times of nonbreeding and it was impossible to determine the reproductive status of most adult female birds sampled. Therefore, it was 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 variable in this study was that a small percentage of the samples from young nestlings were breast down rather than breast feathers. Gariboldi et al. (2001) found a significant  $(P \le 0.05)$  correlation between mercury concentrations in blood, down, and feathers of wood storks (Mycteria americana) (n=300) at four of five sites in the southeastern USA. There is some debate over whether the mercury in down feathers originates mainly from the egg (Becker et al., 1994) or from dietary accumulation (Gariboldi et al., 2001) and this could be variable between species.

The mercury concentrations found in marabou stork nestlings from Kampala 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 and Kampala are higher than the concentrations found at Lake Mburo. The lack of any significant difference  $(P \ge 0.05)$ in mercury concentrations between the total fish eagle population from Entebbe, including adults (which would be expected to have higher levels than nestlings), and the nestling marabou stork population from Kampala, may be caused by species differences in accumulation or greater dietary exposure in marabou stork nestlings. Marabou storks have a longer fledging period than do fish eagles (165 days for marabou storks [Pomeroy, 1977] and 75 days for fish eagles [Sumba, 1988]) and therefore have a longer period of feather growth and potential accumulation of mercury in the growing feather with age. This pattern was also seen in white stork (Ciconia ciconia) chicks (Goutner and Furness, 1998) and is supported by the positive correlation between feather mercury concentration and body weight in marabou stork nestlings (Fig. 2). Routes of mercury exposure, in addition to diet, may contribute to mercury concentrations in these birds. Atmospheric concentrations of mercury also may 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.

Mercury concentrations found in crosssection samples of Nile tilapia from both sites in this study were generally lower than those recovered from seven other studies that measured mercury concentrations in Nile tilapia from various sites around Lake Victoria (Campbell et al., 2003). However, the mean weight of fish in these studies was generally greater than our sample fish. Sampling and analytical techniques also varied from ours, because the main interest of these studies often was assessment of potential human exposure to mercury through fish consumption. In addition, some studies were conducted close to known mercury point-source emissions emanating from gold ore processing centers in Tanzania, on the southern shores of Lake Victoria (van Straaten, 2000). No comparisons can be made between the fish from our study sites because of the small sample size from Lake Mburo and the difference in size of the sampled fish from each lake. The concentrations of total mercury in these studies and our samples are below international marketing limits of 500 ng/g wet weight in the European Union and the USA (Campbell et al., 2003).

The higher mercury concentrations in fish eagles from Entebbe may reflect greater dietary exposure than occurred with the fish eagles at Lake Mburo. Adult African fish eagles are extremely territorial (Brown, 1980); therefore, mercury concentrations in nestlings and adults should reflect environmental contaminant concentrations around the nest site and confluent fishing territory. Anthropogenic activity in the Kampala-Entebbe region would be expected to result in greater environmental mercury release than at Lake Mburo, which is surrounded by a national park. Anthropogenic mercury emissions associated with biomass burning are a possible explanation for the higher mercury concentrations in feathers and fish samples returned from Entebbe and Kampala compared with feathers from Lake Mburo. When plants burn they release mercury assimilated from the soil into the atmosphere (Freidli et al., 2001). The main source of household energy within the Lake Victoria region is wood and charcoal (Ogutu-Ohwayo et al., 1997), with 95% of per capita energy consumption in 1994 being wood fuel (National Environment Management Authority, 2000). In addition, it is estimated that 73.8% of the 25,000 tonnes of solid waste generated monthly in the Kampala region is composed of organic matter and vegetables. In 1996, only about 20% was disposed of in landfills by the regulatory authorities (Matagi, 2002). The remaining 80% was disposed of by residents in whatever way they could, including by burning (Matagi,

2002). Although these figures have improved, urban waste management still poses a serious problem in the Kampala region (National Environment Management Authority, 2000). Urban agriculture is still widely practiced in the Kampala district and also contributes to biomass burning (National Environment Management Authority, 1997). Fifty percent of global fires seen by satellite observations are located in sub-Saharan Africa (Dwyer et al., 2000) and this prompted Campbell et al. (2003)to state that biomass burning is a highly significant potential source of total mercury to African lakes. However, atmospheric deposition rates of mercury in the Lake Victoria catchment need to be measured to help determine the significance of biomass burning as a mercury source. Other potential sources of mercury associated with human population growth in the Kampala-Entebbe region include soil erosion (mercury in soil oxyhydroxide particles can be released upon reduction in water [Roulet et al., 1998]). Soil erosion is also the principal manifestation of land degradation in Uganda (National Environment Management Authority, 2000) and is a significant problem in the Kampala-Entebbe region (Matagi, 2002). The use of diesel fuel in Uganda increased from 125,621 m<sup>3</sup> in 1997 to 207,183 m<sup>3</sup> in 2001 (Uganda Bureau of Statistics, 2002). The rapid increase in use of petroleum products due to increased vehicle numbers and traffic congestion is another potential source of mercury contamination to Lake Victoria that requires quantification (National Environment Management Authority, 2000; Matagi, 2002; Campbell et al., 2003). Kampala contains the major industrial areas in Uganda, including 93% of Uganda's chemical industries, as well as abattoirs, soap factories, breweries, textile industries, and a tannery (Matagi, 2002). In 1996, Uganda imported 190,668 tonnes of chemicals, of which 40,585,527 kg was classified as organic chemicals or inorganic compounds of metals (Matagi, 2002). Although the contribution of these industries

to mercury emissions has not been quantified, it is estimated that only 10% of the 61% of industrial wastes entering municipal streams and sewers in Kampala undergo any form of treatment before eventually entering Lake Victoria (National Environment Management Authority, 2000; Matagi, 2002).

#### Persistent organic pollutants

Evaluation of reproductive parameters has been used to determine effects of environmental contamination on the health of raptor populations, such as the bald eagle in North America (Wiemeyer et al., 1993; Elliot and Norstrom, 1998). Concentrations of 4,4'-DDE found in adult fish eagle plasma samples from Lake Victoria at Entebbe were below plasma concentrations linked to low productivity levels in bald eagle nestlings from the Great Lakes region of North America (Bowerman et al., 2003). Smith and Bouwman (2000) recorded a range of chlorinated hydrocarbon residues in blood plasma in four species of raptors from the North-west Province of South Africa. Concentrations of DDE ranged from 0.0015 to 0.0066 ppm (uncorrected wet weight) except for Lanner falcons (Falco biarmicus), an orniphagous species, which 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 concluded that raptors are in no immediate danger of reproductive impairment because of the present DDE residue concentrations.

Although no studies of POP concentrations in avifauna from the Ugandan portion of the Lake Victoria catchment area are known, Ejobi et al. (1996a, b) found residues of DDT and its metabolites, as well as  $\alpha$ -HCH, dieldrin,  $\beta$ -HCH, and lindane, in cow milk and human breast milk from the Kampala region. Mean concentration of p,p'-DDE in muscle fillets of tilapia (weighing 633±208 g) from Lake Malawi, an East African rift valley lake, taken in 1996–97 were 0.46±0.28 ppb (n=6) (Kidd et al., 2001). Concentrations in fish sampled in our study appear equivalent to or lower than most other reports from the region (Mitema and Gitau, 1990; Lalah et al., 2003). However, comparisons are difficult because of variations in sampling technique, including species, size of fish, tissue sampled, analytical method, and, importantly, time when sampling occurred in relation to possible pesticide use.

The fact that no plasma DDT residues were found at the limits of detection for fish eagles, marabou storks, or Nile tilapia sampled in this study may indicate that at the time of sampling there was little ongoing exposure to this pesticide. This is supported by the African fish eagle being a largely piscivorous tertiary avian predator, in which bioaccumulation of organochlorine compounds through the food chain would lead to high residue concentrations (Blus et al., 1996). Eventually residues of DDT in wildlife decline with time after a ban is imposed on the use of the pesticide. However, the highly persistent nature of DDE means that residues will continue to be found for a considerable period of time (Smith and Bouwman, 2000). This would explain the DDE residues found in biota in this study. Other factors that may account for the lack of organochlorine compounds found in the species sampled include the higher rates of dissipation of organochlorine compounds in tropical compared to temperate climates (Wiktelius and Edwards, 1997). This is thought to be due to high rates of volatilization (Yeadon and Perfect, 1981) due to persistently high temperatures and solar radiation intensity. Heptochlor and lindane have been shown to degrade rapidly in tropical environments (Lalah et al., 2003). Hartley and Douthwaite (1994) made the comparison between the 10-20 yr needed for recovery of some raptor populations in England to occur after a decline in organochlorine use to concentrations in songbirds at Siabuwe, Zimbabwe, which decreased quickly in the year after cessation of spraying operations. Importantly, the samples collected only give an indication of residue concentrations at one point in time and significant variation may occur with changes in physiologic condition, egg laying, breeding status, and climate changes (Cooke et al., 1982; Newton and Wyllie, 1992; Smith and Bouwman, 2000).

The source of the DDE found in adult fish eagles from the Entebbe-Kampala region could be due to atmospheric deposition, past local use, or both. The global transport of organochlorine compounds such as DDT has been well established (Tatsukawa et al., 1990) and air samples collected from the shores of Lake Victoria at Kakira, just north of Kampala, revealed the presence of chlorinated compounds, including DDT (Wejuli and Magunda, 2002). Average concentrations were 155.5 pg/m<sup>3</sup> for total DDT, 45.7 pg/m<sup>3</sup> for dieldrin, and 0.4 pg/m<sup>3</sup> for heptachlor. The authors concluded that preliminary analysis of these data indicated that the presence of these chemicals in air samples may be due to regional dry particle deposition to surfaces (water, soil, and vegetation) and precipitation rather than local use (Wejuli and Magunda, 2002). Surveys of local use of these chemicals (regardless of registration status) within the Kampala-Entebbe region would be required to confirm this.

It is surprising that no dieldrin residues were found in the fish or birds sampled. Dieldrin is highly persistent in the environment (Blus et al., 1996), has a history of use in the Kampala region (Ejobi et al., 1996a, b), has been found in air samples collected from the shores of Lake Victoria near Kampala (Wejuli and Magunda, 2002), and is registered for restricted use in Uganda (United Nations Environment Programme, 2001a, b; Orme and Kegley, 2003). It is possible that actual usage has declined in the regions sampled or that the specific uses of dieldrin (control of banana weevils and termites and selective treatment of tree trunks for tsetse control in the country [Ejobi et al., 1996a]) or atmospheric deposition have not led to high levels of exposure in the species studied.

No scientific study was made of African fish eagle productivity (the number of young per occupied nest) or success (percent of occupied breeding areas fledging at least one young). However, general observations made were that out of 10 nests climbed that contained fish eagle chicks (five at Lake Mburo and five at Entebbe), 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 fledged two chicks. At Lake Mburo the average of three counting sessions by boat yielded a result of 74 adults and 15 immature fish eagles. The percentage of immature eagles at both study sites was approximately 20%. 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 biology and reproductive cycle of the species at the study area. This was beyond the resources available for this study, but given the residue concentrations reported here, it seems that contamination by these chemicals and mercury is unlikely to significantly affect productivity of African fish eagles at either of the sites studied. With other African countries, Uganda has requested a specific exemption from the United Nations Environmental Programme (United Nations Environment Programme, 2001b) to allow the use of DDT for mosquito control. Ugandan ecotoxicological studies are therefore relevant and warranted

### ACKNOWLEDGMENTS

We wish to acknowledge the following individuals and organizations for assistance with this project: A. Norris, S. Waigo, M. Okott, T. Grubb, J. Stuht, K. Strause, A. Parker, M. Wilson, J. Brandenburg, R. Miller, the toxicology laboratory staff at the Diagnostic Center for Population and Animal Health at Michigan State University (particularly K. Stuart and Z. Yang), the Uganda Wildlife Authority, the Uganda National Council for Science and Technology and the Uganda Wildlife Education Centre. Airfares were subsidized through the British Airways Assisting Conservation program. This study was conducted under approval of the Michigan State University All University Committee on Animal Use and Care (AUF 02/01-027-00). The Uganda National Council for Science and Technology and the Uganda Wildlife Authority granted research permits for this project. This paper forms part of the project "African Fish Eagles (Haliaeetus vocifer) and Marabou Storks (Leptoptilos crumeniferus) in Uganda: Use as Biomonitors of Environmental Contamination" funded through Morris Animal Foundation grant DO1ZO-78).

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Received for publication 13 June 2003.