THE PATHOLOGY AND ROLE OF BACTERIAL AND ENDOPARASITIC DISEASES IN MASS MORTALITY OF LESSER FLAMINGOS IN KENYA

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DECLARATION

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To my late mother Janes Nyanduko, a woman of great vision who molded my life and worked hard to educate me and to my dear wife Jane and my dear daughter Irene for their love and moral support.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
As	Arsenic
BHC	Benzene hexachloride
Cd	Cadmium
Cr	Chromium
Cu	Copper,
DDD	1,1-dichloro-2,2-bis(p-chlorophenyl) ethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DVS	Director of Veterinary Services
Fe	Iron
Hg	Mercury
HPAI	Highly Pathogenic Avian Influenza
IBA	Important bird areas
IMVIC	Indole methyl red voges proskaur citrate
ISIS	International Species Information System
IUCN	International Union for Conservation of Nature
KWS	Kenya Wildlife Service
LF	Lesser flamingo
MC-LA	Mycrocystin-Leucine (L), Alanine (A)

MC-LF	Mycrocystin-Leucine (L), Phenylalanine (F)
MC-LR	Mycrocystin-Leucine (L), Arginine (R)
MC-RR	Mycrocystin-Arginine (R), Arginine (R)
MC-YR	Mycrocystin-Tyrosine (Y), Arginine (R)
ND	Newcastle Disease
NH_3-N	Ammonia compounds
NMK	National Museums of Kenya
NO_3-N	-Nitrates
PCV	Packed Cell Volume
Sb	Antymony,
SPSS	Statistics package for social sciences
TSI	Triple sugar iron agar
VIL	Veterinary Investigation Laboratories
WBC	White Blood Cells
WBC	White Blood Cells
ZN	Ziehl-Nielsen
Zn	Zinc

ABSTRACT

The lesser flamingo (*Phoeniconaias minor*) is the most abundant waterbird species in Kenya and a major attraction for ecotourism in many parts of Africa. Mass deaths in the species have become more frequent in recent years and are believed to contribute to declining numbers of the species. Previous studies into the mass deaths have suggested various toxicological and infectious causative agents but most of these studies have been conducted during outbreaks. This study examined pathology and bacterial and endoparasitic diseases in lesser flamingos during non-outbreak periods and compared these with those of outbreak periods, through retrospective study. The study also assessed environmental factors that could contribute to disease in the species.

The study was done in Lakes Bogoria and Nakuru in Kenya. During 4 field visits to the lakes, a total of 57 lesser flamingos comprising 17 that were found dead, 18 sick and 22 healthy ones were examined clinically and by necropsy and samples taken from them were analyzed for haematology, histopathology, bacteriology and parasitology. In addition, tissue samples from 134 lesser flamingos collected over a period of 6 years (1997-2004) and preserved in 10% buffered formalin were processed and examined for histopathological lesions. Environmental data on rainfall, water quality and waterfowl populations for the lakes was collated over periods of 3 to 10 years and analyzed to determine trends that could be associated with disease occurrence.

Weakness, coma and death in good body condition were common signs observed in lesser flamingos during the field survey. They were accompanied by heterophilia and lymphopaenia. Congestion, haemorrhage and extensive necrosis in the visceral organs associated with infiltration with heterophils and mononuclear inflammatory cells were major and consistent histopathological lesions in lesser flamingos examined during the field survey and those examined retrospectively. These lesions were most severe in samples obtained during the mass mortality period. Abscesses and marked lymphocytic infiltration into various organs were observed in healthy as well as sick lesser flamingos. Mycobacterial granulomas were demonstrated in 2/57 lesser flamingos examined during the field survey and 1/134 of those examined retrospectively. *Pasteurella multocida* and *Salmonella gallinarum* were isolated from livers and spleens of 4/46 (9%) and 1/46 (2%), respectively. One of the birds from which *P. multocida* was isolated was healthy. Two cestodes, *Cladogynia phoeniconiadis* and *Gynandrotaenia stammeri* were found in 50/53 and 1/53, respectively of the birds. One nematode, *Striatofilaria phoenicopteri*, was found in 2/53 of the birds. Lesions of helminthiasis comprising extensive burrowing by the worms into the intestinal mucosa accompanied by thickening of the latter were observed in one bird at histology.

Major fluctuations in water quality parameters, waterfowl populations and weather patterns were observed in the study sites. Conductivity of water in Lake Nakuru, recorded monthly over a period of three years, showed a strong and statistically significant negative correlation with lake depth ($r = -0.834$, $p = 0.01$). Concentration of nitrate compounds in the lake water increased after the rains and high levels of *Arthrospira sp*, the primary food for lesser flamingos, were recorded

following the rise in concentration of nitrogen compounds. The lesser flamingo population had a positive and statistically significant correlation with the concentration of *Arthrospira* sp. ($r = 0.968$, $p = 0.032$). The population of lesser flamingos in lakes Nakuru and Bogoria were negatively correlated ($r = -0.503$, $p=0.497$) and mass mortality events of the lesser flamingo were shown to coincide with concentrations of 500,000-800,000 birds in Lake Nakuru.

This study underscores that the causes of mass mortality in lesser flamingo remain as sub-clinical infections in the host and flare up to acute severe disease with high mortalities when suitable conditions prevail. The causes of such mortalities have a multifactorial perspective. Pathogenic bacteria, namely *P.multocida*, *Mycobacterium spp* are present in the lesser flamingo population as sub-clinical infections that can flare up to cause mass mortalities when the population is subjected to environmental stressors. This is supported by the range of pathological lesions observed during the study. Isolation of *S.gallinarum*, a pathogen of domestic poultry, points to possible disease transmission at the lesser flamingo-poultry interfase.

CHAPTER 1

INTRODUCTION

1.1 Background

The lesser flamingo (LF) (*Phoeniconaias minor*) is the most abundant waterbird species in Kenya and a major attraction for ecotourism in many parts of Africa (Nasirwa, 2000; Owino *et al*., 2001; Owino *et al*., 2002; Harper*et al.*, 2003). It is the main consumer of the prolific algae in the saline Rift Valley lakes of East Africa and a key bioindicator species of aquatic ecosystem health as well as a 'flagship' species for the wetlands (del Hoyo 1992; Koyo and Owino 2010)).

The LF is the smallest and most numerous of the six species of flamingo found in various parts of the world. On average, it has a height of 80 cm and weight of 2.5 kg. The LF primarily occurs in Africa with majority of the population in Eastern Africa and the minority in Southern and Western Africa (Brown *et al*., 1982; Childress *et al.*, 2007). The birds are obligate filter feeders and mostly inhabit the alkaline-saline lakes where they primarily feed on the microscopic cyanobacteria *Arthrospira fusiformis* formerly known as *Spirulina platensis* (del Hoyo 1992).

The LF in the wild has an average lifespan of almost 40 years with an estimated generation length of 22-24 years and an age of 5-7 years at first-breeding (Brown, 1973; Howard, 1997;

Underhill *et al.,* 1999).The birds lay eggs on conical mounds of mud nests, topped with a slight depression. The LF lays eggs once a year with a clutch size of one (rarely two eggs), an incubation period of 28-30 days, fledging success of 41-43% and chick survival rate of 15-20% (Childress *et al.,* 2007). There are only three regular breeding sites for lesser flamingo in Africa, namely; Lake Natron in Tanzania (East Africa); Etosha Pan in Namibia and Sua Pan in Botswana, both in Southern Africa (Berry, 1972; Katondo and Mwasanga, 1997).Their long life enables the lesser flamingo to sustain large populations despite their low recruitment rates (Howard, 1997).

Various threats have been identified as possible causes of the declining population of LF in Africa. Among these are altered hydrology of the Rift Valley lakes associated with anthropogenic degradation of their watersheds and catchment basins (Shivoga *et al*, 2007); breeding failure due to human induced disturbances of the breeding sites (Simmons, 1996); and frequent mass deaths caused by infectious and non-infectious diseases (Koyo and Owino 2010).

Mass deaths of LF have become more frequent in East Africa in recent years and they are an important threat to survival of the species. Major die-offs occurred in 1993, 1995, 2000, 2002, 2004, 2006 (Motelin et al., 1995; Kock *et al*., 1999; Beasley *et al*., 2004; Lugomela *et al.,* 2006; Manyibe *et al.,* 2007). The mass deaths show a seasonal and cyclic pattern that suggests that infectious diseases could play an important role in their occurrence.

Previous investigations have implicated infectious and toxicological diseases, acting in combination with various environmental stressors, to be the causes of flamingo mortalities (Tuite 1974; Sileo et al, 1979; Motelin et al., 1995; Kock *et al*., 1999; Beasley *et al*., 2004; Lugomela *et al.,* 2006; Manyibe *et al.,* 2007). However, most of these investigations have been short-term responses to the mass deaths and baseline assessment of the health of the species during inter-epidemic periods is lacking. Although pathogenic bacteria implicated in the deaths of lesser flamingos have been isolated from the tissues of these birds during outbreaks, their prevalence during non-outbreak periods is not known. This makes it difficult to interpret their causal roles. Similarly lesions consistent with toxins have not yet been demonstrated in the tissues of dead and sick birds during outbreaks although various toxic substances have been isolated.

There is therefore need to re-examine disease among other risk factors in order to gain a better understanding of the cause(s) of the mass deaths. This will help in designing appropriate conservation strategies for the species.

1.2 Hypotheses

Pathogenic bacteria, endoparasites and environmental factors play an important role in the mass mortalities of lesser flamingos.

1.3 Research objectives

Overall objective

To examine the pathology and role of bacterial and endoparasitic diseases, among other risk factors, in the frequent mass deaths of lesser flamingos in East Africa.

Specific objectives

1. To characterize the pathological lesions found in the lesser flamingo and assess their prevalence and severity in tissues collected during the monitoring phase and in those collected during previous outbreaks.
2. To determine the various pathogenic bacteria affecting the lesser flamingo in Lakes Nakuru and Bogoria and determine their prevalences during dry and wet seasons.
3. To determine the species of endoparasites found in the lesser flamingo in Lakes Bogoria and assess their prevalences and intensity during dry and wet seasons.
4. To determine the environmental factors that influence prevalence of disease.

1.4 Justification for the study

Previous investigations into the role of infectious diseases in the mortality of lesser flamingos have been triggered by the occurrence of mass deaths. Baseline health assessments and monitoring during inter-epidemic periods have largely been lacking, making it difficult to

interpret the significance of the various infectious agents and pathological lesions encountered during the epidemics.

There have not been any standardized protocols or unified approaches into investigations of mortality in lesser flamingos in East Africa. Networking between researchers from different disciplines has been limited. Investigations have therefore not been holistic and synergistic, and have tended to be biased towards the special interests of the researchers involved. Consequently, the roles of multiple stressors in causing the mortalities have not been easy to define and conclusions drawn from the investigations have reflected the underlying biases. Even estimates of mortality during epidemics are not reliable as they are not based on standardized scientific protocols.

There is therefore need for sustained monitoring of the lesser flamingos to determine the background levels of various infectious agents and their associated pathological lesions. Such data would be useful in determining the contribution of these agents to mortality of the birds during epidemics. There is also need to evaluate clinico-pathological parameters of the lesser flamingos and their usefulness in predicting the health status of the species. As much as possible, the monitoring should be combined with other environmental surveys in order to provide a more holistic picture of the interplay of factors that influence the health of flamingos.

The seasonal and cyclic nature of the mass deaths of lesser flamingos in East Africa suggests that infectious diseases could play an important role in their occurrence. However, the prevalence of

these diseases and their interactions with other risk factors are poorly understood. The proposed study seeks to address this gap in order to contribute to a better understanding of the cause of the frequent mass deaths of lesser flamingos. The knowledge generated from the study will help in designing effective disease control strategies and in improving conservation of the species.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Biology of the lesser flamingo

2.1.1 General ecology and behaviour of the lesser flamingo

Six species of flamingo are known in the world, namely; the lesser flamingo (*Phoeniconaias minor*), the greater flamingo (*Phoenicopterus ruber roseus)* the Caribbean flamingo (*Phoenicopterus ruber ruber*), the Chilean flamingo (*Phoenicopterus chilensis*), the Andean flamingo (*Phoenicoparrus sandinus*) and James' flamingo (*Phoenicoparrus jamesi*). These species are classified under three genera in the order Phoenicopteriformes (Norton, 2003).

The lesser flamingo is the smallest but most numerous of the six species of flamingoes with an average weight of 2.5 kg and average height of 80 cm. It is found in East Africa, Southern Africa, West Africa and parts of Asia (Berry, 1972; Brown *et al.*, 1982; del Hoyo, 1992; Mundkur, 1997; Williams and Velásquez, 1997). The largest population of this species is found in East Africa inhabiting the Rift Valley lakes of Ethiopia, Kenya and Tanzania (Fig 1). Major among these lakes are: Abijatta, Metahara, Aranguade and Chituin Ethiopia (Desta, 1997); Bogoria, Nakuru, Elmentaita and Turkana in Kenya (Githaiga, 1997; Nasirwa, 1997) ; and Manyara, Natron, Momela, Empakai, Ngorongoro Crater, Burungi, Eyasi, Balangida Lelu and Ndutu in

Map not to scale

Figure 1: Locations of major Saline-Alkaline lakes inhabited by lesser flamingo

Tanzania (Arinaitwe, 1999) Periodic refuges for the species in Kenya include Lakes Amboseli, Logipi, Nyaima, Oloidien, Shompole, Simbi, Solai, Sonanchi and Suguta (Githaiga, 1997; Nasirwa, 1997). In Tanzania, occassional sightings of the species have also been documented at Lakes Singida, Rukwa, Kitangiri, Babati, Basotu and Magadi (in Serengeti National Park) (Arinaitwe, 1999). In Southern Africa, lesser flamingo numbers are greatest in Etosha Pan and Sua Pan but they also feed in other inland and coastal wetlands including Walvis Bay, Sandwitch Harbour, Kamfers Dam, Wadrif Salt Pan, Berg River Estuary and Langebaan Lagoon (Williams and Velásquez, 1997; Taylor *et al.*, 1999).

The lesser flamingo is highly nomadic and occurs in large flocks of hundreds to millions, often mixed with greater flamingo (*Phoenicopterus rubber roseus*). Childress *et al.*, (2004) reported that the species, in the Rift Valley lakes of East Africa, has unpredictable movement patterns. The birds move frequently between different lakes, covering a cumulative distance of up to several hundreds of kilometers within a few days (Childress *et al.* 2004). Although food availability and drying of the lakes have been suggested as possible causes of the nomadic movements (Simmons, 2000; Tuite, 2000), these do not adequately explain the perennial, unpredictable inter-lake movements (Childress *et al.*, 2004).

Norton (2003) has described the key morphological features of the lesser flamingo. The birds have long, thin legs and can stand on one leg with the head tucked beneath a wing for a considerable period of time. Their neck is proportionately the longest of any bird, containing 17 cervical vertebrae. The bill is adapted uniquely for filter feeding through numerous complex rows

of lamellae.When the chick hatches, the bill is straight and has no lamellae but it slowly becomes curved and develops lamellae with age. The feathers and skin of adult flamingos are various shades of pink and red because of pigments called carotenoids. Young birds are shades of grey and white because they lack the pigments. As they mature, they slowly derive carotenoids from their feed and develop the pink colour. Flamingos are not sexually dimorphic when they are young and sex determination can be difficult for adults, although males are usually larger than females.

The lesser flamingo is a colonial nester, with colonies comprising tens of thousands of individuals. Courtship behaviour, comprising elaborate displays and ritualized movements, is seen from time to time throughout the year often going on for hours in the pre-breeding months. The eggs are laid in conical mounds of mud nests, topped with a slight depression. The flamingos construct these nests in shallow waters. For successful breeding to occur, the water must be sufficiently shallow to avoid the nests being washed away, and sufficiently deep and long-lasting to prohibit terrestrial predators from reaching the breeding colony (Berry, 1972). It is estimated that only 41-43% of total number of eggs that are laid fledge successfully and that only about 15-20% of the fledged chicks survive, the rest dying, mostly within the first three weeks (Berry, 1972). The mean incubation period is 28-30 days and the clutch size is one egg per bird, rarely two (Berry, 1972; Brown, 1973). In the wild, the lesser flamingo has an estimated lifespan of 40 years and generation length of 22-24 years with a probable age at first breeding of 5-7 years (Brown, 1973; Underhill *et al*., 1999). Lesser flamingo pairs do not produce a viable offspring

every year but they are able to sustain large populations because of their longevity ((Howard, 1997).

The Lesser flamingo is an obligate filter feeder and the primary harvester of the prolific algal productivity of the shallow alkaline Rift Valley lakes (Vareshi, 1978; del Hoyo, 1992;). Their food mostly comprises the microscopic cyanobacteria known as *Arthrospira fusiformis* (formally, *Spirulina platensis*) and phytoplankton species known as benthic diatoms (Vareshi, 1978). The flamingoes feed with their head upside down so that the maxillary bill takes on the function of the mandibular bill and vise versa (Jenkins, 1957; Norton, 2003). The feeding requires a series of tongue movements and opening and closing of the beak which allows food items to be filtered by lamellae and eventually ingested (Jenkins, 1957; Norton, 2003). Unwanted items such as mud and saltwater are pushed out by the tongue. Flamingo chicks are hatched without the capability to filter feed (Norton, 2003). They depend on a highly nutritious pink to red liquid known as crop milk, which is produced in the proventriculus and oesophagus of the parents, for their food (Norton, 2003).

2.1.2 Population trends of the lesser flamingo in East Africa

The population of LF in Africa is estimated to be declining over the last three decades. In 1975, the LF population was estimated at 5 million birds and in 1994 it was about 4 million, representing a decline of 21% (Simmons, 2000). In 2002 and 2005 the estimates were 2-4 million and 1.5-2.5 million respectively, indicating further decline (Wetlands international 2002; IUCN-

2005). This trend coupled with increasing environmental threats facing the species and its habitats has led to its listing in the near threatened category of the IUCN Red List on Threatened Species in 2000, up from the category of species of least concern in 1994 (IUCN-2006). Local trends in the population of the lesser flamingo in the Rift Valley lakes of Kenya have similarly shown a decline (Nasirwa, 2000). Although estimates of the flamingo population in Africa are weakened by limitations in underlying methodologies, the apparent declining trend has raised concern among the scientific and conservation communities (Koyo and Owino.2010)

2.1.3 Threats facing the lesser flamingo in East Africa

Lesser flamingos in East Africa face numerous threats. Most important among these are: altered hydrology and pollution of the Rift Valley lakes due to human activity in their watersheds and catchment basins (Githaiga, 1997; Koyo and Owino, 2010), impact on the limited number of breeding sites (Simmons, 1996) and frequent mass mortality (Koyo and Owino, 2010).

2.1.3.1 Altered hydrology and pollution

Lesser flamingo have specialized habitat requirements due to their unique feeding and breeding behaviour (Simmons, 1996 and Vareshi, 1978). In East Africa, they mostly inhabit the saline-alkaline lakes of the Rift Valley (Brown, 1959; Tuite, 2000). The watersheds of these lakes are rapidly being degraded due to increasing human population and change of land use practices (Shivoga *et al*, 2007). Destructive activities such as deforestation, cultivation on riverbanks, and

over-abstraction of rivers are increasing in these areas. These have led to: reduction in both amount and reliability of rainfall; reduction in percolation of rain water, increased flash flooding into the lakes, increased soil erosion, increased seasonality of river flows, increased sedimentation in the lakes, and declining lake levels (Shivoga *et al.*, 2007). These changes alter the chemical and physical characteristics of the lakes which impacts negatively on the productivity of the algae species which the lesser flamingo feed on (Shivoga *et al.*, 2007; Githaiga, 1997).

Koyo and Owino (2010) have reviewed the anthropogenic changes in the catchment basins that impact negatively on the Rift valley lakes. Increasing human settlement, farming, urbanization and industrial development in these basins exposes the lakes to increasing levels of pollution. Several urban centres discharge their wastes into rivers flowing to some of the lakes. Factories such as diatomite mining, timber treatment, canning and milk processing have developed in these areas discharging waste into some of the rivers that feed the lakes. Agrochemicals from horticultural farms, and acaricides and animal waste from livestock farms also get into the lakes through storm water and the rivers. All these processes have led to increased pollution and more frequent eutrophication in some of the lakes. As a result of eutrophication, algal blooms, some of which may be harmful to the lesser flamingos, have become more common.

2.1.3.2 Human impacts on the limited number of breeding sites

Lesser flamingos have only three known regular breeding sites in Africa: Lake Natron in Tanzania is the only breeding site in East Africa while both Etosha Pan (Namibia) and Sua Pan (Botswana) are in Southern Africa (Katondo and Mwasaga, 1997; Berry, 1972; Simmons, 1996). All the three sites are threatened by abstraction of water from the catchment areas and Lake Natron is threatened by a proposed establishment of a soda ash extraction factory which has been kept on halt (Simmons, 1996; Anderson, 2000a, 2000b and 2008; Childress et al., 2008).

2.1.3.3 Frequent mass deaths

Frequent mass deaths in lesser flamingo in East Africa are now recognized as a threat to the sustainability of the population (Childress *et al*., 2007; Koyo and Owino, 2010). The causes of these deaths have not been clearly defined partly due to lack of long-term monitoring and lack of a multidisciplinary approach into the investigations (Koyo and Owino 2010).

2.2 Causes of mass deaths of lesser flamingo in East Africa

2.2.1 Previous investigations

Previous investigations have identified various disease causing agents in association with the mortalities of lesser flamingos (Table 1). They include the following: bacteriological agents, particularly *Mycobacterium avium* (Kock *et al*., 1999; Sileo *et al*., 1979) and *Pasteurella multocida* (Beasley *et al*., 2004; Manyibe *et al*., 2007.); parasitological agents such as cestodes,

Table 1: Possible causative agents of LF mortalities in Kenya 1974-2006

Year	Lake	Month	Associated agents	Citation
1974	Nakuru	July-August	*Mycobacterium avium*	Sileo *et al.,1979*
1993	Bogoria	Mid August – November	*Mycobacterium avium*	Kock *et al., 1999*
	Nakuru,	Mid September	*Mycobacterium avium*	Kock *et al., 1999*
1995	Nakuru	Mid August - September	*Heavy metals, cyanotoxins*	Motelin *et al., 1995*
2000	Nakuru Bogoria	July	*Pasteurella multocida, cyanotoxins, heavy metals*	Beasley *et al., 2004*
2002	Nakuru, Bogoria	July	*Pasteurella multocida, cyanotoxins, heavy metals*	Beasley *et al., 2004*
2004	Nakuru, Elmentaita, Oloidien	September-November	*Pasteurella multocida*	Lugomela *et al.*, 2006, Manyibe et al., 2007
2006	Nakuru, Elmentaita, Oloidien	July -August (small episode in February)	*Pasteurella multocida*	Manyibe *et al*., 2007

nematodes and besnoitia (Johnes and Khalil, 1980; Kastard *et al*., 1981) ; heavy metals, particularly Cr, Fe and Zn, and cyanobacterial toxins, especially mycrocystins and anatoxin-a

(Motelin *et al.*, 1995; Beasley *et al*., 2004; Lugomela *et al*., 2006; Krienitz *et al.*, 2003). Most of the reported deaths have occurred between July and November

Various environmental factors, such as high density of the lesser flamingos, the density of blue green algae, dry season and high ambient temperatures, low water level in the lakes are variously mentioned in association with the mortalities (Sileo *et al.,* 1979; Kock *et al.,* 1999; Manyibe *et al*., 2007).

2.2.1.1 The 1974 die-off of lesser flamingo in Lake Nakuru

The 1974 deaths of lesser flamingos in Lake Nakuru coincided with a sudden decline in the density of blue green algae and a dramatic emigration of flamingos from the lake (Tuite, 1974; Sileo *et al*., 1979). Previously, in September and November 1973, a total of four debilitated flamingos from the lake had been investigated and avian mycobacteriosis had been confirmed in all four (Cooper*et al.,* 1975). Following this, Sileo *et al*. (1979) conducted a four-week survey in Lake Nakuru in April 1974 aimed at determining the prevalence of mycobacteriosis in the lake. Although many debilitated birds were present in the lake at the time of the survey, higher than normal mortalities were not reported until July and August of the same year (Tuite, 1974; Sileo *et al*., 1979). The survey involved necropsy of debilitated birds, identification of endoparasites, and histopathology of tissues from the birds (Sileo *et al*., 1979; Karstad *et al*., 1981; Jones & Khalil, 1980).

Diverse pathological lesions were documented including extensive mycobacterial granulomatous lesions in 37% (19 out of 51) of birds examined (Sileo *et al*., 1979). Proliferative endarteritis associated with at least four different stages or forms of a besnoitia-like protozoan organism was recorded in about 80% (46 out of 57) of the birds examined (Karstad *et al*., 1981). Seven species of cestodes and two of nematodes were described in the flamingos during this investigation (Jones & Khalil, 1980). Although no one condition was singled out as the cause of the mortality, it was associated with nutritional stress due to a sudden decline in the density of blue green algae (Sileo *et al*., 1979).

2.2.1.2 The 1993 and 1995 die-offs of lesser flamingo in Lakes Bogoria and Nakuru

The 1993 mass deaths of lesser flamingos began in August in Lake Bogoria, spread to Lake Nakuru a month later and resolved by mid-November of the same year with arrival of the rains (Kock *et al*., 1999; Motelin *et al*., 2000). The deaths occurred during a drought when ambient temperatures were high, water levels were low, the population of flamingos in Lake Bogoria was considered unusually high and an uncharacteristic algal bloom was present in the lake (Kock *et al*., 1999). Kock *et al*. (1999) estimated a total of 18,500 to have died at both locations by the end of October, based on a flamingo census. Investigations by Kock *et al.* (1999) into the 1993 deaths involved necropsy of affected birds combined with histopathology and bacterial culture from tissues of affected birds. Presence of pathological lesions consistent with acute sepsis in most of the birds and isolation, in pure colonies, of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* from tissues of affected birds led to the conclusion that bacterial

septicaemia was the immediate cause of the deaths (Kock *et al.*, 1999). Presence of granulomatous lesions in about 40 % of the birds examined and isolation of *Mycobacterium avium serovar 1* from tissues of some of the birds confirmed the presence of avian tuberculosis in the lesser flamingo population, suggesting that the disease was endemic within the Lake Nakuru (Kock *et al*., 1999). The investigators argued that an algal bloom that was present during the mass deaths might have caused the proliferation of saprophytic bacteria to high levels that overwhelmed susceptible birds ingesting the contaminated algae. They suggested that avian tuberculosis might have contributed further to the deaths by compromising the immunity of birds that were already chronically suffering from it.

Motelin *et al*. (2000) also investigated the 1993 mass deaths of lesser flamingos in Lakes Bogoria and Nakuru, and a similar episode that occurred in the same lakes between mid-August and September 1995. The latter involved an estimated 15,000 deaths. Investigations by Motelin *et al.* (2000) into both the 1995 and 1993 mass deaths involved necropsy of the sick birds combined with histopathology, haematology, and toxicological analysis of tissues from affected birds. Pathological lesions were essentially identical in both episodes and consistent with those of acute sepsis reported by Kock *et al.* (1999). Heavy metals, pesticides and microcystins were found in tissues of affected birds from both episodes and their role in causing the deaths was suggested (Motelin *et al.* 2000).

2.2.1.3 The 2000 and 2002 die-offs of lesser flamingo in Lake Bogoria

Beasley *et al.* (2004) investigated the 2000 and 2002 die-offs using necropsy, histopathology, bacterial culture and toxicological analysis of tissues from affected birds. Isolation of pure cultures of *Pasteurella multocida* from sick birds along with pathological lesions characteristic of sepsis, including multifocal intrahistiocytic bacilli in inflamed livers and spleens, led to the conclusion that infection with these bacteria was the proximate cause of the deaths. Elevated levels of chromium, iron and zinc and various levels of the cyanotoxins microcystins and anatoxin-A were detected in the tissues of affected birds. The significance of these metals and toxins in causing the mortality was not clear due to lack of lesions that could be attributed to them. Heavy infestation with cestodes and lice in the affected birds were reported in both outbreaks.

Between 2001 and 2003, Krienitz *et al*. (2003 and 2005) investigated the contribution of cyanobacterial toxins to the massive deaths of lesser flamingos in the alkaline Rift Valley lakes. They analyzed the species community and toxin content of cyanobacterial mats at the hot springs on the shore of Lake Bogoria and those of algal blooms in both Lakes Nakuru and Bogoria. In addition, they analyzed for the cyanotoxins in stomach and intestinal contents and liver tissues sampled from two dead lesser flamingos collected at Lake Bogoria. Microcystins (hepatotoxic) and anatoxin-A (neurotoxic) were identified in the cyanobacterial mats growing in the hot springs at Bogoria and in the cyanobacterial blooms in both lakes. Two toxic strains of *A. fusiformis* were identified in both lakes and cyanotoxins were found in the stomach and intestinal contents and in the liver tissues of the two flamingos. They observed that the flamingos they had examined were

in “a posture of opisthotonus” and had“convulsed extremeties”, which they suggested as possible neurological signs caused by the cyanotoxins. Krienitz *et al*. (2005) concluded that cyanotoxins should be included among the possible agents causing mass deaths in flamingos.

2.2.1.4 The 2004 die-offs of lesser flamingo in Lakes Manyara and Big Momela

Mass deaths of lesser flamingos at Lakes Manyara and Big Momela began in mid-June and late-July 2004, respectively, with an estimated 41,000 deaths during the two episodes (Kilewo and Mlengeya, *pers com* 2004; Lugomela *et al.,* 2006). Limnological studies revealed very high concentration of the cyanobacteria *A. fusiformis* in the water in Lake Big Momela during the deaths (Lugomela *et al.,* 2006). Extracts from the algae were injected intraperitoneally into laboratory mice resulting in signs of toxicosis and death (Lugomela *et al*., 2006). Based on these findings, the authors concluded that the deaths in the two lakes were most likely caused by a toxic type of *A. fusiformis* (Lugomela *et al.,* 2006). They argued that Ballot *et al.* (2004) had isolated such atype of *A. fusiformis* from lakes Bogoria and Nakuru and that the prevalence of toxic and non-toxic types was possibly influenced by external or endogenous factors that were not yet understood.

2.2.1.5 The 2004 and 2006 die-offs of lesser flamingo in Lake Nakuru

Mass deaths of lesser famingos in Lake Nakuru in 2004 and 2006 were reported by Manyibe *et al*. *(*2007.). Their investigation involved necropsy and bacterial culture of tissues from affected

birds in both outbreaks and histopathology of samples from the 2006 outbreak. Details of the two die-offs are given below.

2.2.1.5.1 The 2004 die-off of lesser flamingo

The die-off of lesser flamingoes in Lake Nakuru in 2004 occurred during the months of September to November, affecting all ages of birds (Manyibe *et al.,* 2007). Mortalities increased gradually over several weeks before accelerating rapidly to a one-week peak in mid October. Peak mortality occurred during the rainy season and coincided with an increase in the population of lesser flamingoes from 200,000 birds in July to 800,000 birds in September after a recent influx of the birds into the lake (Kariuki, personal communication 2004). Several thousands of flamingos were estimated to have died during the outbreak (Kariuki, personal communication). Two fresh carcasses of white pelicans (*Pelecanus onocrotalus*) were also collected at the lake shore in early October.

The sick lesser flamingos were weak or comatose and a number were lame. Fifty-six necropsies were done including dead, sick and healthy birds. Most of the carcasses were in good nutritional status. Most sick or dead birds had generalized congestion (Fig 2) and hemorrhages (Fig 5) in the internal organs and some had focal necrotic lesions and fibrinous exudation into coelomic cavity (Fig 4). Most birds had heavy infestation of intestines with tapeworms. Spleens and livers were enlarged and there were occasional white foci in the latter (Fig 3). Bacterial culture of swabs from livers and spleens of 18 lesser flamingoes and the 2 pelicans was done and *Pasteurella*

multocida was isolated from both pelicans and from 10 out of 18 flamingoes. *Pseudomonas aeruginosa* was isolated from two lesser flamingos and *Staphylococcus* from one. These findings were consistent with acute bacterial septicaemia.

2.2.1.5.2 Die-off of 2006 of lesser flamingo

The 2006 die-off occurred in two episodes, a small one during the last week of February and a larger one from July to August (Manyibe *et al.,* 2007.). Two Egyptian geese (*Alopochen aegyptiacus*) and one black winged stilt (*Himantopus himantopus*) were also recorded sick or dead at the beginning of the second episode.

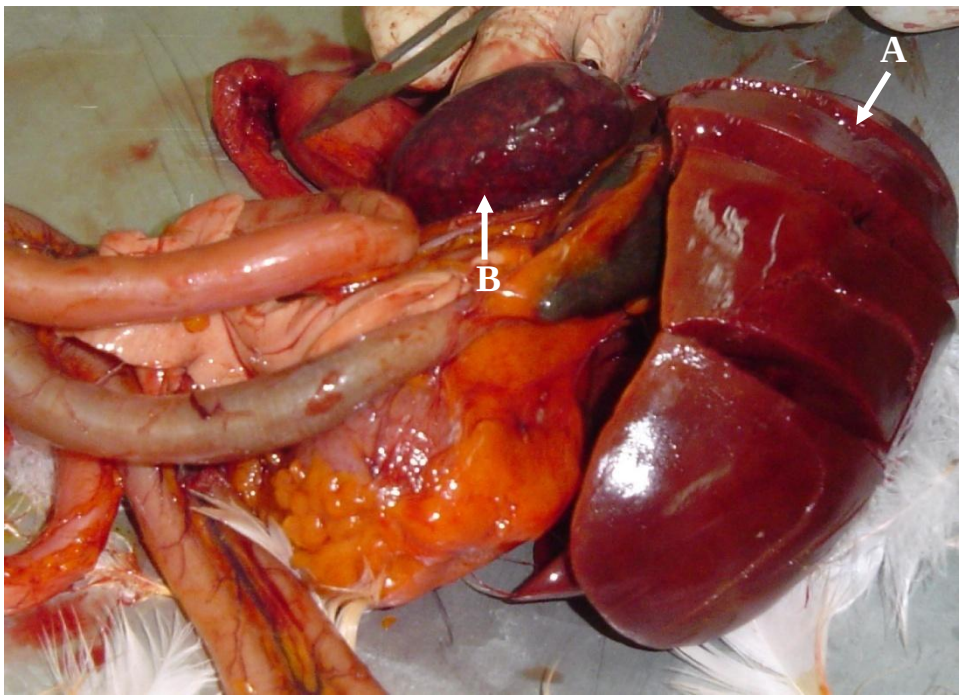

Figure 2: Congestion of liver (A) and spleen (B) of a lesser flamingo sampled in Lake Nakuru during the mass mortality in 2004
(Photo by Thomas Manyibe)

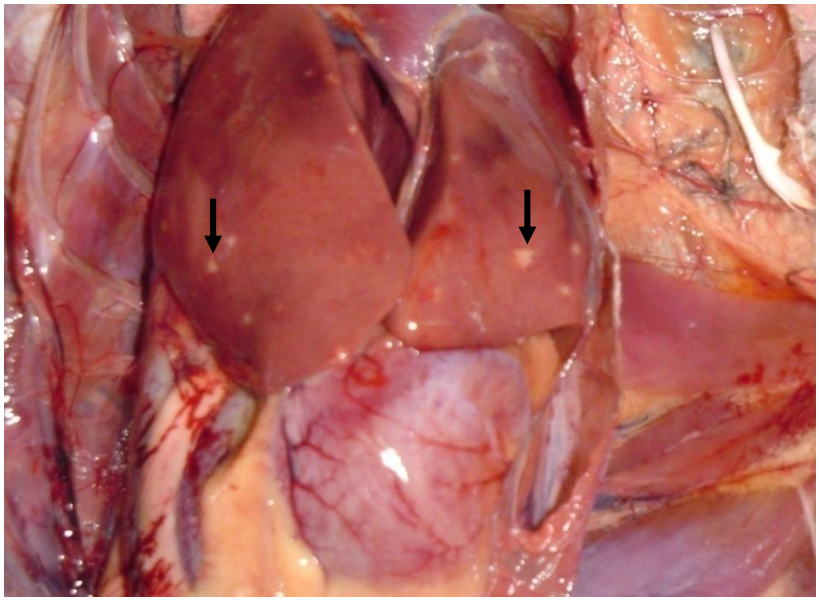

Figure 3: White foci of necrosis (arrows) in liver of a lesser flamingo sampled in Lake Nakuru during the mass mortality in 2004
(Photo by Thomas Manyibe)

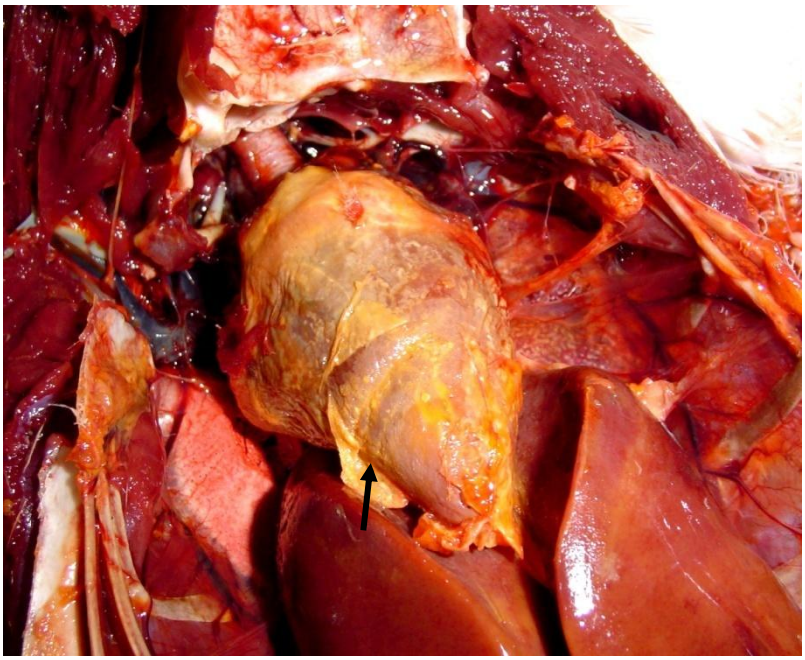

Figure 4: Fibrinous exudate (arrow) on the heart of a lesser flamingo sampled in Lake Nakuru during the mass mortality in 2004
(Photo by Thomas Manyibe)

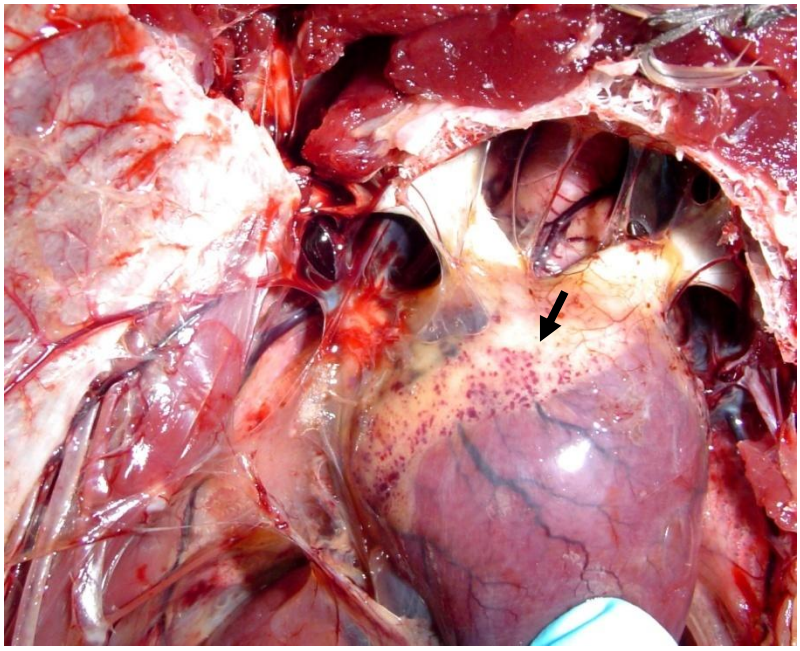

Figure 5: Petecchial haemorrhage in the coronary groove (arrow) of a lesser flamingo sampled in Lake Nakuru during the mass mortality in 2004
(Photo by Thomas Manyibe)

Mortality in lesser flamingoes increased gradually over several weeks before accelerating rapidly to a two-week peak in mid July. Initially, less than 20 flamingo carcasses were recorded in a day but the number increased to a climax of about 5000 carcasses per day. Most of the deaths occurred during the two weeks of peak mortality. The mortality occurred during the dry season coinciding with a very high population of lesser flamingos in the lake estimated at 1.2 million in mid July. Mortality decreased rapidly and stopped in early August with the onset of rains. An estimated 35,000 lesser flamingoes died during the outbreak affecting all ages.

The clinical manifestation in birds during the 2006 die-off was similar to that of 2004 with presence of many weak and comatose birds. Lame birds were frequent especially among

juveniles. Thirteen necropsies of dead or sick birds revealed generalized congestion accompanied with white foci in the livers of some birds, presence of mucohaemorrhagic contents in the intestines, haemorrhages on the heart and thigh muscles, and heavy infestation with tapeworms. One bird had copious caseous exudates in the air sacs accompanied with fungal growth and white focal lesions in the lung parenchyma. Histopathology was done on samples from 4 birds and generalized congestion and haemorrhage in various organs was the main lesion observed. Samples from all the birds were tested for highly pathogenic avian influenza (HPAI) and Newcastle disease (ND) and they were negative. *Pseudomonas aeruginosa* was isolated from livers, spleens, kidneys and lungs of three birds and from water samples from the western shore of the lake.

2.2.2 Role of infectious diseases

2.2.2.1 Bacterial diseases of lesser flamingo

2.2.2.1.1 Avian tuberculosis

Avian tuberculosis, caused by *Mycobacterium avium*, has been reported in lesser flamingos in the Rift valley lakes of Kenya. Kaliner and Cooper (1973) reported the disease in an African fish eagle (*Cuncuma vocifer*) in Lake Nakuru and Cooper *et al.* (1975) reported it in four lesser flamingos from the same lake. Sileo *et al.* (1979) surveyed Lake Nakuru in 1974 and found a 37% prevalence of characteristic mycobacterial granulomas among 51 sick lesser flamingos. Kock *et al*. (1999) found a prevalence of over 40% of mycobacterial granulomas among 42 lesser

flamingos that were examined during the 1993 die-off. Kock *et al*. (1999) isolated *Mycobacterium avium serovar* 1 from tissues with these lesions. Pessier *et al* (2004) described characteristic mycobacterial lesions in one bird collected from Lake Bogoria in 2000. Oaks *et al.* (2006) reported *Mycobacterium avium* in five sick lesser flamingoes in Lake Bogoria in 2005.These reports suggest that *Mycobacterium avium* is endemic in the Rift Valley lakes and other predisposing factors are required for an epidemic to occur.

Friend and Franson (1999) has suggested that mycobacteriosis is likely to be present in a small proportion of wildbirds whenever there are major bird concentrations. The risk of infection by the disease is known to increase with age and duration of exposure (Thoen and Fulton, 2003). Mycobacteriosis in lesser flamingos has been associated with suppressed immunity but the factors responsible for the latter have not been clarified (Harper *et al.,* 2003).

2.2.2.1.2 Fowl cholera

Fowl cholera, caused by *Pasteurella multocida*, was confirmed as the proximate cause of lesser flamingo deaths in Lake Bogoria in 2002 (Beasley *et al., 2004)* and was also associated with mass deaths of the species in Lake Nakuru in 2004 (Manyibe *et al*., 2007).

Fowl cholera is a contagious disease affecting a wide range of domestic and wild birds (Glisson *et al.,* 2003). It usually occurs as a septicaemic disease with high morbidity and mortality but chronic or benign conditions do occur (Glisson *et al.,* 2003). *Pasteurella multocida* has naturally

infected over 100 species of wild birds, its greatest impact having been documented among North American wild fowl (Botzler, 1991). Epizootics may involve thousands of birds with some species suffering proportionately greater mortality than others (Botzler, 1991).

Glisson *et al.* (2003) has described the pathology caused by avian cholera. Many signs caused by the diseases are due to an endotoxin produced by *P. Multocida*. Sick birds are rarely seen among the infected waterfowl due to the rapid death following the onset of signs. At the agonal stage convulsions and torticollis may occur. Dead birds are usually in good body condition. Internal lesions vary but commonly comprise petechial and ecchymotic haemorrhages on the myocardium, mucoid enteritis, focal necrosis of liver and other internal organs.

The epizootiology of avian cholera in the lesser flamingos and other wild and domesticated birds in and around the Rift valley lakes of East Africa is not well understood.

2.2.2.1.3 Other septicaemic or localized infections

Various other bacteria that have been associated with deaths of lesser flamingo in the Rift valley lakes include *Pseudomonas aeruginosa*, *Eschericha coli* and *Staphylococcus aureus*. Both *P. aeruginosa* and *E. coli* have been associated with septicaemia and death of flamingos in Lakes Bogoria and Nakuru in 1993 (Kock *et al.,* 1999) and in lake Nakuru in 2004 and 2006 (Manyibe *et al.,* 2007.).

Barnes (2003) has described *P. aeruginosa* as a ubiquitous organism considered to be an opportunistic pathogen that is capable of producing localised or sepicaemic infections in susceptible poultry. The bacteria can infect birds of any age but mostly young or immunodeficient birds are more susceptible. Concurrent infections with viruses or other bacteria are common and may affect susceptibility to *P. aeruginosa.* Morbidity and mortality are usually 2-10% but can be high, approaching 100%.

Avian pathogenic *Escherichia coli* is recognized as the cause of localized and systemic infections referred to as collibacillosis (Gross *et al.*, 2003). Both systemic collibacillosis, referred to as collisepticaemia, and the localized disease have several distinct clinical forms depending on the parts of the body affected and the process of the infection (Gross *et al.*, 2003). Most, if not all, avian species are susceptible to collibacillosis as a secondary infection due to impaired host immunity (Gross *et al.*, 2003). *Escherichia coli* is present in the intestinal tract of most animals and is shed in faeces in large numbers (Gross *et al.*, 2003).

Andreason (2003) has described *Staphylococcus aureus* infections as being common in poultry, most frequently affecting bones, tendon sheaths and joints, especially the tibiotarsal and stifle joints. Staphyloccocal septicaemia causes acute deaths and resembles avian cholera. Localized infections are characterized by lameness of one or both legs, drooping of one or both wings, reluctance to walk and fever. Birds surviving the acute disease have swollen joints, sit on their hocks and keel bone and are reluctant or unable to stand. Gross lesions comprise osteomyelitis manifested as focal yellow areas of caseous exudates or lytic areas on bones; arthritis,

periarthritis and synovitis manifested as swelling of affected joints due to filling with inflammatory exudate. Gross lesions of Staphylococcal septicaemia consist of necrosis and vascular congestion in many internal organs including liver, spleen, kidneys and lungs. All avian species are susceptible to staphylococcal infections. *Staphylococcus* species are ubiquitous normal inhabitants of the skin and mucus membranes as normal flora, and common environmental contaminants. Some have the potential to be pathogenic and produce disease.

2.2.2.2 Parasitic diseases of lesser flamingo

2.2.2.2.1 Helminth parasites

Seven species of cestodes and two of nematodes have been reported in flamingos from the Rift Valley lakes. The cestodes are *Cladogynia phoeniconaiadis, Flamingolepis tengizi, F. dolguschini, Gynandrotaenia stammeri, Leptotaenia sp.,* and *Phoenicolepis nakurensis*, while the nematodes are *Tetrameres sp.,* and *Striatofilaria phoenicopteri* (Jones and Khalil, 1980). Comparative studies of the prevalence of the helminth parasites and their associated pathology in healthy and sick lesser flamingos have not been reported. Such studies would be useful in assessing the significance of the parasites in the health of the birds.

2.2.2.2.2 Protozoan parasites

Karstad *et al*. (1981) have described besnoitia-like organisms in lesser flamingos at Lake Nakuru occurring in at least four developmental stages in association with arteritis. The arteritis, mostly

affecting the intima, was age related with a prevalence of 90% in adult birds and 70% in immature birds. The arteries most commonly affected were the medium sized vessels of the gastrointestinal tract, particularly the serosal arteries of the small intestine and the pancreatic arteries. Coronary arteries were affected occasionally and aorta rarely. The various forms of the parasite found in the media and intima of large arteries, the tunica muscularis of the small intestine and the lamina propria of the intestines have been described in detail by the authors. They also reported renal coccidiosis, affecting the ureters in 10 out of 60 flamingos and macroscopic sarcocysts in the skeletal muscle of one lesser flamingo during their survey. However, it was not clear whether the renal coccidiosis was related to the besnoitia-like forms in flamingos. The morbidity of these protozoan infections and their effects on flamingo health has not been studied.

2.2.2.3 Viral diseases of lesser flamingo

Other than tests conducted on lesser flamingos for pathogenic avian influenza (HPAI) (Manyibe et al 2007), there have been hardly any reports on investigations of viral diseases in the lesser flamingo population in East Africa.

2.2.3 Role of toxicological diseases

2.2.3.1 Cyanobacterial toxins

Cyanobacterial toxins have been implicated as possible causes of deaths of lesser flamingos in the East African Rift valley lakes (Ballot *et al*., 2004; Ballot *et al*., 2005; Krienitz *et al*., 2003; Krienitz *et al*., 2005; Lugomela *et al*., 2006; Motelin *et al*., 2000). These toxins have also been reported as causes of mortality in wild birds (Matsunaga *et al*., 1999; Carmichael, 1997) and even humans (Carmichael *et al*., 2003) in other parts of the world.

Cyanobacteria (blue-green algae) are unicellular or microscopically filamentous organisms that rely on photosynthesis for energy, have a cell wall like that of gram negative bacteria, and occur in waters of varied organic and ionic composition (Fogg *et al*., 1973). About forty species of cyanobacteria have been implicated in the generation of toxic blooms (Carmichael, 1997). Known toxin producing species may have both toxin-producing and non-toxin-producing strains occurring in the same bloom. Toxicity can vary between clones of the same isolate (Carmichael, 1992). Some strains produce multiple toxins with relative proportion being influenced by the environment (Carmichael, 1992). The factors which trigger algal toxin formation are not precisely known (Carmichael, 1992).

Cyanobacterial toxins are complex compounds that fall into three broad groups of chemical structure; cyclic peptides, alkaloids and lipopolysaccharides (Kuiper-Goodman *et al*., 1999). According to mode of action these toxins include hepatotoxins (microcystins and nodularins),

cytotoxins (cylindrospermospin) and neurotoxins (anatoxin-a, saxitoxins and anatoxin-a(s) (Kuiper-Goodman *et al*., 1999).

Cyanobacterial hepatotoxins (five structural variants of microcystin; MC-LR, MC-RR, MC-LA, MC-LF, MC-YR) and a neurotoxin (anatoxin-a) have been identified in cyanobacterial blooms in Lakes Nakuru and Bogoria and in cyanobacterial mats growing at the shore of the hot springs in lake Bogoria (Ballot *et al*., 2004; Krienitz *et al.*, 2005). Toxic strains of *Arthrospira fusiformis* (the main food for lesser flamingo) have also been isolated from both lakes (Krienitz *et al*., 2005). Other potentially toxigenic cyanobacteria found in the lakes include; *Formidium terebriformis, Spirulina subsalsa, Oscillatoria willei, Synecoccus bigranulatus, Anabaena spp*., *Anabaenopsis abijatae* and *Anabaenospsis arnoldii* (Krienitz *et al*., 2005).

Detection of cyanotoxins in various body parts of birds that died during toxic blooms (Krienitz *et al.*, 2003; Metcalf *et al*., 2006) and experimental mouse bioassays (Lugomela *et al*., 2006) provide circumstantial evidence of the possible role of the toxins in causing mass deaths. However, the characteristic microscopic lesions associated with cyanobacterial hepatotoxicosis (Yoshida *et al.*, 1997) have not been described in the affected birds.

2.2.3.2 Chemical pollutants

Surveys of pollution by trace metals and pesticides in the Rift valley lakes of East Africa have been conducted by various workers since 1970 (Beasley et al., 2004; Kairu, 1994; Kairu, 1996;

Koeman *et al*., 1972; Lincer *et al*., 1981; Motelin *et al*., 2000; Nelson *et al*., 1998;). Among the trace elements that have been surveyed are cadmium, arsenic, lead, mercury, copper, chromium, iron, zinc, tin and selenium (Beasley et al., 2004; Kairu, 1996; Koeman *et al*., 1972; Motelin *et al*., 2000). The pesticides that have been surveyed include dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), 1,1-dichloro-2,2-bis(p-chlorophenyl) ethane (DDD), Dieldrin, Endrin, lindane (benzene hexachloride (BHC)) isomers, aldrin, heptachlor, and heptachlor-epoxide (Kairu, 1994; Koeman *et al*., 1972; Lincer *et al*., 1981; Motelin *et al*., 2000).

In a preliminary survey of pollution of Lake Nakuru, Koeman *et al.* (1972) analyzed the levels of trace metals (As, Sb, Cu, Zn, Cd and Hg) in kidney and livers of two lesser flamingos and one white pelican, and in one whole *Tilapia grahami*. Copper and zinc appeared to be the most prominent elements but it was not possible to determine conclusively whether the levels of these elements found in the tissues were in the toxic ranges. Motelin *et al.* (2000) reported presence of trace metals in tissue livers and kidneys of lesser flamingos collected from Lakes Nakuru and Bogoria in 1993, 1995 and 1997/1998 during die-offs that affected the species in these areas. Arsenic, lead, mercury, copper, cadmium, chromium, iron, zinc and selenium were found in the tissues of the birds in various amounts. Although Motelin *et al.* (2000) implicated the metals as a possible cause of mortality, alongside other toxicants, they did not compare the levels they found with those reported to be toxic or elevated in other species. They also did not report pathological lesions specific to acute metal toxicosis. Nelson *et al.* (1998) found a wide range of trace elements in sediments obtained from 11 sites in Lake Nakuru. They described a model of

ingestion by lesser flamingos of the trace elements associated with *A. fusiformis* and indicated that the birds were potentially exposed to toxic levels of some trace elements.

Beasley *et al*. (2004) have discussed the levels of trace elements that they found in tissues collected from Lakes Nakuru and Bogoria during lesser flamingo die-offs in 1993, 1995, 2000 and 2002. Arsenic, cadmium, copper, mercury, lead and selenium were below the elevated or toxic levels reported for other species. However, iron, chromium and zinc were within the elevated or toxic ranges reported for other species. The high levels of these three trace elements were not associated with any lesions consistent with metal poisoning (Sileo *et al*., 2003 and Carpenter *et al*., 2004). Certain physiological factors and disease conditions have been reported to cause accumulation of trace metals in tissues (Puls, 1994; Wardsworth *et al*., 1983; Lowenstein and Munson, 1999; Borch-iohnsen *et al*., 1991). The current information is therefore not enough to enable accurate conclusions to be made regarding the significance of these apparently high levels of trace metals to the health of lesser flamingos.

Surveys of pesticides in the Rift Valley lakes since 1970 have shown presence of low levels of chlorinated hydrocarbon pesticides, especially DDE, in tissues of birds, fish and other fauna (Koeman *et al.*, 1972; Lincer *et al*., 1981; Kairu, 1994). Firm conclusions on the toxicological significance of the levels of pesticides found have not been drawn since no specific deleterious effects have been associated with them. However, the researchers state the need to minimize the use of environmentally persistent pesticides, continue monitoring the pesticide levels in the lakes

and conduct long-term studies to determine the effects of the pesticides on avifauna in the lakes (Koeman *et al*., 1972; Lincer *et al*., 1981; Kairu, 1994).

2.3 Other species of birds found in the lesser flamingo habitats

Over 70 species of waterfowl are found in Lakes Bogoria and Nakuru; the latter lake usually hosts more species (Imboma et al., 2009). About 50-60 % of these species are resident and the rest are migrant (Zimmerman *et al.,* 1999). Resident species are more or less constantly within the lakes and respond to local conditions by local movements. Migrant species move into the lakes and then go back to their countries of origin at specific seasons in response to prevailing conditions in those countries.

Two types of migrant waterfowl species exist in the lakes, namely; the Palearctic and Afrotropical migrants. Palearctic migrants originate mostly from regions with temperate climates including parts of Europe, Russia, Asia, Mediterranean basin and the Sahara among others. Afro-tropical migrants originate from Sub-Sahara Africa and Madagascar. Palearctic migrants comprise 20-30 % of the species in the lakes (Zimmerman*et al.*, 1999).

CHAPTER 3

3. MATERIALS AND METHODS

3.1 Study sites

The study was done in Lakes Nakuru and Bogoria both of which are saline-alkaline lakes found in the Rift Valley of Kenya. The lakes are described below.

3.1.1 Lake Nakuru

Lake Nakuru (Fig. 6) is a small, shallow, alkaline-saline lake in Kenya's Rift Valley. It is located at 36^{o} 05' E, 00^{o} 24' S and stands at an altitude of 1860m above sea level. It has a surface area of 44 km^2 with an average depth of 1.8 m but which is highly variable, ranging from complete dryness to 4m (Vareschi, 1985; Ramsar Convention 2005 a). The lake lies within a national park that is 188 km^2 within a closed basin of about 1800 km^2 (KWS, 2002; Ramsar convention, 2005a). The lake has an average water pH of 10.5, conductivity that oscillates from 9,000 to 160,000 µS and mean salinity of 45% (Vareschi, 1978). The main ions are sodium, bicarbonate and carbonate. The lake's high alkalinity, conductivity and other physical-chemical parameters make it uninhabitable to many aquatic species (Vareschi, 1978). However, the few that have adapted to these harsh conditions have made the lake to be one of the highest producers of biomass among Kenya's Rift Valley saline lakes (Vareschi, 1982). The lake has large densities of the cyanobcterium (*Arthrospira fusiformis* that often occurs as a single species bloom that can

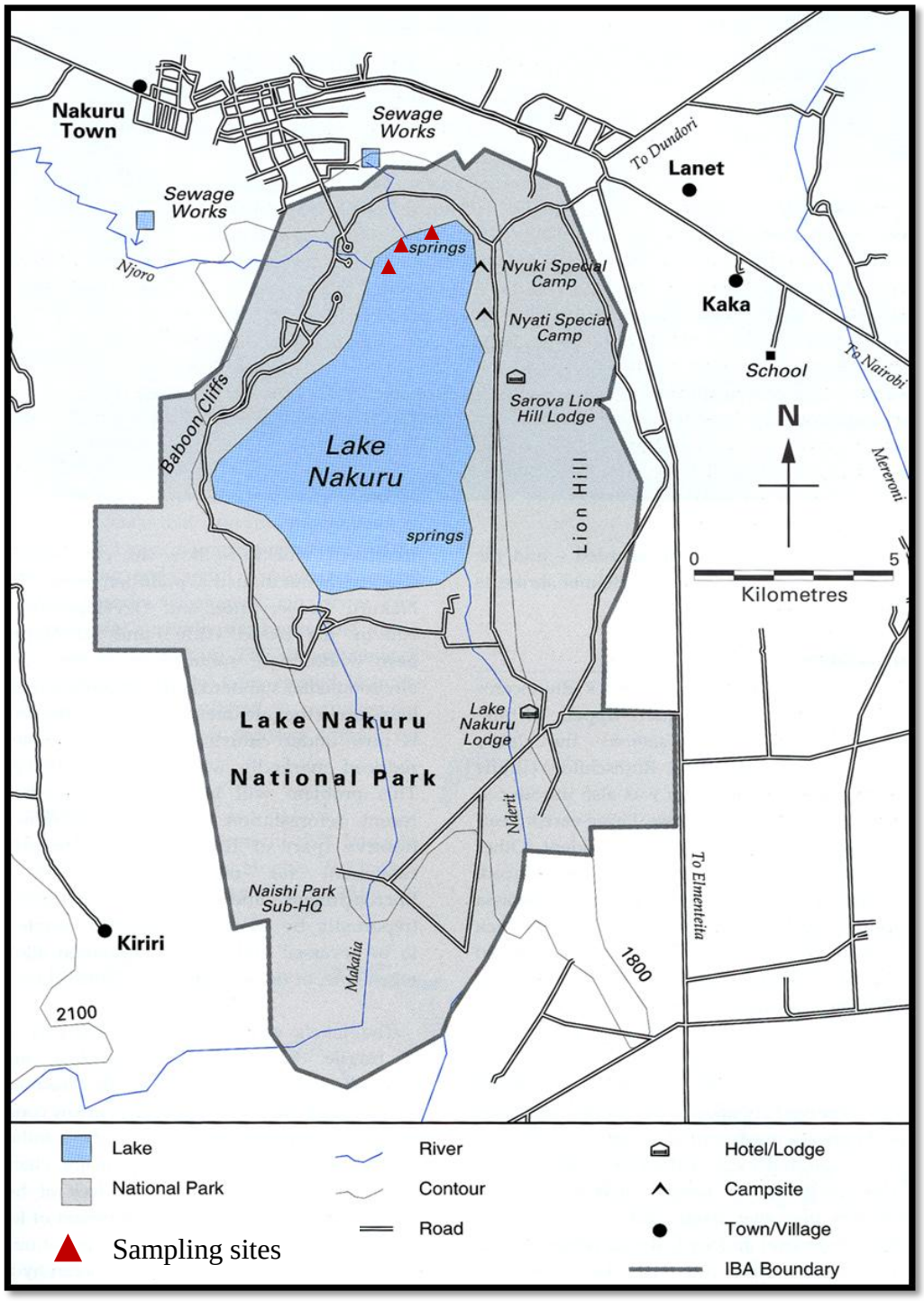

Figure 6: Map of Lake Nakuru showing detailed geographic features
(adapted from Bennun and Njoroge, 1999).

support over 1 million LF (Vareschi, 1982, 1985). The introduction of the fish species *Sarotherodon alcalicus grahami* in 1960s has enriched waterbird diversity bysupporting hundreds of fish eating water birds, most notably the Great White Pelican (*Pelecanus onocrotalus*) and Great Cormorant *(Phalacrocorax carbo*) Bennun, 1993).

The mean annual rainfall at Nakuru averages 750mm and falls within the periods of November to December and April to May. The rainfall has tri-modal peaks centered on April, August and November (KW, 2006; Bennun & Njoroge, 1999) and it is influenced by the Inter-Tropical Convergence Zone (ITCZ). The climate ranges from cold, hot and humid to arid and semi-arid. The maximum and minimum-recorded temperatures in the area are 33^{o}C and 12^{o}C, respectively (KWS, 2002; Bennun & Njoroge, 1999).

Lake Nakuru is supplied by five seasonal rivers: Makalia, Nderit, Naishi, Njoro and Larmudiac and by treated wastewater from Nakuru Town. There is also some recharge from the natural springs on the north end of the lake. Most of the rivers are tapped for irrigation and other forms of use before they reach the lake (KWS, 2002).

Lake Nakuru is located close to Nakuru Town, which is an important local centre of industry and agriculture (Shivoga *et al*., 2007). Effluents from the town's two sewage treatment plants are discharged into the lake, causing considerable danger of contamination (Mathooko, 2001; KWS, 2002). Within the catchment of the lake, land fragmentation continues to occur through urban development, settlements, agriculture and infrastructure development with severe ecological

consequences to the lake (Shivoga *et al*., 2007). The Lake is a designated wetland of international importance under the Ramsar Convention and an Important Bird Area (IBA) (Bennun & Njoroge, 1999; Ramsar Convention, 2005a).

3.1.2 Lake Bogoria

Lake Bogoria (Fig. 7) is an alkaline saline lake in the Rift Valley. It stretches between 36o 4'- 36o 7' E and 0^o 10'- 0^o 20'S, and it is about 65 km to the north of Nakuru at an altitude of 970m above sea level (KWS, 2007; Vareschi, 1978). The lake has a surface area of 33 km2 (33,000 ha) and lies within a National Reserve of 107 km2 (Bennun & Njoroge, 1999). The lake is narrow (16 km long and 3km wide) and shallow with a maximum depth of 10.2m (Vareschi, 1978; Harper et al., 2003). It has an average water pH of about 10.2 and conductivity ranging from 48,000 uS / cm to 88,000 uS/ cm (Githaiga, 1997. The dominant salts are sodium carbonate and sodium hydrogen carbonate.

The climate of Lake Bogoria area is dry and semi arid with annual rainfall that varies from 600mm to 1000mm and evaporation of up to 1600mm (KWS, 2002; Bennun & Njoroge, 1999). The area experiences two rainy periods annually (April-May and October-November), associated with Inter-Tropical Convergence Zone and an additional July-August peak attributed to westerly air flows at this time of year (KWS, 2002; Bennun & Njoroge, 1999). The area is characterized by high ambient temperature of 36°C-38°C (KWS, 2007; Bennun & Njoroge, 1999).

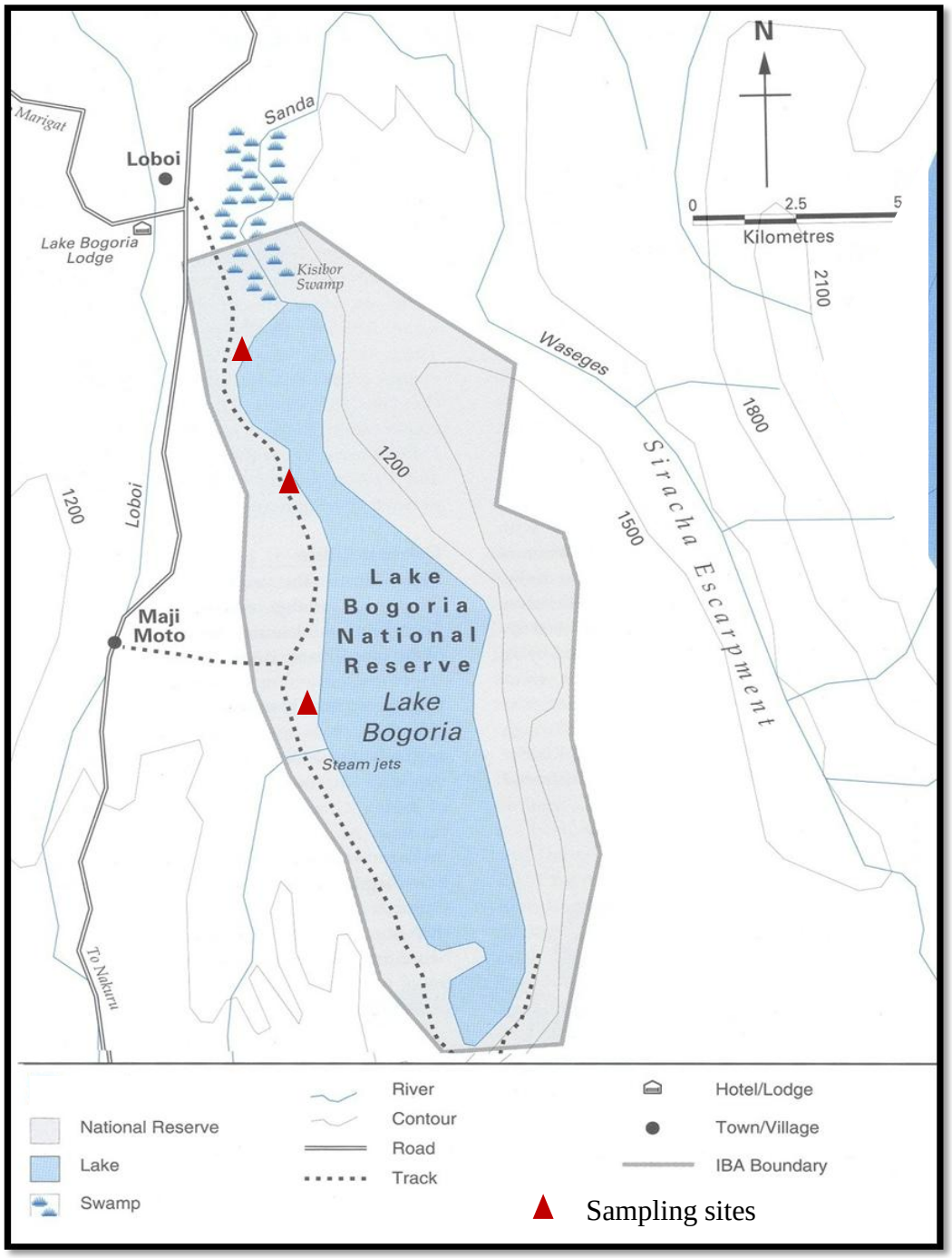

Figure 7: Map of Lake Bogoria showing detailed geographic features
(adapted from Bennun and Njoroge, 1999).

Lake Bogoria is mainly supplied by the Loboi swamps and by hot geysers found at the shore of the lake. The geysers shoot up to 4m high at temperatures of between 140°C and 300°C and they contribute significantly to water balance in the lake. Irrigation and diversion of water from the Loboi swamps has contributed to reduced inflow to the lake (Githaiga, 1997).

Lake Bogoria National Reserve has more than 350 species of waterbirds it is the only reliable feeding site for the LF in East Africa (Bennun & Njoroge, 1999). It occasionally hosts up to one million LF especially when the other lakes in the region are affected by drought (Howard, 1997; Childress, 2005). The lake is a designated wetland of international importance under the Ramsar convention and an Important Bird Area (IBA) (Bennun & Njoroge, 1999; Ramsar Convention, 2005b).

3.2 Study design and sample size determination

Purposive sampling procedure was used and the sample size was calculated using the method as described by Pfeiffer (2002) for estimating the level of disease occurrence. The study sites were chosen because of being the most important habitats for the flamingo species in Kenya. The estimated number of lesser flamingos in the lakes is usually over 10,000, which is considered an infinite population Sample size was therefore determined using the formula below:

$N = 1.96^2 \times p\ (1-p)/d^2$ where N= sample size, p = disease prevalence, d = precision (Pfeiffer, 2002)

Since the disease prevalence was unknown, the value of p was set at 0.5. The value of d was set at 0.05. The minimum sample required was therefore 384 birds.

3.2.1 Assumptions of the study:

The study design was based on the following assumptions:

1. The method of trapping (described in section 3.5 below) would ensure random capture of birds from the accessible population regardless of individual bird characteristics.
2. The lesser flamingo population would remain adequate for successful capture during the scheduled field visits.
3. The weather at the lakes would be condusive for successful capture of birds during the scheduled visits.
4. There would be a number of dead or sick birds at the lakes during scheduled visits

3.3 Field visits and preliminary assessments.

3.3.1 Field visits

Field visits were made quarterly to the lakes to sample the lesser flamingo and record prevailing environmental conditions during both the dry and the wet seasons. Since the birds are nomadic and their populations shift unpredictably between lakes, the visits were planned based on abundance of the birds and accessibility of the trapping sites as reported by the field officers that the researcher was liaising with. Scheduling of the visits was also influenced by logistical

considerations such as availability, from the collaborating institutions, of personnel and transport for the capture of lesser flamingos.

3.3.2 Gathering of historical data

Key informant interviews targeting the research officers from the respective wildlife management authorities (Lake Nakuru National Park and Lake Bogoria National Reserve) were conducted during the visits. Information gathered through the interviews included the following: recent population trends and movement patterns of lesser flamingo and other water birds; any cases of dead or sick birds observed; any drastic changes in rainfall and temperature; any changes in water level in the lake, water flow at the lake inlets and any changes in lake algae. The form for gathering field data that was developed for this study is in Appendix 1. Supporting data from research records was gathered whenever possible.

3.3.3 General observation of lesser flamingos and their environment.

The lake shores were patrolled to determine the following: level and colour of water in the lake; flow of water in the inlets; relative abundance of lesser flamingo; relative abundance of other birds, age structure of the flamingos; presence of sick or dead birds; approximate duration of carcasses and any signs of sickness observed (Appendix 1).

3.3.4 Estimation of population of lesser flamingo and other water birds

The population of lesser flamingo was estimated during each visit using the techniques described by Rose and Scott (1997) and Bibby *et al.* (1998). The estimation was conducted from ground position by two experienced bird specialists from the NMK who had been regularly involved in the biennual waterfowl censuses that are conducted by the institution in collaboration with KWS. Other bird species within a radius about 300m off the lake shore were identified and their populations also estimated.

3.3.5 Capture and examination of the birds

After the general observation of the lesser flamingo population, some birds were captured randomly using the method described by Childress and Jarrett (2005). Traps consisting of rectangular wire mesh grids with multiple nooses made of polyethylene fishing line were placed under water a few metres from the shoreline in areas where many birds congregate while feeding (Figs. 8 and 9). Three to four traps were placed and monitored from a distance for trapped flamingos, indicated by signs of struggle. The success of capture was variable depending on the concentration of the birds and accessibility of trapping sites. Protective clothing (hood, gloves, coveralls and gumboots/wading suit) was worn while handling the birds to minimize the risk of exchange of zoonotic diseases (Evans and Carey, 1986).

The trapped birds were freed from the snares and handled humanely as described by Gaunt *et al.*, 1999. The heads were covered with a hood to eliminate visual stimuli and to minimize stress to the bird. Weak birds were captured by hand. Several capture sites were selected in each lake where possible. Captured birds were examined individually for evidence of external injuries, other visual or palpable abnormalities, and presence of external parasites.

Birds were weighed using the Pesola® Lightline Spring Scales (Pesola AG, Rebmattli 19, CH-6340 Baar, Switzerland). They were classified into different age groups using physical identification features as described by Sileo *et al.*, 1977; Zimmerman *et al.*, 1999; Childress *et al*., 2005; and Hockey *et al*., 2005. These features included colour of the bill, iris, head, neck, legs, back and plumage (Table 2).

The body condition of the birds was scored using a method adopted from Gregory and Robins (1998) (Figure 10). The bird was palpated to grade the protuberance of the keel, the development of the breast muscles alongside the ventral ridge of the keel, and the convexity or concavity of the breast muscle contour.

Figure 8: A trap made of wire mesh and fishing line nooses being prepared for capture of lesser flamingo in Lake Nakuru during the study

Figure 9: A KWS ranger wading through the mud to lay a trap for capture of lesser flamingo during the study

Table 2: Morphological characteristics for determining age in lesser flamingos

Age classes	Physical identification features
Juvenile [< 1 yr old]	Bill: Dark grey Iris: Brown Head and neck: Grey Back: Grey or white Legs: Grey legs and feet Plumage: Greyish. Underwing coverts- First pink at 6 months
Immature [1-3 yrs old] **A**	Bill: Light red Iris: Brown or orange Head and neck: White or pink Back: White or pink Legs: Grey or pink Plumage: Whitish with no red in wings
Adult (Breeding) [>3 yrs old]	Bill: Dark red, black tipped Iris: Yellow to orange Head and neck: Pink Back: Pink Legs: Bright red legs and feet Plumage: Deep pink, deeper reddish pink on breasts,
Adult (Non-breeding)	Same as above but body plumage much paler and whiter.

Ref: Sileo *et al.*, 1977; Zimmerman *et al.*, 1999; Childress *et al.*, 2005; and Hockey *et al.*, 2005

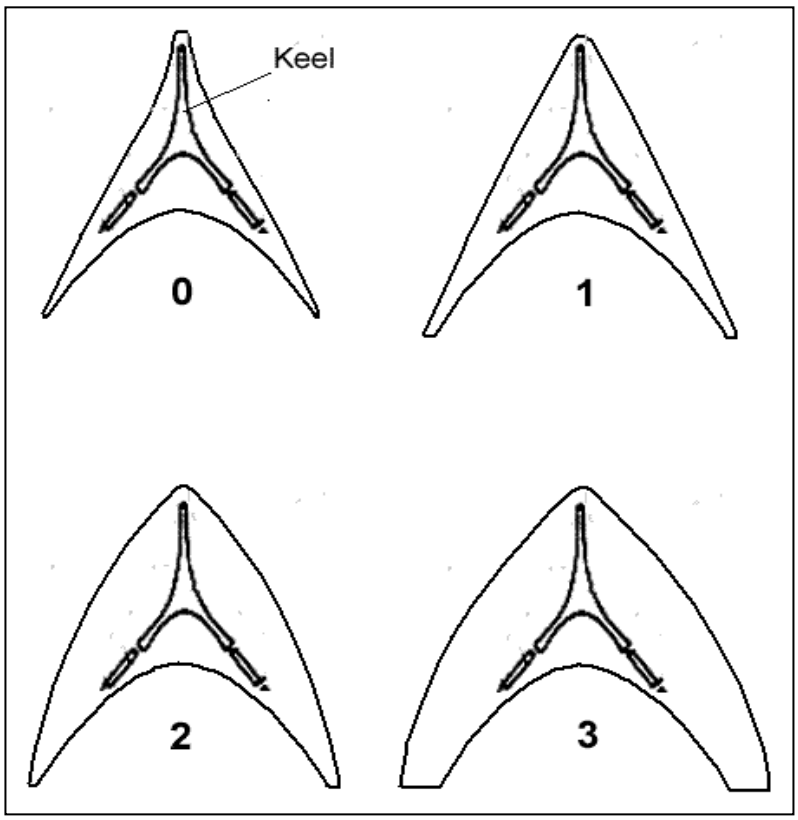

Figure 10: Criteria used to score body condition of lesser flamingo during the study
(Adopted from Gregory and Robins 1998)

Key

Body condition scoring criteria

0- Emaciated: Prominent ridge on the keel with limited overall breast muscle and a concavity of the breast muscle alongside the keel

1- Poor: Greater development of breast muscle which is not concave and feels more or less flat. The keel is still prominent.

2- Fair: Moderately developed convex breast muscle. The keel is less prominent.

3- Good: Well developed relatively plump breast. Smooth over the keel.

3.4 Assessment of lesser flamingo pathology

3.4.1 Haematology

Blood was drawn from the right jugular, the caudal tibial or the cutaneous ulnar veins using 23 gauge hypodermic needles and 2ml syringes (Morton *et al.,* 1993; Campbell, 1994). Samples were immediately transferred into a microcollection tube coated with heparin and shaken gently to allow proper mixing with the anticoagulant. Meanwhile, the last drop from the syringe was placed on a glass slide and a thin blood smear was prepared. The smear was dried by waving it in the air and it was then stored in a slide box for further processing in the laboratory. All samples were labeled with species, location, date, age and a unique identification number of the bird using a waterproof indelible marker pen.The whole blood was transported in a cool box to the laboratory where it was analyzed to determine absolute cell counts and centrifuged to obtain plasma. The blood samples were transported to the laboratory within 4 hours after sampling.

In the laboratory, blood smears were fixed in absolute methanol and stained using the Diff Quik Stain (American Scientific Products, Division of American Hospital Supply Corporation, McGraw Park, IL 60085), within three hours after preparation of the smears (Campbell, 1994). Differential white cell counts were conducted based on a count of 300 cells while examining for haemoparasites at the same time.

The Phloxine-B semi-direct technique was used to determine the total leucocyte count in whole blood (Campbell, 1994; Walberg, 2001). Absolute heterophil and eosinophil counts were

determined using a hemacytometer with improved Neubauer ruling. The total leucocyte count was then computed using the absolute heterophil and eosinophil count and the differential white blood cell count as described by Campbell (1994).

3.4.2 Gross post-mortem assessment.

Necropsy of birds taken from Lake Nakuru was conducted in Nakuru Provincial Veterinary Investigation Laboratory. Necropsy of birds taken from Lake Bogoria was done in the field, in a designated area that was cordoned off. The live birds were euthanized using 0.2-0.5 ml of 20% pentobarbitone sodium injected via occipital sinus as per guidelines given by Gaunt *et al.* (1999). Necropsy was done using the standard procedures described elsewhere (Charles and Sandra 1980; Olafson, 1954) and also a necropsy protocol that was adopted from the Envirovet Summer Institute training (Appendix 1). Samples at autopsy were taken for laboratory analysis using a check-list adopted from the same source (Appendix 2).

Gross lesions were recorded in a necropsy sheet (Appendix 3). The whole liver, spleen and where possible bursa of Fabricius of each bird was weighed using the Pesola® Lightline Spring Scales (Pesola AG, Rebmattli 19, CH-6340 Baar, Switzerland).

Tissue samples for histopathology were collected from various organs as per the check-list, including: liver, pancreas, kidneys, lungs, spleen, brain, heart, muscle, tongue, oesophagus, proventriculus, gizzard, bursa of Fabricius and intestine. Samples for histopathology were about 1cm thickbut long and wide enough. The samples were preserved in 10% buffered formalin at a

tissue to formalin ratio of 1:9. Screw-capped plastic bottles with a wide mouth and capacity of about 250ml were used. The remaining pieces of liver, kidneys and brain were kept in zip-lock plastic containers and transported in a cool box to the laboratory at KWS where they were stored in a freezer at -20^{o}Cas part of the tissue bank.

3.4.3 Histopathology

Tissue samples in 10% buffered formalin were processed for histopathological study using the standard procedure described by Culling (1974). The tissues were trimmed into 3-5mm pieces that were processed using an automatic tissue processor and embedded in paraffin wax (Fisher Tissue Manton, Fisher Scientific Co.).

The tissues were first dehydrated in alcohol baths of gradually increasing concentration namely, 50%, 70%, 96%, and 100%. They were then cleared in xylene for 3 hours to displace the alcohol before being infiltratred with paraffin. Infiltration was done in glass jars containing wax that was heated to 56-60^{0}C. Infiltrated tissues were transferred into a final molten wax bath in a clearly labeled mould of paper-boat, which was then cooled to form a block. Small blocks of embedded tissue were trimmed and attached to blocks of wood using heat (blocking). Each blocked tissue was clearly labeled with the laboratory identification number and stored in a box until it was sectioned.

Tissue sections were prepared at a thickness of 5 microns using a rotary microtome. Sections were floated on a warm water bath to straighten out. Each section was then transferred to an egg

albumin pre-coated microscope slide by dipping the clean slide in the water bath, and pulling it out in such a way as to allow the section to adhere to the albumin coated side. The slides were clearly labeled using a diamond pen. They were then dried on an electrically warmed rack at 60 ^{o}C for 30 minutes to adhere the sections on to glass slides.

The sections were stained with haematoxylin and eosin using the procedure described by Culling (1974). In outline, the sections were: de-waxed using xylene; hydrated by passing them through baths of alcohol of decreasing concentration; stained with haematoxylin; differentiated by washing in acid alcohol; blued by washing in tap water; counterstained with eosin; blued further by washing in tap water; dehydrated by passing through baths of alcohol of increasing concentration; cleared in xylene and then mounted on slides using DPX mountant and coverslips of an appropriate size for the sections.

Slides were examined at scanning power magnification (x4), followed by low power (x10), then by dry high power (x40) and finally by emersion high power (x100) magnifications using the Leica® DM 500 model of compound microscope with an inbuilt camera. Lesions were scored as mild, moderate, marked or severe using a criteria based on size, number or intensity as illustrated below:

1. Mild:

a) One to two small (about 0.5mm diameter or less) focal lesions in the examined tissue.

b) For cellular infiltration, less than ten inflammatory cells per ten fields examined under high (x 100 objectives) power.

2. Moderate:
    a) One to two medium sized focal lesions (up to 1.0 mm diameter) in the tissue or three to five small lesions.
    b) For cellular infiltration, an average of 1-2 inflammatory cells per field in a random count of ten fields under high (x100 objective) power.
3. Marked
    a) One or two large sized focal lesions (up to 3mm), three to four medium sized lesions or five to 10 small ones.
    b) For cellular infiltration, an average of 3-5 inflammatory cells per field in a random count of ten fields under high (x100 objective) power.
4. Severe
    a) More than two large sized focal lesions, more than four medium sized lesions or more than 10 small lesions
    b) For cellular infiltration, an average of more than 5 inflammatory cells per field in a random count of ten field under high (x100 objective) power.

3.4.4 Retrospective histopathology.

Tissue samples of lesser flamingos that were collected from previous studies in 1997, 1999, 2000, 2001 and 2004 and preserved in paraffin wax blocks at KWS and University of Nairobi laboratories were retrieved, sectioned and stained with haematoxylin and eosin and examined under the microscope as described in section 3.5.3. The samples obtained in 2004 had been taken during mass mortality of the species. The other retrospective samples were taken during other

studies that involved trapping of healthy birds as well as opportunistic sampling of sick and dead ones.

3.5. Assessment of pathogenic bacteria

Samples for bacteriology were taken from sick and healthy birds as well as from fresh carcasses. Samples were obtained immediately after opening the birds from large internal organs, mostly liver and spleen and in a few cases from lung and the surface of coelomic cavity. The surfaces of organs were flamed and the organs then deeply incised using sterile surgical blade. The swab was inserted into the incision to soak in the organs tissue fluid then transferred into transport media near a Bunsen flame to avoid contamination. The swab was inserted into the transport medium in bijou bottle and the bottle closed. The cap of the bottle was flamed before closing. The samples were then transferred to the laboratory and stored at -4°C until they were tested.

Samples in transport media were inoculated into blood agar and McKonkey agar plates using the standard procedure and incubated for up to 72 hours. At the same time direct smears were made from the transport media and stained with Gram stain for examination of cellular morphology of bacteria. Plates were examined for colony growth at 24 hr, 48hr and after 72 hrs. Based on colony characteristics and cellular morphology of bacteria growing in either of the media, mixed colonies were sub-cultured to obtain pure colonies. Respective characterization was done using biochemical tests and, where necessary, serological tests as per the techniques described by Cheesbrough (2000) and Merchant and Parker (1983). Impression smears and histological tissue sections from organs with granulomatous lesions were stained using the Ziehl-Neelsen procedure

as described by Cheesbrough (2000) and Bartholomew 1981 and examined for *Mycobacterium spp.*

3.6. Assessment of endoparasites

Collection and quantification of endoparasites was done using methods described by Doster and Goater (1997). Different parts of the gut were slit open longitudinally and placed with their ingesta in separate 250 ml containers half-filled with 70% alcohol. The containers were shaken to ensure that the worms were exposed to the fixative and then transported to the laboratory for analysis. In the laboratory, the containers were further agitated to detach the worms from the intestine and the suspension was passed through a sieve of 250 microns to retain the worms. The worms were transferred into a petri dish, sorted by type (nematodes or cestodes) and counted.

Whole mount preparations of cestodes were stained in aceto-carmine (ACETO 3100®, Kobian Kenya Ltd.) and identified by examining the hooks, the scolex, the shapes of the egg pockets and genital organs under the dissecting microscope using the techniques as described byMcLaughling, 2000 and Doster and Goater, 1997. Nematodes were cleared in lactic acid and identified by examining the spicules, bursal rays, caudal alae and the oesophagus under the dissecting microscope using the techniques as described the same authors.

3.7 Assessment of environmental factors.

3.7.1 Water quality assessment during visits

The conductivity and pH of water at the bird capture sites was measured during the field visits using a pH meter. Water samples from the same sites were taken in glass bottles and submitted to the Nakuru Water Testing Laboratory for determination of concentration of *Arthrospira.spp*.

Additional water samples were also taken from the same sites in sterile universal bottles and transported in a cool box to the bacteriology laboratory for bacteriological testing at the Department of Microbiology Parasitology and Pathology, Faculty of Veterinary Medicine, University of Nairobi.

3.7.2 Collation of restrospective environmental data

The mean monthly rainfall as well as mean monthly physicochemical and biological qualities of Lake Nakuru from 2008 to 2010 was collated from KWS records.

3.7.2.1 Mean monthly rainfall

The mean monthly rainfall of Lake Nakuru that was collated from KWS records had been detrmined based on daily readings from rain gauges located in 7 stations around the lake, namely; Naishi, Main Gate, Lanet, Nderit, Nganyoi, Pwani and Zakaria. The mean monthly total from the seven stations was computed as the monthly mean for the lake.

3.7.2.2 Physicochemical qualities of water

Monthly measurements of lake level that were collated from KWS had been determined from the readings of a water gauge located at lake-centre. Monthly measurements of conductivity, level of nitrates, level of ammonium compounds and concentration of arthrospira in lake water had been determined based on monthly analysis of water at taken from lake-centre. Conductivity had been determined using a pH meter. Level of nitrates had been determined using the cadmium reduction method as described by Eaton *et al.,* (2005). Level of ammonium compounds had been determined by flow injection analysis method using Nessler's reagent as described by Krug et al., (1979). The concentration of arthrospira had been detrmined using the Sedgewick-Rafter counting chamber as described by Kimberly (1999).

3.7.2.3 Populations of lesser flamingos and other water birds

Data on population estimates of water birds in the lakes from 2000 to 2010 that was collated from NMK records had been determined using the on-the-ground counting method as described by Rose and Scott (1997) and Bibby *et al.* (1998). The data was based on records of the January and July waterbird counts that are conducted joinly by NMK and KWS.

3.8 Data analysis and presentation

The data was entered into Ms Excel® spreadsheet then exported to SPSS® statistical package for analysis. Descriptive statistics were computed for the characteristics under study and analysis of variance (ANOVA) was done to test for significant differences.Values of $P< 0.05$ were considered significant (Mason *et al*., 2003).

Mean score of severity of pathological lesions, based on the scoring criteria described in section 3.4.3, was used as a composite indicator of the degree of severity of lesions in the individual birds and the frequency of the lesions in the group. Higher mean scores indicated lesions that were relatively more severe in individual birds and relatively more frequent in the group. Lower mean scores indicated lesions that were relatively mild in individual birds or those that were severe in individual birds but relatively less frequent in the group.

Data sets on selected environmental parameters were plotted on combined charts to show patterns, trends and relationships. Relationships were tested using correlation and regression analysis (Mason *et al.,* 2003).

CHAPTER 4:

4. RESULTS

4.1 Preamble

The results of the assessment of pathological lesions, pathogenic bacteria and endoparasites in the lesser flamingo population and the analysis of underlying environmental factors are presented in this chapter.

A sample size of 384 birds was required for determination of prevalences of pathological lesions, pathogenic bacteria and endoparasites in the population of lesser flamingo (Section 3.2). This was, however, not attained due to logistical constraints related to capture of the birds (Section 5.3). Consequently, a sample size of 57 birds was obtained from the two study sites during four visits that included the dry and wet seasons. Clinical signs and gross postmortem lesions were determined in all the birds. Histopathological lesions and endoparasites were determined in 53 birds and presence or absence of pathogenic bacteria was determined in 46 birds. These sample sizes, though too low for determination of prevalence, were adequate, at 95% confidence limits, to detect presence of disease attributes that had a prevalence of 5-6 % or more in the population (Pfeiffer, 2002). The formula for calculating the sample size required to detect presence of disease in infinite populations is given below:

n= (log (1-ß))/ [log (1-d/N]

(n=sample size, ß=level of confidence, d=number of diseased, N=population size)

The population of lesser flamingo at the study sites was over 10,000 and was therefore considered infinite.

Frequencies of pathological lesions, pathogenic bacteria and endoparasites, rather than the prevalences of these attributes, were therefore computed and are presented in the results. The frequencies indicate those attributes that were most commonly encountered and that can therefore be considered to be the most prevalent in the population.

Haematology was conducted only for samples that could be analysed within four hours of collection. This was only feasible for 14 samples collected in Lake Nakuru during the dry season. Similarly ests for *Arthrospira spp* in lake water were conducted but produced un-reliable results and were therefore not included in the analysis (Section 5.3)

Environmental factors measured in Lakes Bogoria and Nakuru during the study and those collated from secondary data are presented at the end of this chapter. Differences in parameters observed during the field visits compared to those from secondary data for corresponding months are largely due to the fact that the measurements of the two sets were made at different times Population of lesser flamingo, for instance, is quite dynamic and estimates made a few days apart can have a big difference.

4.2. Clinical findings

Fifty seven (57) lesser flamingos were examined during the study: 22 were healthy, 17 were found dead and 18 were sick. Sick birds had clinical signs that were either obvious before they were captured or were noticed after they were captured and examined. Birds with no clinical signs were considered healthy. The distribution of the healthy, sick and dead birds by season and location is summarized in Table 3.

Twenty (20) of the birds were examined during the dry season and 37 during the wet season. Ten (10) of the sick birds were examined during the dry season and 8 of them during the wet season. Twelve (12) of the dead birds were examined during the wet season 5 of them during the dry season. Seventeen (17) of the healthy birds were examined during the wet season and 5 of them during the dry season. Birds from Lake Bogoria were all examined during the wet season.

Table 3: Distribution by season, location and condition of the 57 lesser flamingos that were examined during the study

Season	Status	Location		Total
		Lake Bogoria	Lake Nakuru	
Dry	Healthy		5	5
	Dead		5	5
	Sick		10	10
	Total		20	20
Wet	Healthy	16	1	17
	Dead	12	0	12
	Sick	6	2	8
	Total	34	3	37

Weakness and impaired activity was observed in 16 of the 18 sick birds: 10 were observed in Lake Nakuru during the dry season and 6 in Lake Bogoria during the wet season (Table 4 and Figure 11). Weak birds tended to stay near the shore, were often isolated from the rest of the flock, were unable to fly and in three cases comatose (Figure 12). Poor body condition was observed in 6 birds: five in Lake Nakuru during the dry season and one in Lake Bogoria during the wet season.

Diarrhoea was observed in 4/18 birds: two of these were strong birds in good body contition that were captured randomly in Lake Nakuru during the wet season; one was a bird in good body condition with a compound fracture of the wing observed in Lake Bogoria during the wet season and the remaining was a bird in poor body condition observed in Lake Nakuru during the dry season. Diarrhoea was manifested by matting of feathers around the cloaca and discharge of copious watery stool.

Two (2/18) birds examined during the dry season had drooping of the wing (s), unilateral in one and bilateral in the other (Figure 13). A wing fracture was observed in 1/18 bird examined during the wet season in Lake Bogoria. All 18 birds were infested with lice.

Table 4: Frequency of clinical signs in lesser flamingo by season and location during the study

Clinical observation	Frequency				
	Dry season		Wet season		Overall
	Nakuru Mar 09	Nakuru-Jul 09	Bogoria-Nov 09	Nakuru-Mar 10	
1. Weak, impaired activity or coma	2/2	8/8	6/6	0/2	16/18
2. Poor body condition	1/2	4/8	1/6	0/2	6/18
3. Diarrhoea	0/2	1/8	1/6	2/2	4/18
4. Drooping wings	0/2	2/8	0/6	0/2	2/18
5. Wing fracture	0/2	0/8	1/6	0/2	1/18
5. Presence of lice	2/2	8/8	6/6	2/2	18/18

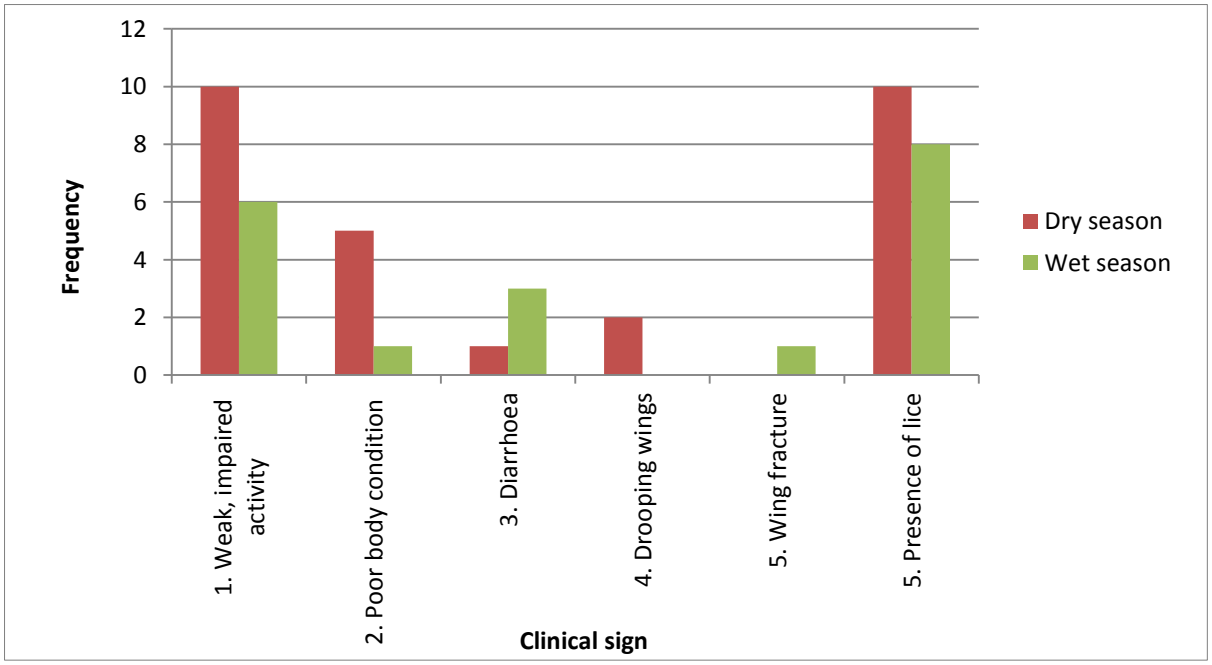

Figure 11: Frequencies of clinical signs in the wet and dry seasons during the study.

Figure 12: A mature lesser flamingo found comatose in Lake Nakuru during the dry season

Figure 13: A sick juvenile lesser flamingo with both wings drooping observed in Lake Nakuru during the dry season

4.3 Pathology

4.3.1 Haematology

Total white blood cell counts (WBC) (cells/µl), absolute lymphocyte, heterophil, eosinophil, monocyte and basophil counts (cells /µl) and total solids were determined for 12 lesser flamingos. Differential cell counts (%), cellular morphology, and presence or absence of haemoparasites were determined for 14 birds. Packed cell volume (PCV) (%) was determined for 13 birds. All the birds for which the various parameters were determined were sampled in Lake Nakuru during the dry season. They comprised 5 mature healthy birds and 9 sick ones. The sick ones comprised 3 juvenile, 3 immature and 3 mature birds. All the healthy birds were in good body condition while among the sick ones, 3 were in good body condition, 5 in poor body condition and the body condition of one was inadvertently not scored.

Total, absolute and differential white blood cell counts were highly variable both in the sick and healthy groups. One healthy bird, LNF4, had an unusually high total white blood cell count of 36x 10^3 cells/ µl compared to the mean for the healthy group of (14.7±10.8) x 10^3 cells/ µl. This bird also had a relatively high lymphocyte count of 31.1 x 10^3 cells/ µl compared to the mean for the healthy group of 18.0 x 10^3 cells/µl. One sick lesser flamingo, LNF13, had a severe leucopaenia of 1.64 x 10^3 cells/ µl with an absolute lymphocyte count of 0.9 x 10^3 cells/ µl, absolute neutrophil count of 0.4 x 10^3 cells/ µl and relatively high differential monocyte count of 10%. The mean haematological parameters of the sick and healthy groups of birds are given in Table 5 and illustrated in Figure 14.

Table 5: Mean haematological values of birds sampled in Lake Nakuru during the the dry season

Parameter	**Clinical status**	
	Healthy**(X±δ (n))**	Sick**(X±δ (n))**
Packed Cell Volume (%)	44.0±6.3 (4)	41.2±10.6(9)
Total solids (plasma proteins) (g/dl)	4.2±1.4 (3)	4.2±1.1 (9)
Total white blood cell count x 10^3	22.8±12.5 (4)	10.6±7.8 (8)
Differential heterophil count (%)	15.4±5.9 (5)	39.0±26.9 (9)
Absolute heterophil count (cells/µl) x 10^3	2.7±1.0 (4)	4.9±4.8 (8)
Differential lymphocyte count (%)	75.6±7.3 (5)	50.0±23.4(9)
Absolute lymphocyte count (cells/µl) x 10^3	18.0±10.6 (4)	3.2±1.9 (8)
Heterophil:Lymphocyte ratio	1:5	4:5
Diferrential granulated lymphocyte count (%)	4.3±2.9 (3)	0.0 (2)
Absolute granulated lymphocyte count (cells/µl) x10^3	1.9±0.4 (2)	0.0(2)
Differential monocyte count (%)	3.8 ± 3.6 (5.0)	4.9±3.6 (9)
Absolute monocyte count (cells/µl) x 10^3	0.8±1.0 (4)	0.4±0.5 (8)
Differential eosinophil count (%)	2.2±1.6 (5)	5.9±5.4 (9)
Absolute eosinophil count (cells/µl) x 10^3	0.48±0.36 (4)	0.44±0.63 (8)
Differential basophil count (%)	0.40±0.55 (5)	0.22±0.29 (9)
Absolute basophil count (cells/µl) x 10^3	0.0(4)	0.0 (8)

Key

X=Mean; **δ =** standard deviation; **n** = Number of birds on which the mean was calculated

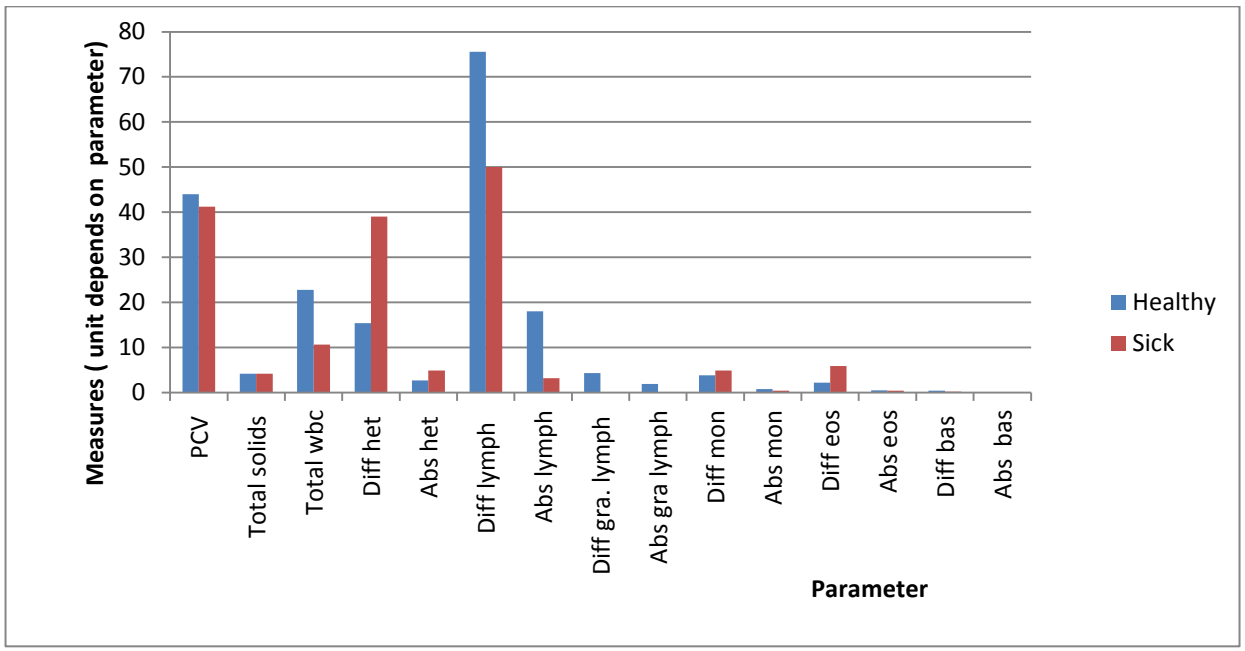

Figure 12: Mean haematological values of lesser flamingos examined in Nakuru during the dry season

Key

PCV= Packed cell volume (%)

Diff mon = Differential monocyte count(%)

wbc = white blood cells

Diff het =Differential heterophil count (%)

Abs het =Absolute heterophil count cells/ µl

Diff lymph =Differential lymphocyte count (%)

Diffgra.lymph= Differential granulated lymphocyte count (%)

Absgra.lymph=Absolute granulated lymphocyte count(cells/ µl)

Abs lymph= Absolute lymphocyte count cells/ µl

Diff mon = Differential monocyte count (%)

Abs mon= Absolute monocyte count (cells/ µl)

Diff eos = Differential eosinophil count (%)

Abs eos = Absolute eosinophil count (cells/ l)

Diff bas = Differential basophil count (%)

Abs bas = Absolute basophil count (cells/ µl)

The mean packed cell volume (PCV) of 4 healthy birds was 44.0±6.3 %. It did not differ significantly from that of the 9 sick birds, which was 41.2±10.6 %. Five of the sick birds that were in poor body condition had a PCV of 34.0±7.3 %: this was significantly lower, at 95% confidence limits, than that of 48.7±5 % for the 3 sick birds in good body condition (p=0.029). There were no significant differences in mean PCV values between different age groups of the sick birds (p=0.664).

The mean value of total solids for 3 healthy birds was 4.2±1.4 g/dl, which was not significantly different from that of the 9 sick birds of 4.2±1.1 g/dl (p=0.956). This parameter did not differ significantly between groups of sick birds with different body conditions (p=0.605) or different ages (p=0.709).

The mean total white blood cell count of 4 healthy birds was 22.8±12.5 x 10^3 cells/ µl. This was notably higher than that of 8 sick birds at 10.6±7.8 x 10^3 cells/ µl but the difference was just above the margin of statistical significance (p=0.063). There were no significant differences in mean white blood cell count between different body condition (p=0.811) and age categories among sick birds (p=0.720).

The mean absolute heterophil count of 4 healthy birds was 2.7±1.0x10^3cells/µl. This was lower than that of the 8 sick birds of 4.9±4.8x10^3cells/µl but the difference was not statistically significant (p=0.411). Mean differential heterophil count of 5 healthy birds was also lower (15.4±5.9 %) but not significantly different (p=0.082) from that of 9 sick birds of 39.0±26.9%.

There were no significant differences in mean absolute heterophil counts between groups of sick birds with different body scores (p=0.136) or ages categories (0.596). Similarly there were no significant differences in mean differential heterophil counts between groups of sick birds with different body scores (p=0.085) or age categories (p=0.662).

The mean absolute lymphocyte count of 8 sick birds was 3.2±1.9x 10^3 cells/ µl, which was significantly lower than the mean for 5 healthy birds of 18.0±10.6x 10^3 cells/ µl (p=0.002). The mean differential lymphocyte count of 9 sick birds was 50.0±23.4 %, which was also significantly lower than that of 5 healthy birds of 75.6±7.3 % (p=0.037). .The mean absolute lymphocyte count of the 3 sick birds that were in good body condition (2.1±1.4 x 10^3cells/ µl) was lower than that of the 4 healthy birds in good body condition (18.0±10.6x 10^3 cells/ µl) with a marginal level of significance (p=0.053). Mean absolute lymphocyte counts did not differ significantly between groups of sick birds with different body scores (p=0.241) or between different age categories (p=0.533). Similarly, mean differential lymphocyte counts did not differ significantly between groups of sick birds with different body scores (p=0.131) or age categories (p=0.267). The heterophil to lymphocyte ratio derived from the respective differential cell counts was 1:5 in the healthy birds compared to 4:5 in the sick birds, representing a four-fold increase in the ratio in the latter group.

The mean absolute monocyte count of 4 healthy birds (0.8±1.0x 10^3 cells/ µl) did not differ significantly from that of 8 sick birds of 0.4±0.5x 10^3 cells/ µl (p=0.558). The mean differential monocyte count of the 5 healthy birds (0.8±1.0 %) did not differ significantly from that of 9 sick

birds of 0.4±0.5 % (p=0.595). The mean absolute monocyte count did not differ significantly between groups of sick birds with different body scores (p=0.544) or age categories (p=0.932). Similarly mean differential monocyte count did not differ significantly between groups of sick birds with different body scores (p=0.153) or age categories (p=0.694).

The mean absolute eosinophil count of 4 healthy birds (0.48±0.36x10^3 cells/ µl) was not significantly different from that of 8 sick birds of 0.44±0.63x 10^3 cells/ µl (p=0.921). The mean differential eosinophil count of 5 healthy birds (2.2±1.6 %) similarly did not differ significantly from that of 9 sick birds (of 5.9±5.4 % (p=0.170). Mean absolute eosinophil count did not differ significantly between groups of sick birds with different body scores (p=0.703) or age categories (p=0.614). Similarly mean differential eosinophil count did not differ significantly between groups of sick birds with different body score (p=0.647) or age categories (p=0.345).

The mean absolute basophil count was 0.0 cells/ µl for the 4 healthy birds as well as for the 8 sick birds. Mean differential basophil count of the 4 healthy birds (0.40±0.55 %) did not differ significantly from that of 9 sick birds of 0.22±0.29 % (p=0.622). Mean differential basophil counts also did not differ significantly between groups of sick birds with different body scores (p=0.729) or age categories (p=0.422).

The morphology of the red blood cells was normal with 3-5% reticulocytes in 5/14 birds. White blood cell morphology was normal except for granulated lymphocytes observed in 2/14 birds (Figures 15 to 183). Blood parasites were not found.

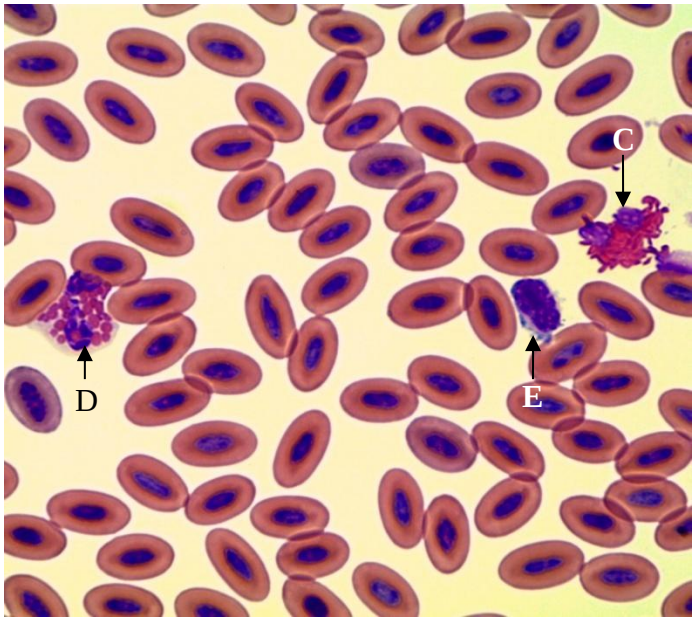

Figure 13: Morphology of normal eosinophil (C), heterophil (D) and small lymphocyte (E) of an adult lesser flamingo examined in Lake Nakuru during the dry season
Diff Quick Stain, x1000

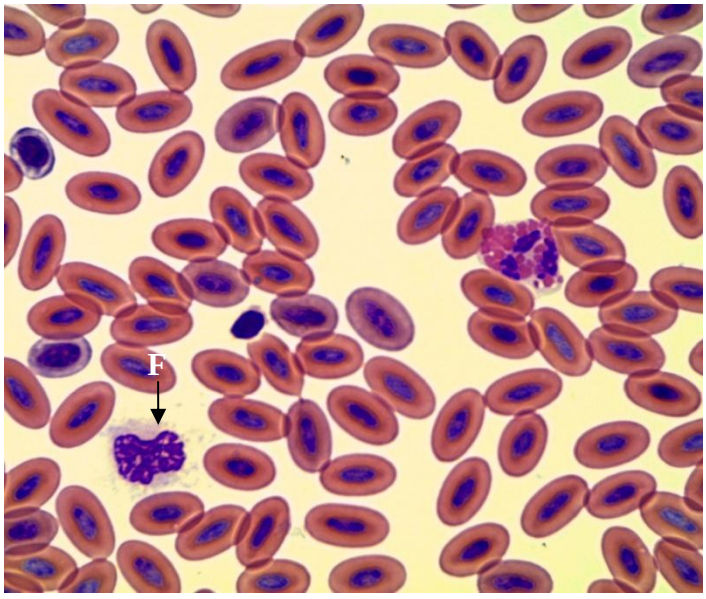

Figure 14: Morphology of a normal medium lymphocyte (F) of an adult lesser flamingo examined in Lake Nakuru during the dry season
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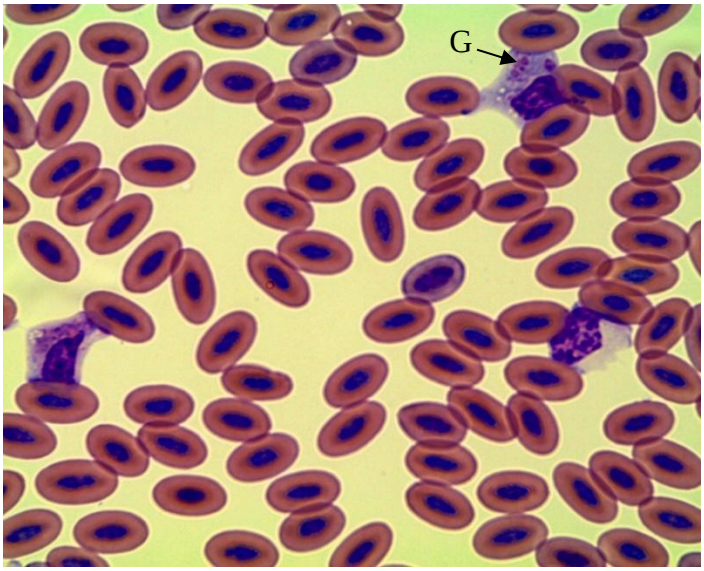


Figure 15: Morphology of granulated lymphocyte (G) of an adult lesser flamingo examined in Lake Nakuru during the dry season
Diff Quick Stain, x1000

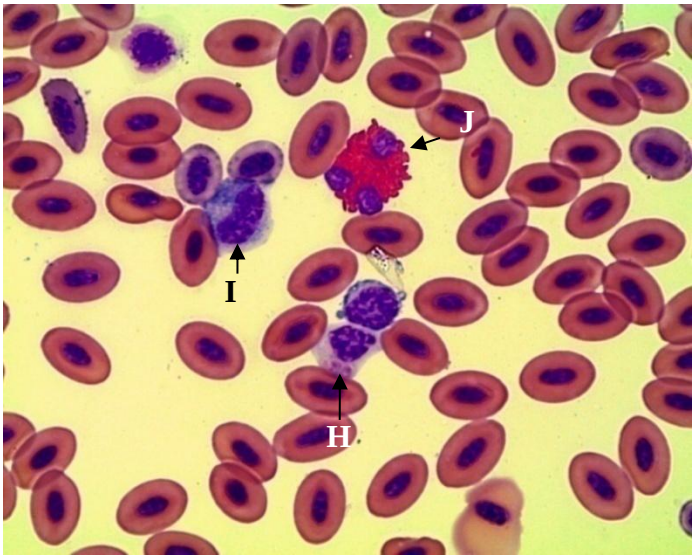


Figure 16: Morphology of granulated lymphocytes (H), normal lymphocyte (I), and normal heterophil (J) of an adult lesser flamingo examined in Lake Nakuru during the dry season
Diff Quick Stain, x1000

Key

4.3.2 Gross postmortem findings

The types and frequencies of gross postmortem lesions that were observed in the 57 birds that were examined during the study are shown in Table 6. Congestion of visceral organs was observed in 30/57 of the birds, mostly those that were sick or found dead (Figure 19). The most affected organs were liver, kidneys, lungs, spleen and intestinal mucosa. Haemorrhage was present in the visceral organs in 11/57 of the birds, all of which were sick or found dead. It was petecchial and ecchymotic type in the coronary fat of the heart of 5 birds and it was diffuse in the intestinal mucosa of 2 birds, causing ingesta in the lumen to be bloody (Figure 20).

Fibrinous exudate (Figures 21 and 22) covering the visceral organs and/or the lining of the coelomic cavity was present in 7/57 birds all of which were sick or found dead. It was variously accompanied with adhesion of the heart to the coelomic cavity in 4 birds, flabbiness of the myocardiumin one bird, rough pericardia in 2 birds and cloudiness of the airsacs in 3 birds.

Necrotic foci were observed in the visceral organs of 5/57 birds, 4 of which were sick or found dead and one was healthy. The necrotic foci, which were 1-3mm in diameter, occurred in livers of 2 birds and spleens of 3 birds. Multiple nodular granuloma-like lesions of varying sizes (1-30mm) occurred in the liver, spleen and lungs of 2/57 birds extending to the ovaries and lining of the coelomic cavity in one of the birds (Figures 23 and 24). The bursae of Fabricius were enlarged to various degrees (up to 50mm x 20mm) in 12/57 birds, 7 of which were healthy and 5 were sick or found dead (Figures 23 and 24). Fractures and joint abscesses occurred in 4/57 birds.

Table 6: Frequencies of gross postmortem lesions found in lesser flamingos during the dry and wet seasons

Lesion	Frequency				
	Healthy birds	Sick or found dead	Dry season	Wet season	Overall frequency
Congestion of visceral organs	2/22	28/35	14/20	16/37	**30/57**
Haemorrhage	0/22	11/35	2/20	9/37	**11/57**
Fibrinous exudation into coelomic cavity	0/22	7/35	5/20	2/37	**7/57**
White necrotic foci on visceral organs	1/22	4/35	2/20	3/37	**5/57**
Multiple nodular lesions on visceral organs	0/22	2/35	1/20	1/37	**2/57**
Enlarged bursa of Fabricius	7/22	5/35	6/20	6/37	**12/57**
Fractures and joint abscesses	0/22	4/35	3/20	1/37	**4/57**

Tapeworm infestation occurred in the small intestine. It was accompanied by severe congestion and haemorrhage in one bird and it caused localized bulging along the length of the intestine in another bird (Figure 25). Small roundworms (1-2cm) were found under the skin around the thoracic area over the crop (Figure 26).

The frequencies of gross postmortem lesions observed during the dry and wet seasons were not significantly different when tested using the student's t-test for proportions ($p>0.095$).

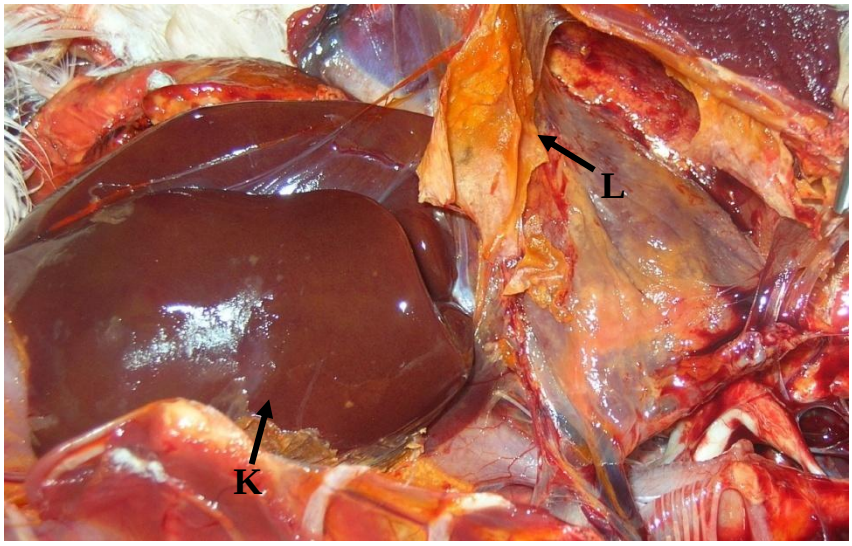


Figure 17: Congested liver (K) and fibrinous exudation (L) in a sick lesser flamingo examined in Lake Nakuru during the dry season

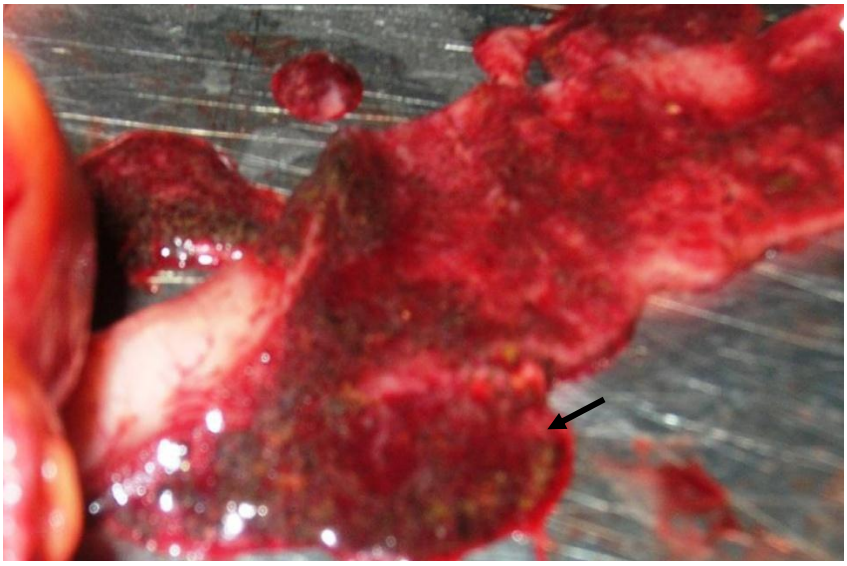


Figure 18: Haemorrhage in the intestine of sick lesser flamingo examined in Lake Nakuru during the dry season

Key: C = Bloody ingesta in the mucosa of the intestine

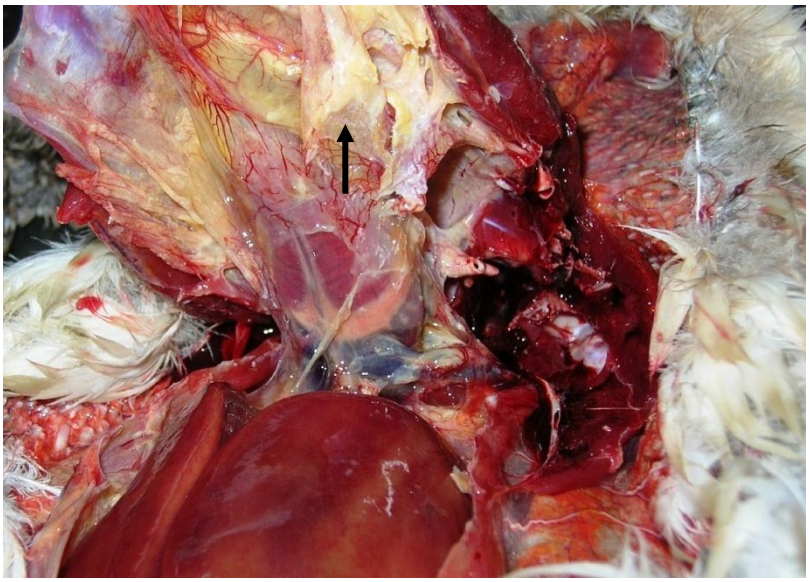


Figure 19: Fibrinous exudation around the heart (arrow) in a lesser flamingo examined in Lake Nakuru during the dry season

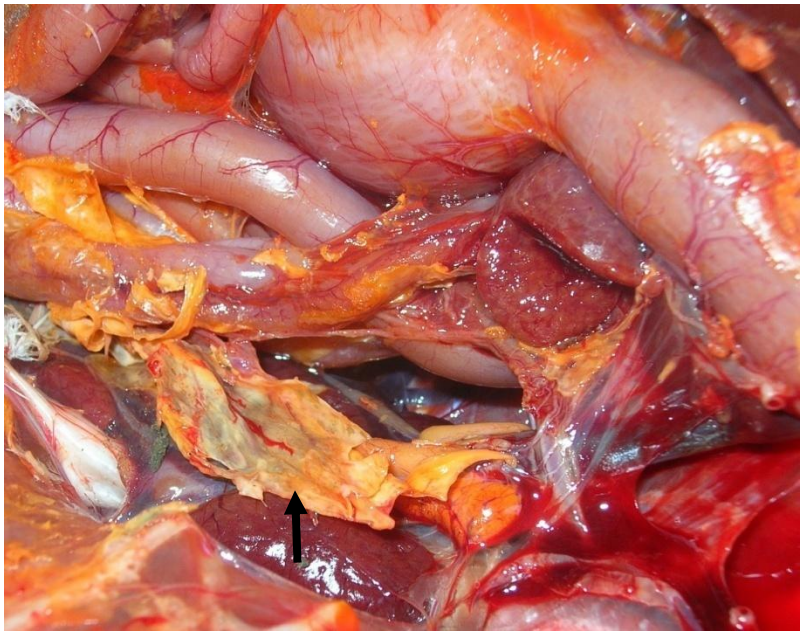


Figure 20: Fibrous exudation over the intestines (arrow) of a lesser flamingo examined in Lake Nakuru during the dry season

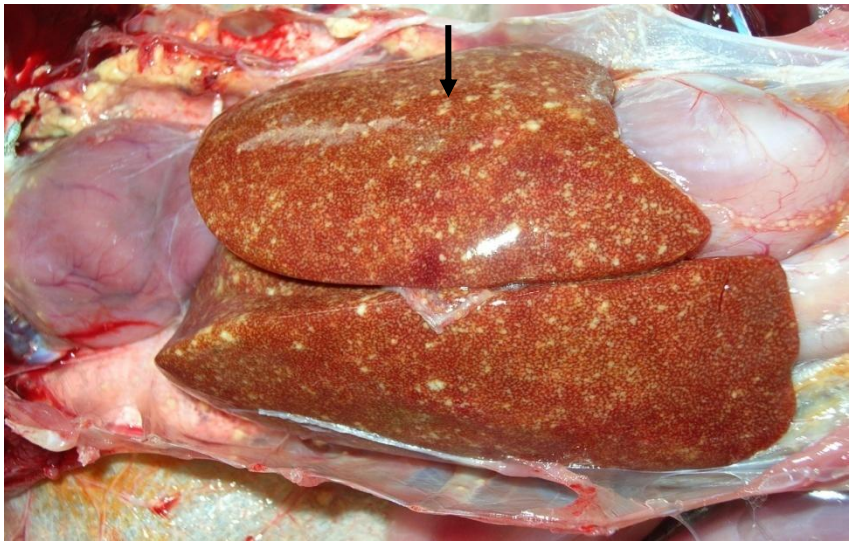


Figure 21: Granulomatous lesions in the liver (arrow) of a lesser flamingo examined in Lake Nakuru during the dry season

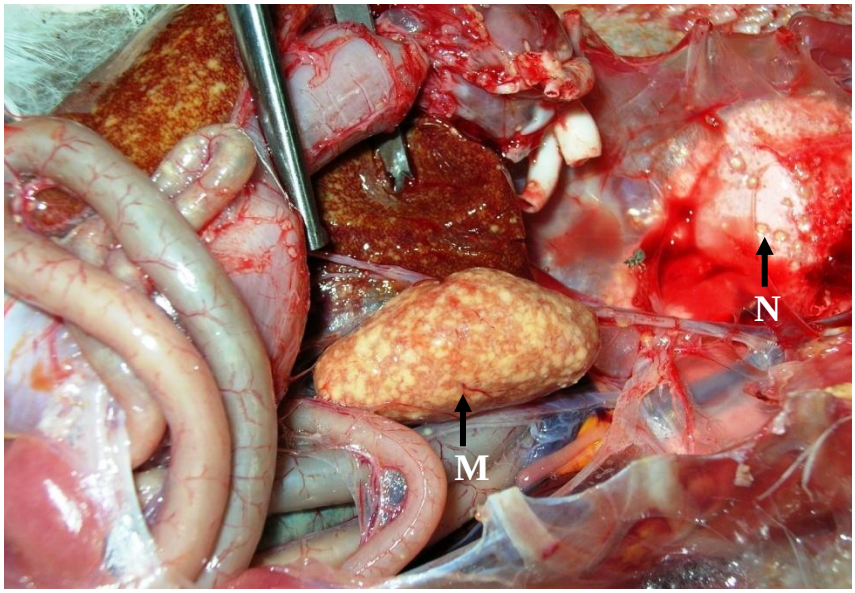


Figure 22: Granulomatous lesion in spleen (M) and lungs (N) of a lesser flamingo examined in Lake Nakuru during the dry season

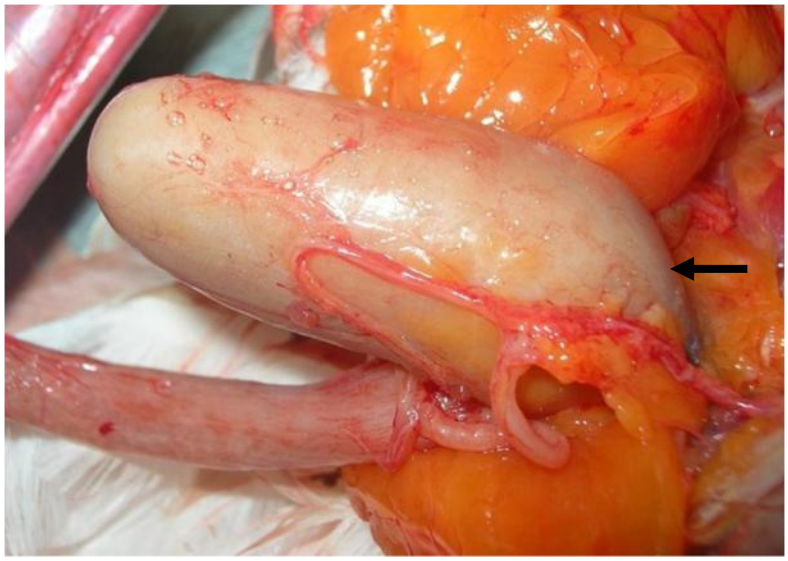
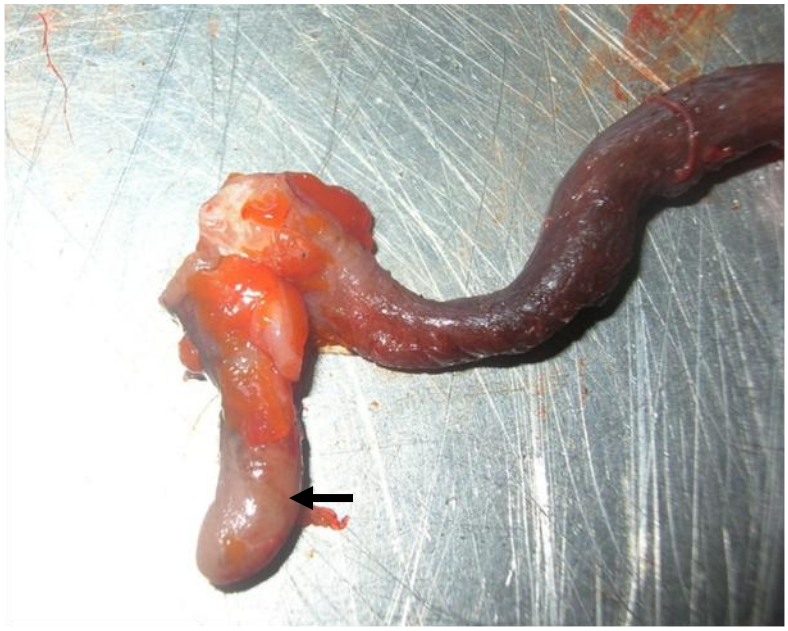


Figure 23: Enlarged bursa of Fabricius (arrow) in a mature lesser flamingo observed in Lake Nakuru during the dry season



A

Figure 24: Normal bursa of Fabricius (arrow) in a mature lesser flamingo observed in Lake Nakuru during the dry season

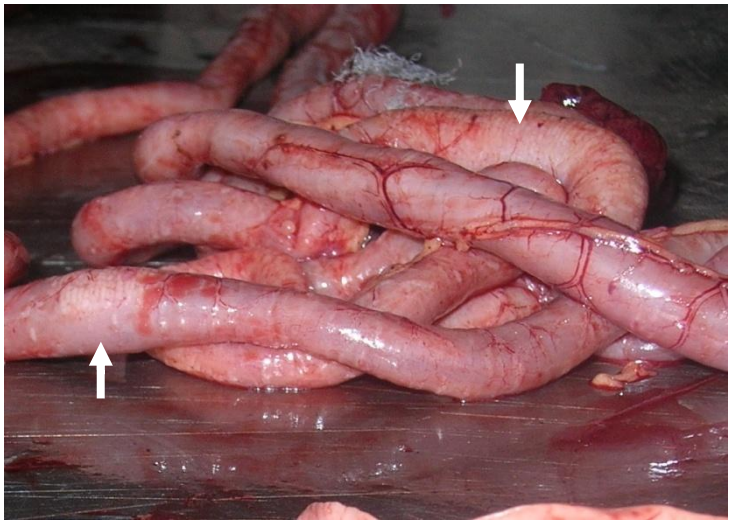


Figure 25: Congestion on the serosal surface and bulging (arrows) along an intestine infested with tapeworms in a lesser flamingo sampled in Lake Nakuru

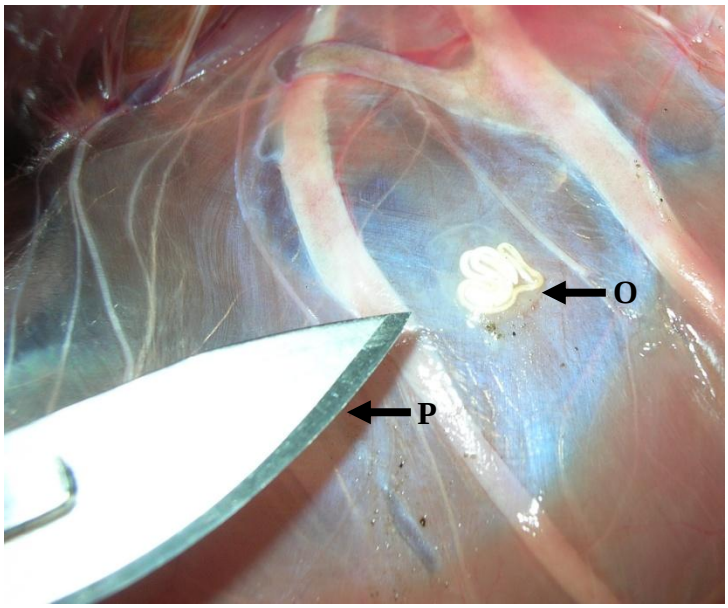


Figure 26: Roundworms (O) in sub-cutaneous tissue of the thoracic area of one bird sampled in Lake Bogoria during the wet season (scaple blade (P) for perspective)

The mean liver and spleen mass indices were higher in the sick and dead birds than in the healthy ones (Table 7 and Fig 27). The indices were computed using the following formula:

Organ mass index= (A/B) x 100

Where, A = weight of organ at necropsy B = Weight of bird at necropsy.

The mean liver mass index of sick birds was significantly higher than that of the healthy birds ($p=0.015$). That of the dead birds was even higher ($P<0.0001$) There was, however, no significant difference in liver mass index between sick and dead birds ($p = 0.574$). There was marginal statistical significance in the differences between the mean spleen mass indices of the health birds and that of the sick birds ($p=0.060$). The same same was true for the difference between the mean spleen mass index of the healthy birds and that of the dead birds ($p=0.069$). The mean spleen mass indices of dead and sick birds were, however, not significantly different ($p = 0.857$). These differences in mean organ mass indices shows that livers and (to a lesser degree) spleens were congested in sick and dead birds. The mean bursa mass index of healthy birds was not significantly different from that of sick birds ($p=0.097$) or that of the dead birds ($p=0.384$). This shows that enlargement of bursa was not directly related to death or sickness in the birds but may be due to an underlying factor.

Table 7: Mean organ mass indices of health, sick and deadbirds examined in Lakes Nakuru and Bogoria during the study

	Healthy**(X±δ (n))**	Sick **(X±δ (n))**	Dead **(X±δ (n))**	P Value
Liver mass index	3.0±0.6 (n=20)	4.4±2.4 (n=16)	4.9±1.6 (n=13)	0.004
Spleen mass index	0.11±0.03 (n=20)	0.16±0.12 (n=16)	0.17±0.06 (n=13)	0.06
Bursa mass index	0.28±0.23 (n=19)	0.08±0.05 (n=4)	0.20±0.06 (n=7)	0.150

Key

X-Mean **δ =** standard deviationn = Number of birds on which the mean was calculated

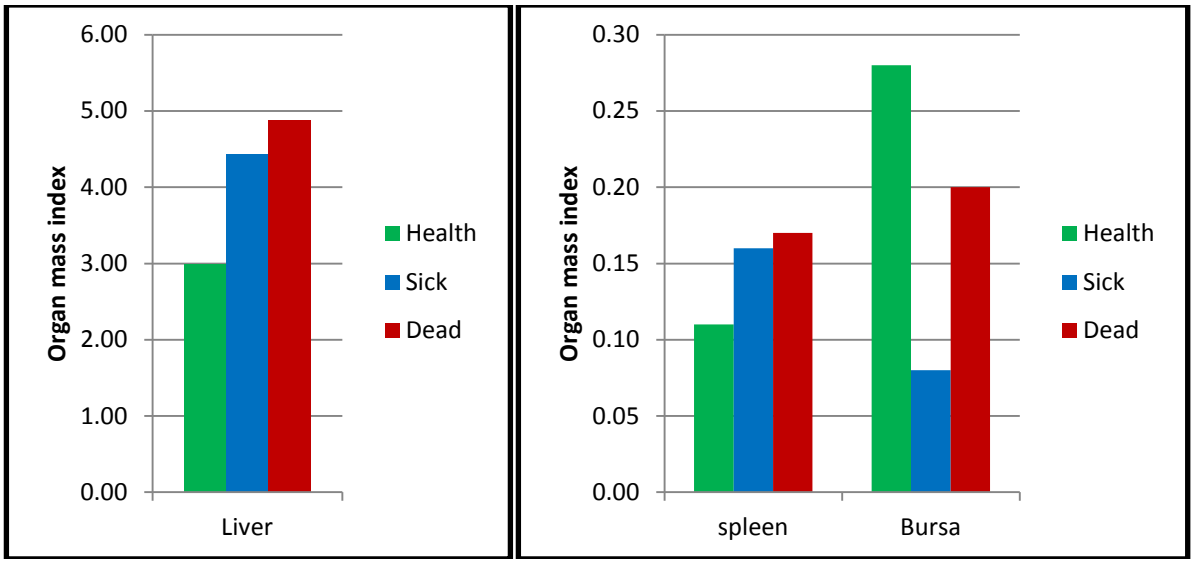


Figure 27: Mean organ mass indices of lesser flamingos examined in Lakes Bogoria and Nakuru during the dry and wet seasons

4.3.3 Histopathology

4.3.3.1 Lesions in samples collected during the field survey

The frequency of lesions in 53 lesser flamingos that were examined at histopathology is summarized in Table 8. The mean score of severity of lesions, using the scoring criteria explained in section 3.8, is given in Table 9. Congestion of the blood vessels in the visceral organs was the most frequent lesion observed. This was present in the tissues of 49/53 birds, mostly affecting liver, lung, spleen and kidneys. It was accompanied by focal to extensive haemorrhage in 40/53 birds. Moderate and mild levels of congestion and haemorrhage were present in healthy birds. However, the mean score of severity of congestion was significantly higher in the sick and dead birds than in healthy ones at 95% confidence limits ($p=0.037$ and $p=0.002$, respectively)

Focal necrosis with abscessation was found in 12/53 birds affecting visceral organs most frequently the liver, lungs, kidneys and spleens (Figures 28-31). Seven of the affected birds were healthy, 4 were found dead and one was sick. There was, however, no significant difference in mean score of severity of the lesion among the groups ($p=0.109$). Extensive necrosis was present in the spleens of 5/53 birds that were sick or found dead (Figures 32-33).

Granulomatous lesions were present in 3/53 birds. They were severe in two sick birds (LNF14 and LB33) that were in poor body condition and moderate in one bird (LN15) that was found dead in fair body condition. In the two birds, the granulomas were numerous multifocal to

coalescent foci of different sizes found in many organs including liver, lung, spleen, kidney and gastrointestinal tract. Each granuloma comprised a homogenous eosinophilic mass at the centre followed by a zone of nuclear debris, dead cells and giant cells, a zone of numerous fibroblasts, followed by a broader zone with different types of inflammatory cells including giant cells, macrophages and heterophils (Figure 34 and 35). Ziehl-Neelsen staining of the histopathological sections demonstrated numerous acid-fast coccobacillary rods in amorphous arrangements in liver, spleen, lungs, intestines and kidneys often coinciding with the lesions (Figures 36 and 37).

Table 8: Frequency of microscopic lesions in 53 lesser flamingos examined during the study.

Lesion	Group			Overall
	Healthy	Dead	Sick	
Congestion	18/21	17/17	14/15	49/53
Haemorrhage	12/21	15/17	13/15	40/53
Focal necrosis	7/21	4/17	1/15	12/53
Granulomatous lesions	0/21	1/17	2/17	3/53
Perivascular lymphocytic infiltration	13/21	7/17	11/15	31/53
Lymphocytic hyperproliferation in spleen and bursa	6/21	2/17	2/15	10/53
Changes in bursa (necrosis and cystic formations)	6/21	3/15	3/15	12/51
Parasites in tissues	5/21	1/16	2/15	8/52
Gastrointestinal parasite sections	4/21	1/16	2/15	9/52
Thickening of walls of blood vessels	2/21	4/17	3/15	9/53
Pericarditis (heterophil infiltration into pericardium)	0/21	1/17	3/15	4/53
Proliferation of reticuloendothelial tissue in spleen	6/21	0/17	0/17	6/53

Table 9: Mean score of lesions in 53 lesser flamingos examined during the study.

Lesion	Group		
	Healthy **(n=21)**	Dead **(n=17)**	Sick **(n=15)**
Congestion	2.0±1.1	3.1±0.9	2.8±1.2
Haemorrhage	1.2±1.3	2.9±1.3	2.5±1.4
Focal necrosis	0.8±1.2	0.7±1.2	0.1±0.3
Granulomatous lesions	0.0±0.0	0.1±0.5	0.5±1.4
Perivascular lymphocytic infiltration	1.6±1.4	1.1±1.4	1.9±1.5
Lymphocytic hyperploriferation in spleen and bursa	0.6±1.0	0.3±0.8	0.3±0.8
Changes in bursa (necrosis and cystic formations)	0.4±0.7	0.5±1.1	0.7±1.4
Thickening of walls of blood vessels	0.1±0.3	0.2±0.4	0.2±0.4
Pericarditis-heterophil infiltration into pericardium	0.0±0.0	0.2±1.0	0.4±0.9
Proliferation of reticuloendothelial tissue in spleen	0.6±1.1	0.0±0.0	0.0±0.0

Key: 0=Absent 1=Mild 2=Moderate Marked=3 4=Severe

n = Number of birds on which the mean was calculated

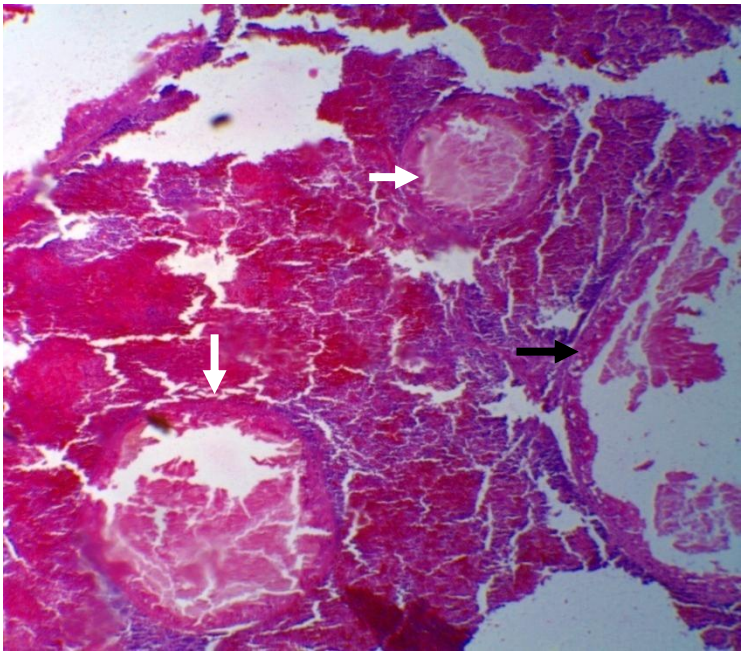


Figure 28: Abscesses (see arrows) in the spleen of a healthy lesser flamingo (LBF25) examined during the study
Haematoxylin and Eosin x 100

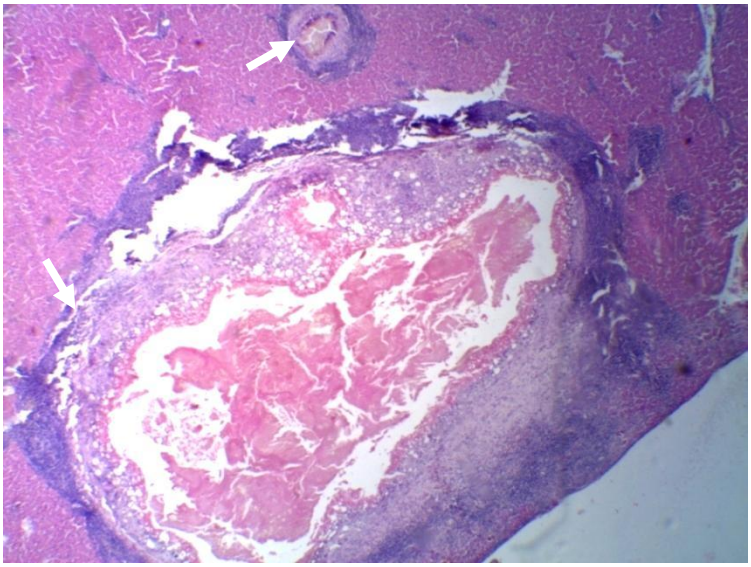


Figure 29: Abscesses (see arrows) in the liver of a healthy lesser flamingo (LNF2) examined during the study
Haematoxylin and Eosin: x100

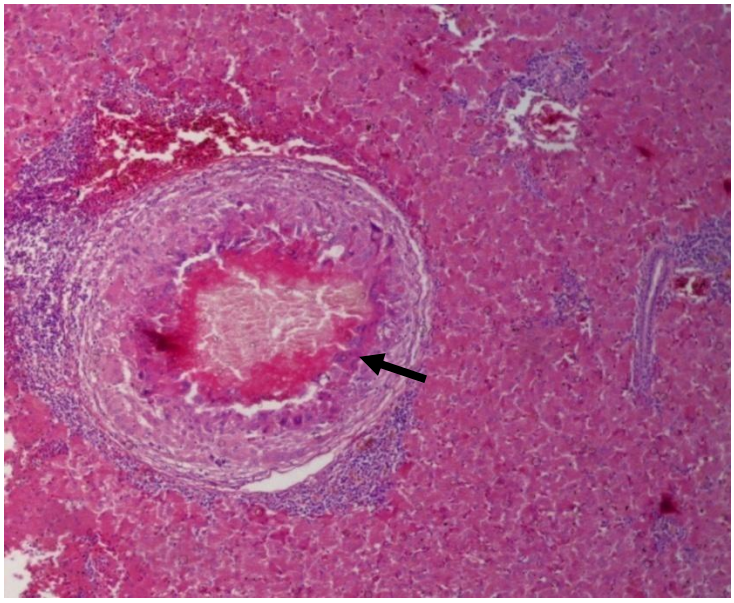


Figure 30: Abscess (see arrow) in liver of a healthy lesser flamingo (LBF10) examined during the study
Haematoxylin and Eosin: x100

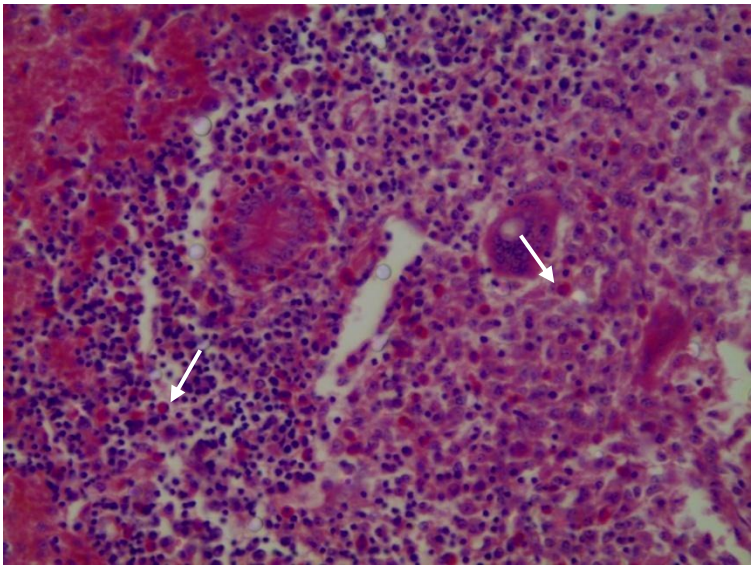


Figure 31: Higher magnification of Figure 30 showing an area of dense heterophil infiltration (see arrows)
Haematoxylin and Eosin: x400; Staining: H&E

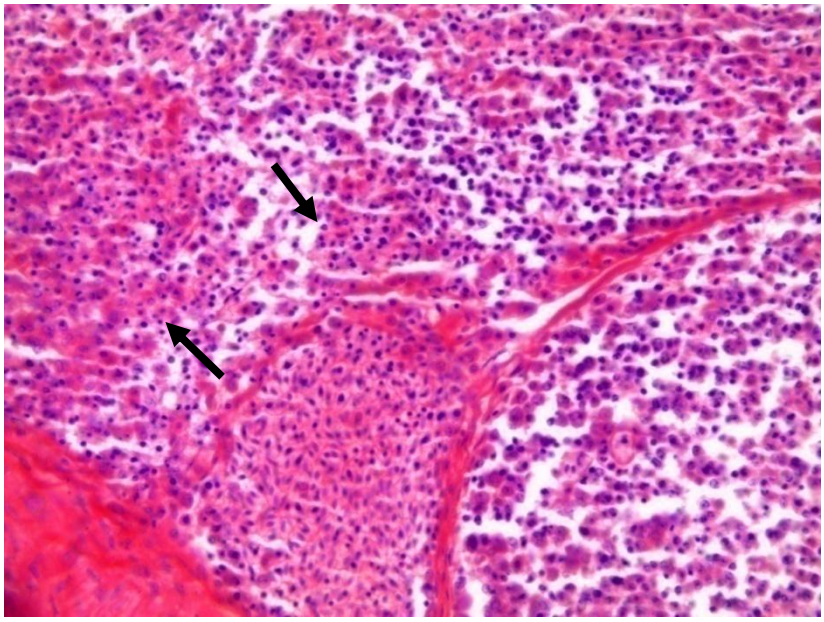


Figure 32: Extensive necrosis, with numerous nuclear debris (see arrows), in the spleen of a lesser flamingo (LBF17) found dead during the study
Haematoxylin and Eosin x400

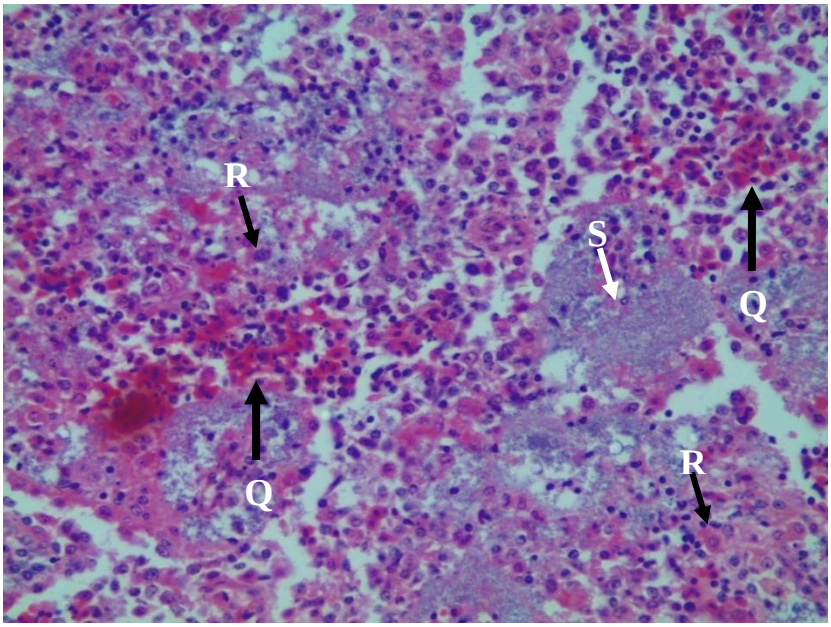


Figure 33: Extensive necrosis with focal infiltration by heterophils (Q) and macrophages (R) in the spleen of a lesser flamingo (LBF2) found dead during the study.
Few foci of saprophytes (S) also present
Haematoxylin and Eosin x400

Infiltration of the pericardium with numerous heterophils was observed in 4/53 birds, three of which were sick and one dead. It was extensive and widespread in one bird (Figures 38 and 39) but focal in the rest. Thickening of the walls of arteries in various organs especially the spleen was observed in 9/53 birds. The periarteriolar lymphoid sheath of the affected spleen was depopulated of lymphocytes (Figures 40 and 41). Two of the birds affected by this lesion were healthy, 3 were sick and 4 were found dead. The mean score of severity of the lesion in the three groups of birds were, however, not significant ($p=0.502$)

Lymphocytic infiltration into the liver, kidney or lung was observed in 31/53 birds, 14 of which were healthy, 11 were sick and 7 were found dead. In severe cases, lymphoid aggregations were large and formed follicle-like lesions in the periportal areas of the liver (Figure 42). Such lymphoid aggregations also occurred in the renal pelvis and in the interstitium of the lungs (Figure 43). Mean score of severity of lymphocytic infiltration in healthy, sick and dead birds did not differ significantly ($p=0.334$).

Lymphoid hypercellularity of the spleen, bursa of Fabricius or peyers patches and gut associated lymphoid tissue was observed in 10/53 birds of which 6 were healthy, two were sick and two were dead. The organs had numerous densely populated lymphoid follicles with many germinal centres and dense population of lymphocytes between the follicles. These were often accompanied by proliferation of reticuloendothelial tissue. The mean score of severity of lymphoid hypercellularity in healthy, sick and dead birds did not, however, differ significantly ($p=0.529$).

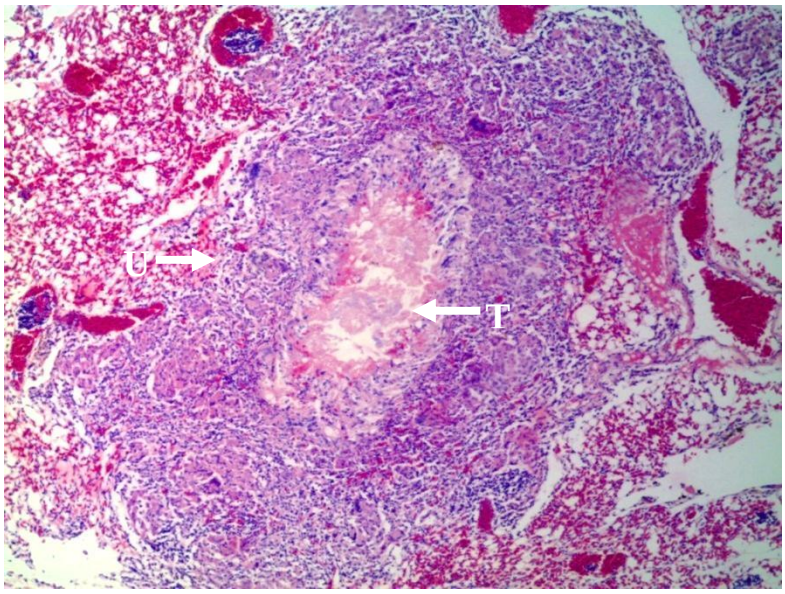


Figure 34: Granulomatous lesion with homogenous necrotic mass (T) surrounded by a zone of cellular infiltration (U) in the lungs of a lesser flamingo (LBF33) examined during the study
Haematoxylin and Eosin x400

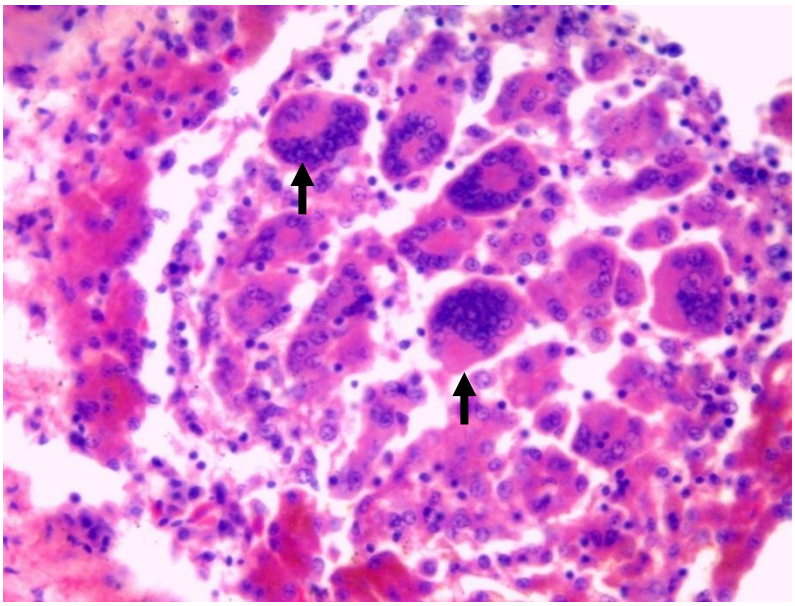


Figure 35: Higher magnification of Figure 34 showing several giant cells (see arrows)
Haematoxylin and Eosin: x1000; Staining: H&E

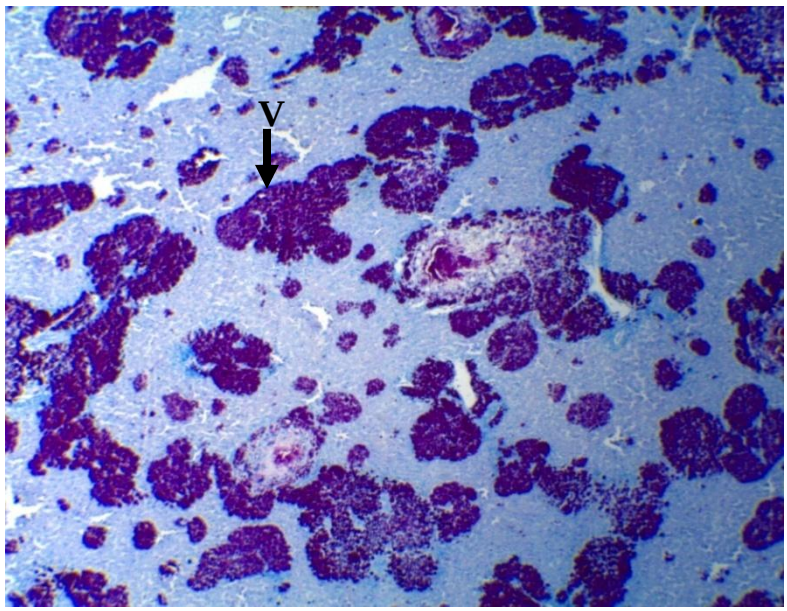


Figure 36: Multiple purple staining Z N positive foci (V) in liver tissue of a lesser flamingo LB33 examined during the study
Haematoxylin and Eosin x 400

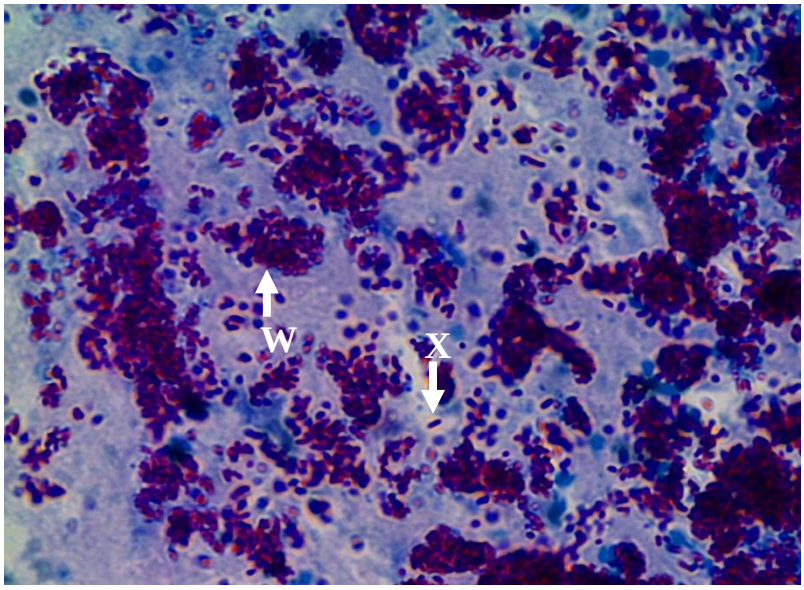


Figure 37: Acid fast bacteria demonstrated in clumps (W) and separate (X) in a lesser flamingo LB33 using Ziehl Neelsan staining
Ziehl Neelsan x 1000

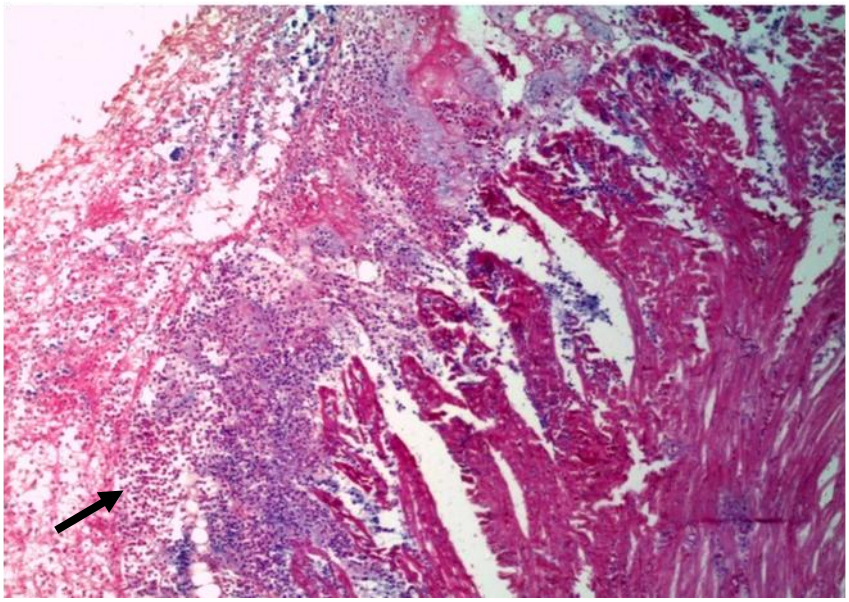
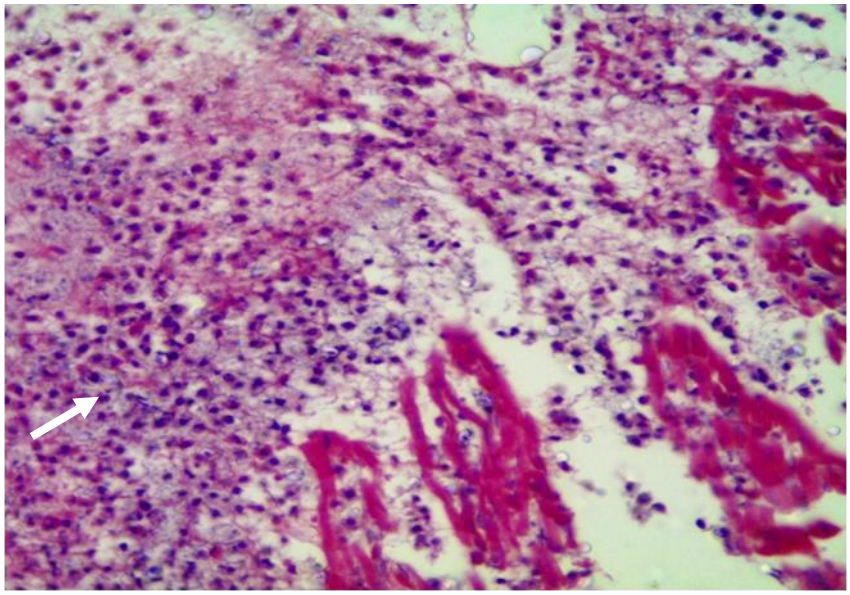


Figure 38: Dense infiltration of heterophils (see arrow) into the pericardium of a sick lesser flamingo (LB 32) examined in Lake Bogoria during the study
Haematoxylin and Eosin x 100



A

Figure 39: Higher magnification of Figure 38 showing an area of dense infiltration with heterophils (arrow)
Haematoxylin and Eosin x 400

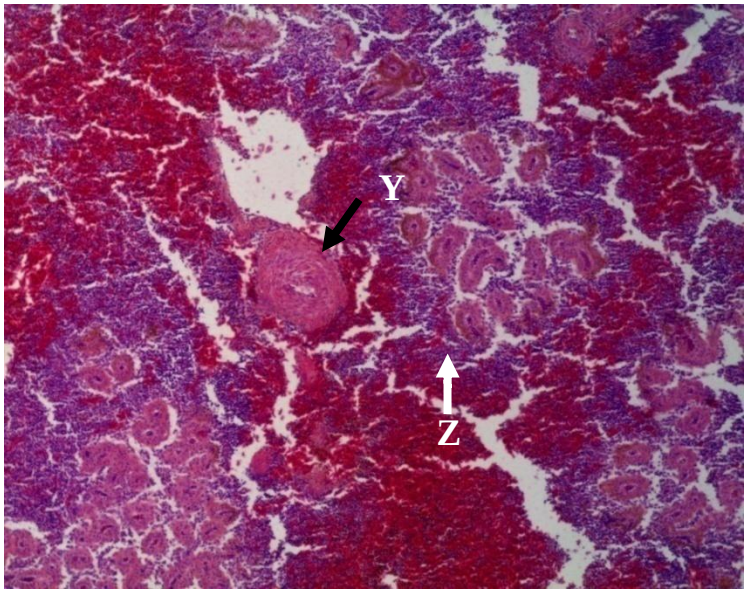


Figure 40: Thickened artery (Y) and lymphoid depopulation (Z) in the spleen of a healthy lesser flamingo (LBF 23) examined in Bogoria during the study
Haematoxylin and Eosin x 400

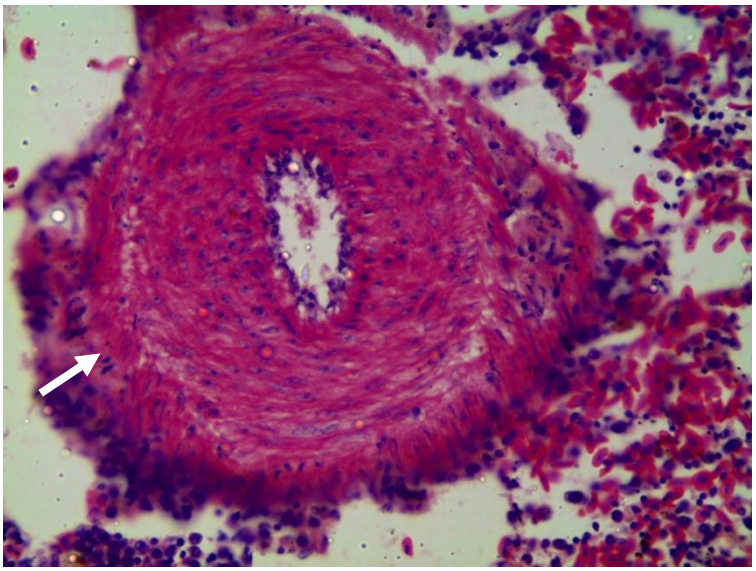


Figure 41: Higher magnification of Figure 40 showing thickened artery (arrow) and periarteriolar lymphoid depopulation in the spleen
Haematoxylin and Eosin x 1000

Necrosis and cystic lesions in the bursa of Fabricius was present in 14/51 birds. They affected healthy, sick and dead birds (Figures 44 and 45). In severe cases there was total replacement of lymphoid tissue with homogenous eosinophilic masses or empty cyst-like vacuoles. The mean score of severity of these lesions in healthy, sick and dead birds did not differ significantly ($p=0.734$).

Sections of protozoan parasites were observed in the serosal wall of the intestine of 1/53 bird that was sick (Figures46 and 47). Sections of tissue helminth parasites were found in thymus of 2/53 birds (Figures 48 and 49) both of which were healthy

Sections of gastrointestinal helminth parasites were observed in the mucosa of the intestines in 9/53 birds (Figures 50-53); in some cases the parasites had burrowed into the mucosa and there was thickening of the epithelial lining (Figure 50). Four of the affected birds were healthy, 4 sick and one dead.

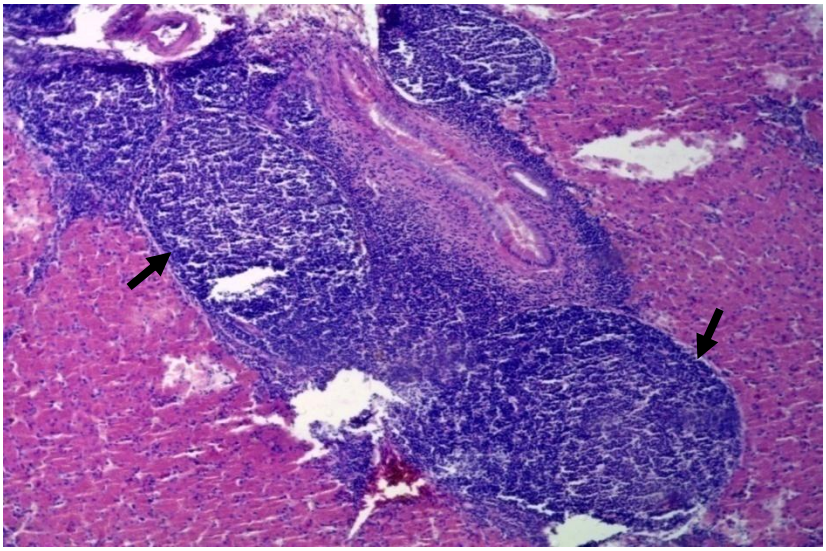


Figure 42: Dense infiltration of liver with lymphocytes (arrows) in healthy lesser flamingo (LNF 4) examined in Lake Nakuru during the study
Haematoxylin and eosin x100

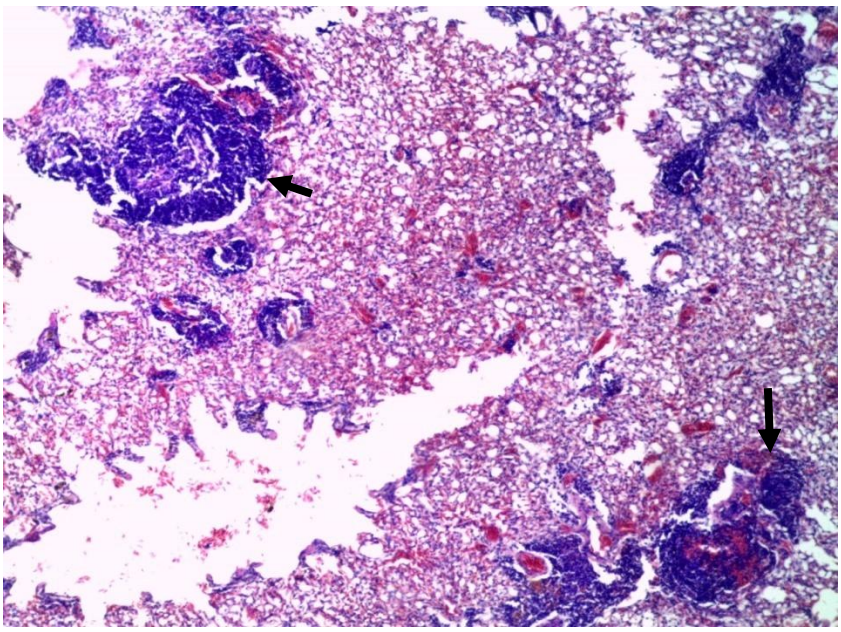


Figure 43: Dense infiltration of lung with lymphocytes (arrows) in a healthy lesser flamingo (LNF10) examined in Nakuru during the study

Haematoxylin and eosin x x100

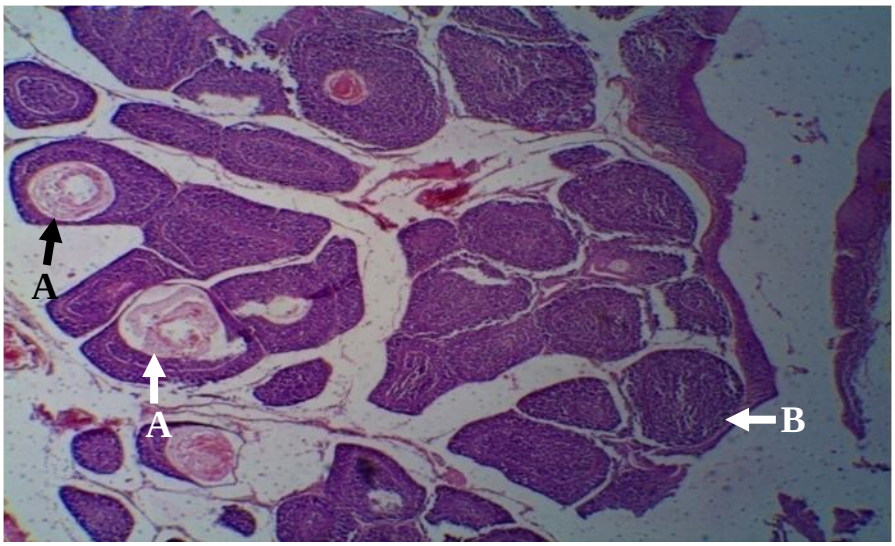


Figure 44: Necrotic changes (A) in Bursa of Fabricius of a sick lesser flamingo (LBF34) examined in Bogoria during the study
Normal follicle (B) shown
Haematoxylin and eosin x 100

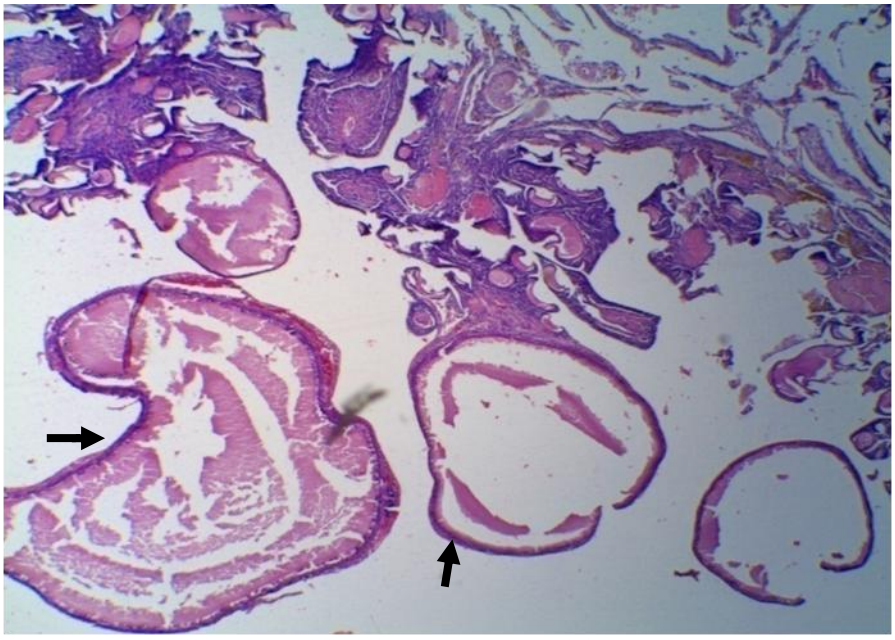


Figure 45: Cystic changes (arrows) in Bursa of Fabricius of a sick lesser flamingo (LBF34) examined in Bogoria during the study
Haematoxylin and eosin x 100

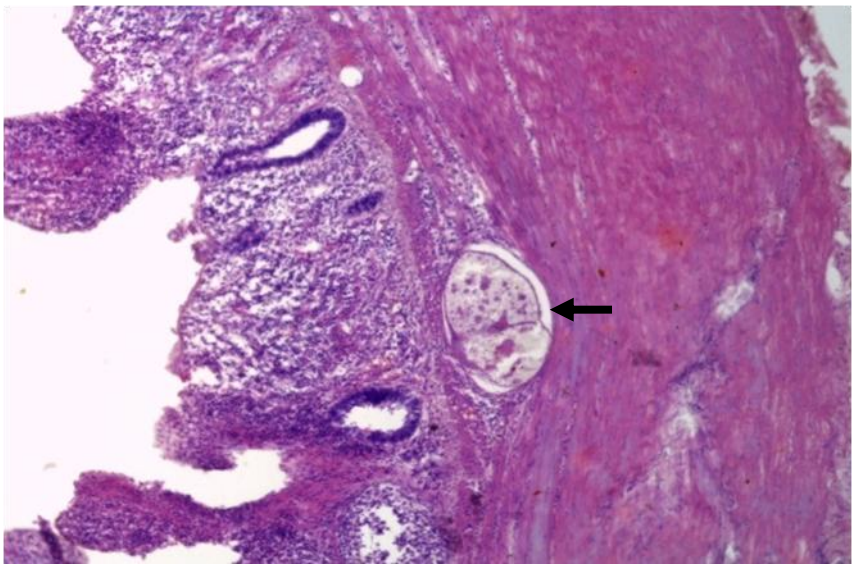


Figure 46: Section of protozoan parasite in the muscular layer of the intestine of a healthy lesser flamingo (LNF4) examined in Nakuru during the study
Haematoxylin and eosin x 100

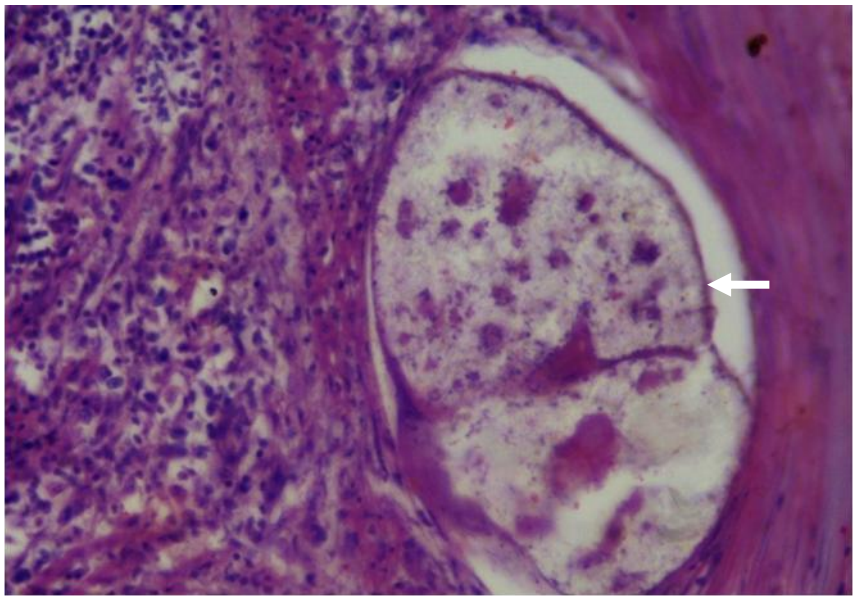


Figure 47: A higher magnification of Figure 48 showing the section of protozoan parasite (arrow).
Haematoxylin and eosin x 100

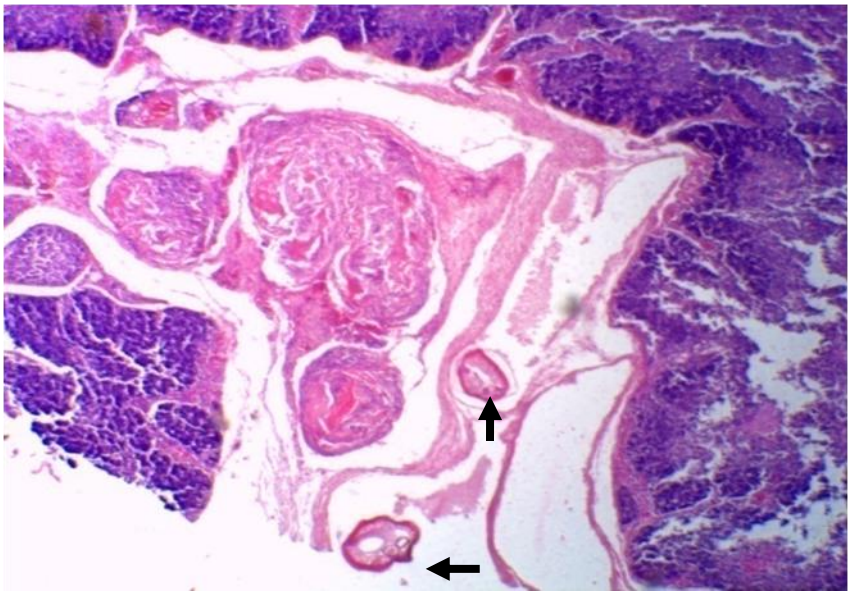


Figure 48: Sections of aberrant helminth parasites in the thymus of healthy lesser flamingo (LBF22) examined in Lake Bogoria during the study
Haematoxylin and eosin x 100

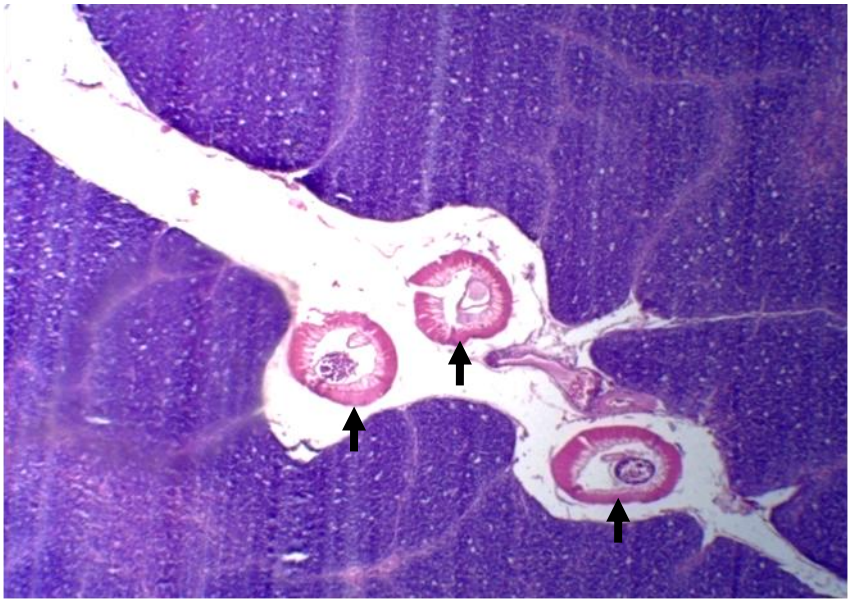


Figure 49: Sections of aberrant helminth parasites in the thymus of healthy lesser flamingo (LNF4) examined in Lake Nakuru during the study
Haematoxylin and eosin x 100

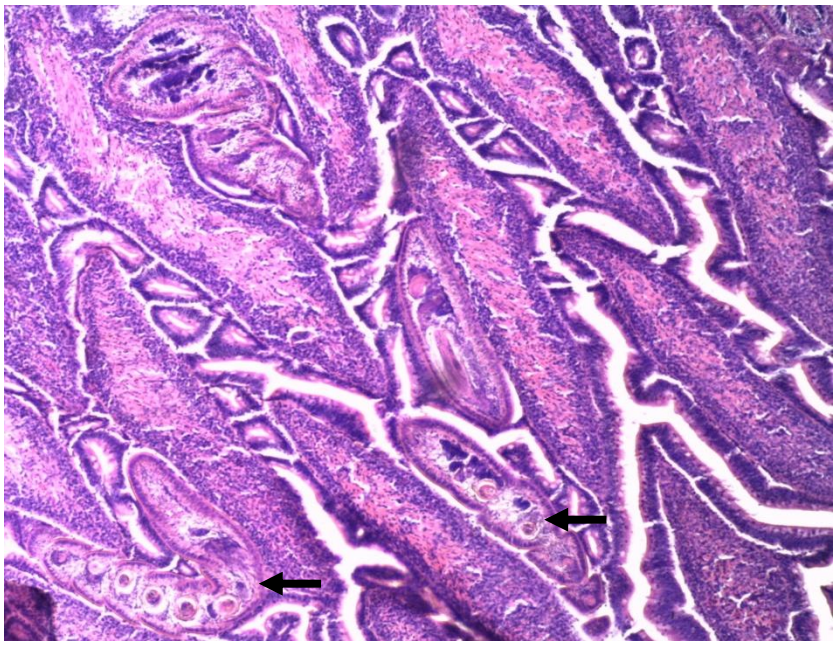


Figure 50: Sections of helminth parasites in the mucosa of small intestine of a sick lesser flamingo in poor body condition (LBF14) examined in Bogoria during the study
Haematoxylin and eosin x 100

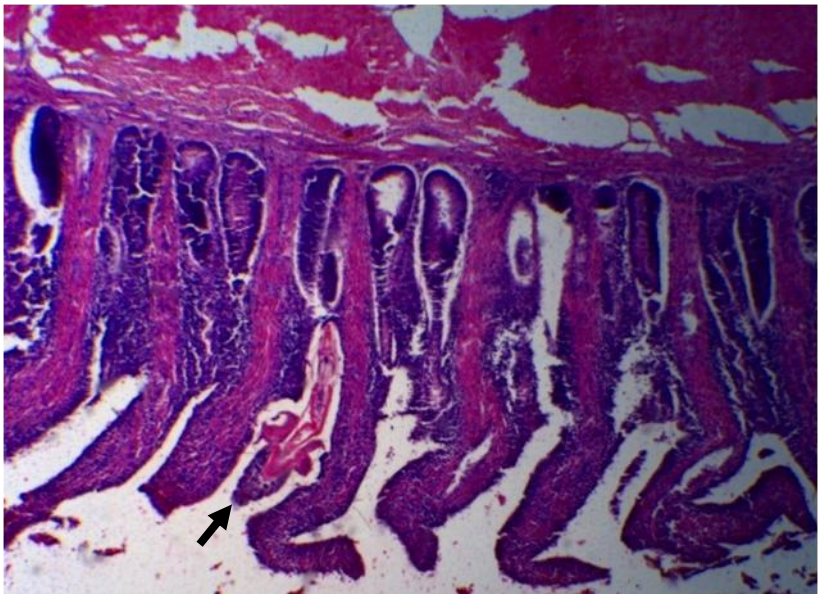


Figure 51: Parasite (arrow) in the lumen of intestine of one healthy lesser flamingo (LBF14) examined in Lake Bogoria during the study
Haematoxylin and eosin x 100

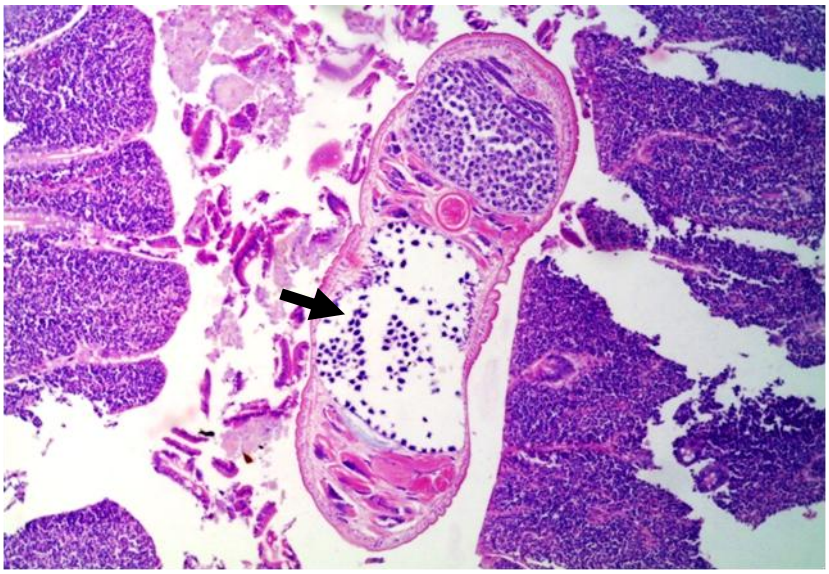


Figure 52: Section of helminth parasite in the intestinal mucosa of a lesser flamingo (LNF15) found dead in Lake Nakuru during the study
Haematoxylin and eosin x 100

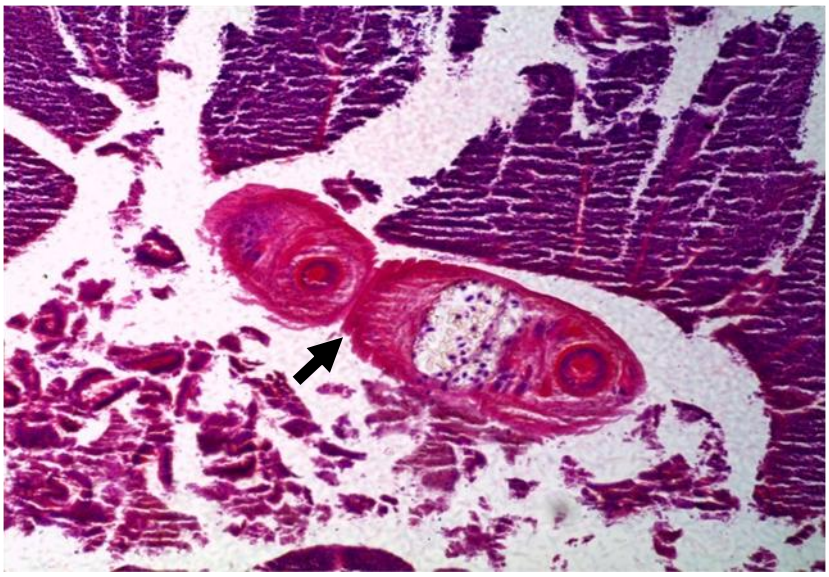


Figure 53: Parasite in the lumen of intestine of one healthy lesser flamingo (LBF14) examined in Lake Bogoria during the study
Haematoxylin and eosin x 100

4.3.3 2 Lesions observed in retrospective samples.

Tissue samples from a total of 134 lesser flamingos that had been examined previously were studied for histopathological lesions. Eighty one (81) of these birds had been sampled in 1997, 1998, 1999, 2000, and 2001 as part of a long-term toxicological study that involved capture of birds: these are considered as samples from non-outbreak periods Fifty-three (53) of the birds were sampled during the mass mortality in 2004.

The predominant gross postmortem lesion that had been observed in birds sampled in 2004 was generalized congestion and haemorrhage of visceral organs (section 2.2.1.5). The 22 cases of 1997 were drawn from 61 birds in which the following gross lesions had been observed: congestion and haemorrhage in visceral organs affecting muscles, heart, brain, liver, kidneys, spleen) in 19/61 birds, widespread granulomatous lesions in 3/61 birds, necrotic foci in 3/61 birds, fibrinous matting of visceral organs in 3/61, trauma in 8/61 and presence of tapeworms in 14/61. Similar background data was not available for the samples taken in 1998, 1999, 2000 and 2001. Table 10 shows when and where samples were taken.

Table 10: Number of lesser flamingos examined retrospectively during the study

	Location	Year						Total
		1997	1998	1999	2000	2001	2004	
Number of birds	Bogoria	18	21	12	7	19	0	**77**
	Elmentaita	1	0	0	0	0	0	**1**
	Nakuru	3	0	0	0	0	53	**56**
Total		**22**	**21**	**12**	**7**	**19**	**53**	**134**

The frequency of occurrence of lesions examined retrospectively is summarized in Table 11. The frequency of occurrence of severe congestion and haemorrhage, severe cellular infiltration and severe necrosis in different organs is illustrated in Figure 54. Congestion was observed in tissues from 108/134 birds and it was common in all the years. Haemorrhage was observed in tissues of 79/134 birds, mostly those sampled in 2004, 2001 and 1997. All the 53 birds sampled in 2004 had congestion and haemorrhage that was severe in one or more organs in 49/53. Congestion and haemorrhage was most frequent and most severe in the lung, liver, kidney and spleen (Figures 55 and 56). Haemorrhage was multi-focal, extensive, diffuse or a combination of the different types. It was accompanied with pulmonary oedema in 12/53 birds sampled in 2004.

Infiltration of various tissues with heterophils, lymphocytes and macrophages was present in 75/134 birds. Marked or severe lymphocytic infiltration into the peri-portal areas of the liver and sometimes into the renal pelvis was observed in these tissues from 31/81 birds sampled during the non-outbreak period (1997, 1998, 1999, 2000 to 2001) and was more frequent in tissues from 1997 and 2001 samples. Infiltration of organs with heterophils was observed in 44/53 birds sampled in 2004 and it was severe in 31/53 birds (Figure 57). Infiltration of organs with macrophages and lymphocytes, mostly accompanying heterophils, was observed in 31/53 birds sampled in 2004 and it was severe in 21/53 birds (Figure 58). The spleens, intestines, proventriculus, livers and kidneys showed the most prominent inflammatory cell infiltration.

Table 11: Frequency of lesions in tissues of lesser flamingos examined retrospectively during the study.

Lesion	Frequency of occurrence						
	1997	1998	1999	2000	2001	2004	Total
Congestion	11/22	11/21	9/12	7/7	19/19	51/53	108/134
Haemorrhage	14/22	1/21	0/12	0/7	11/19	53/53	79/134
Cellular infiltrations	10/22	3/21	2/12	1/7	15/19	44/53	75/134
Necrosis	6/22	0/21	0/12	0/7	0/19	40/53	46/134
Granulomatous lesions	1/22	0/21	0/12	0/7	0/19	0/53	1/134
Parasite sections	3/22	0/21	0/12	0/7	0/19	6/53	9/134

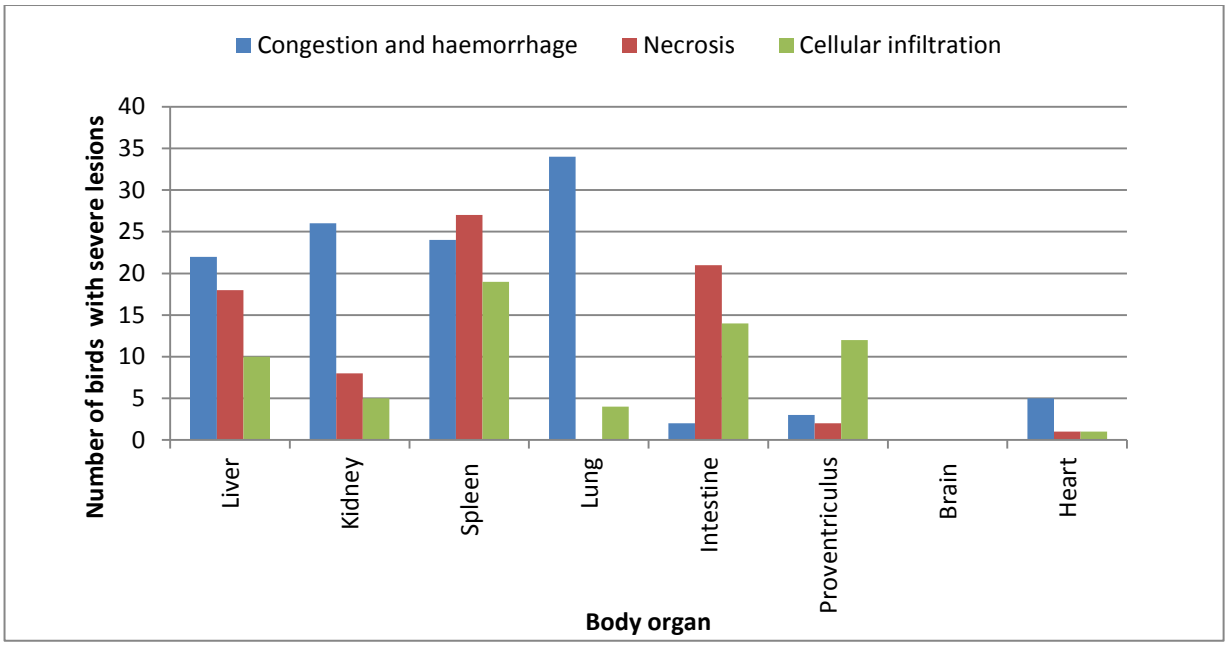


Figure 54: Frequency of severe lesions in organs of 53 lesser flamingos sampled in 2004 mortality

Livers were diffusely infiltrated with heterophils and macrophages into the parenchyma but these inflammatory cells were more numerous at the peri-portal areas. In the intestines and proventriculus, infiltration with heterophils and macrophages were most frequently observed in the mucosae and in the gut associated lymphoid tissues of the organs. In the spleens, infiltration with these inflammatory cells occurred in the entire parenchyma but it was more marked below the capsule and around the trabeculae. In the kidneys, inflammatory cell infiltration was mostly localized in the renal pelvis.

Necrotic lesions were observed in 46/134 birds. Six (6/81) of these were handled during the non-outbreak periods (all in 1997) and 40/53 during the mass mortality period in 2004. The lesions in the 6 birds sampled during the non-outbreak period comprised multiple foci of necrosis and abscessation in the lungs (Figure 59), livers, spleens and kidneys of 5 birds and necrosis of gut associated lymphoid tissue in the intestine of one bird. The necrotic lesions were densely infiltrated with heterophils and macrophages. The 43/53 birds handled in 2004 had extensive tissue necrosis and degeneration that were severe in 37 of the birds. The spleens, intestines, livers and kidneys were affected most frequently and most severely.

Necrosis in the liver was characterized by marked presence of nuclear debris in the liver parenchyma, swollen hepatocytes with deeply eosinophilic cytoplasm and pyknotic nuclei and heavy infiltration with heterophils and macrophages into the liver parenchyma.

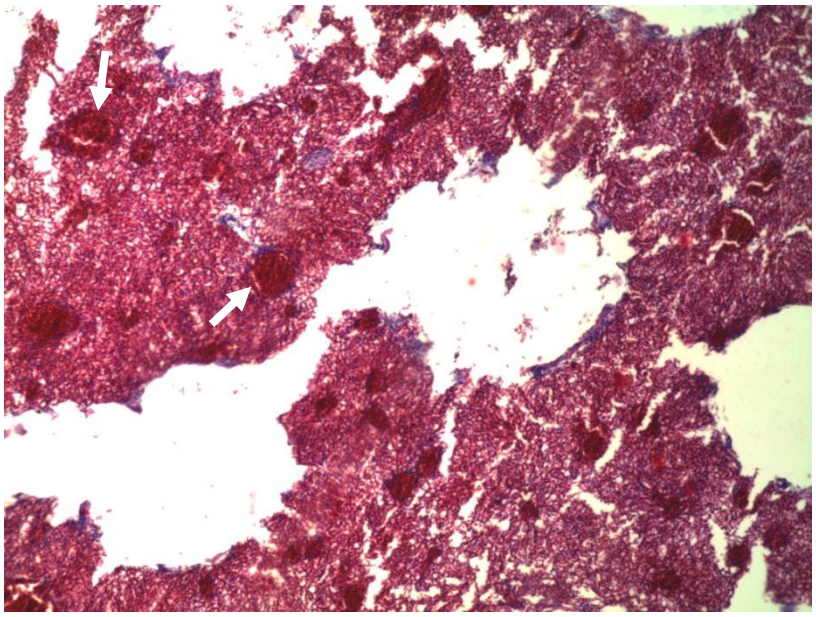


Figure 55: Diffuse haemorrhage andcongestion (see arrows) in the lung of a lesser flamingo (B5/2001) examined retrospectively
Haematoxylin and eosin x 100

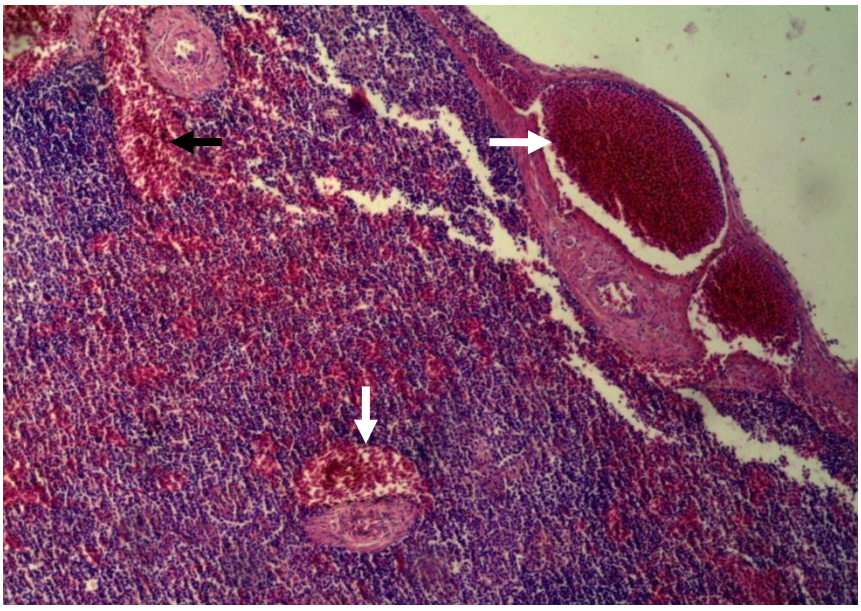


Figure 56: Haemorrhage (see arrows) in the spleen of a lesser flamingo (B12/2001) examined retrospectively
Haematoxylin and eosin x 100

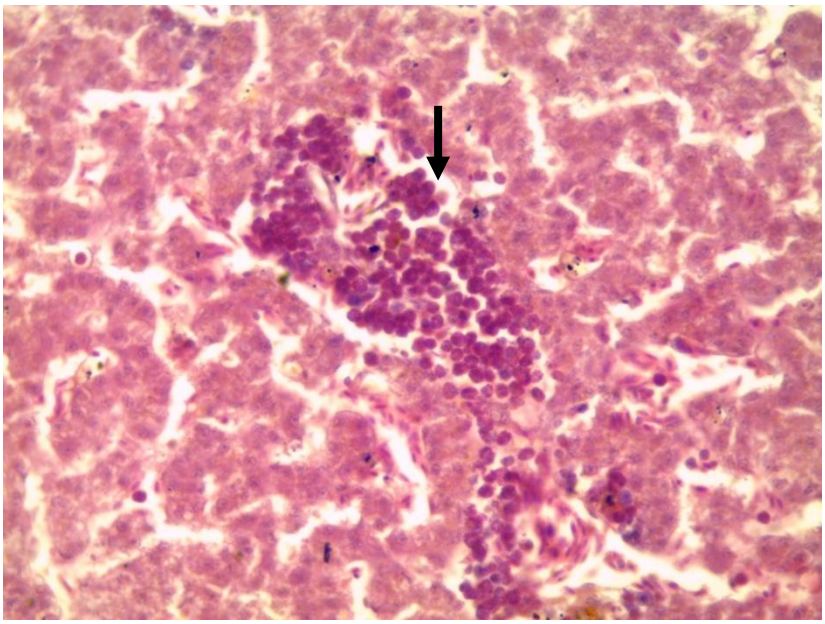


Figure 57: Focus of dense infiltration of heterophil (see arrow) in the liver of a lesser flamingo (LN4/2004) examined retrospectively
Haematoxylin and eosin x 400

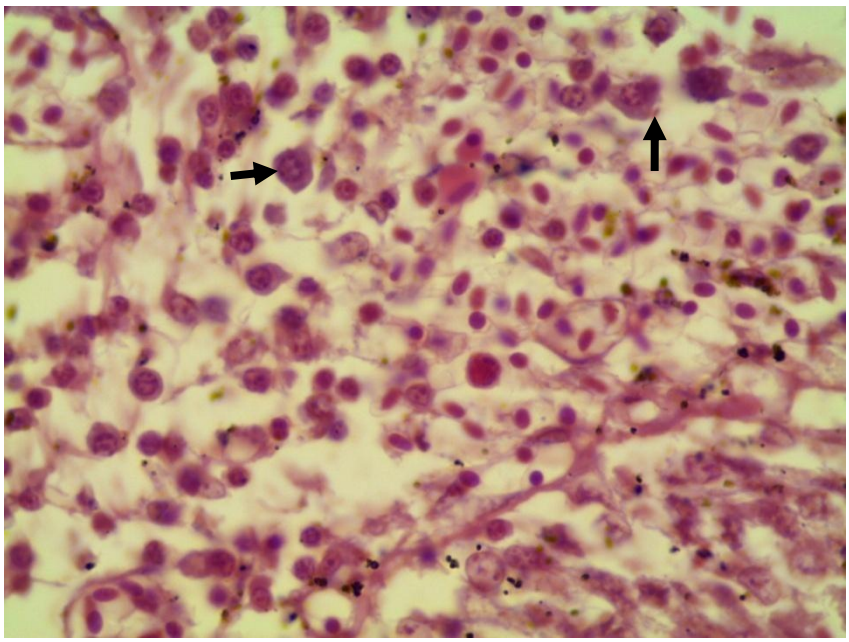


Figure 58: Infiltrationof mononuclear cell (see arrows) into renal pelvis of a lesser flamingo (LN4/2004) examined retrospectively
Haematoxylin and eosin x1000:;

The lesions in the spleen comprised multiple focal areas of complete lymphocellular necrosis (Figure 60) with masses of nuclear debris, marked presence of necrotic cells over the entire parenchyma and severe infiltration of the tissue with heterophils and macrophages. Lesions in the intestines were characterized by: severe necrosis of gut associated lymphoid tissue with presence of nuclear debris; severe necrosis and sloughing off of glandular epithelia and villi and massive infiltration of the mucosa with heterophils and macrophages. Affected kidneys had severe necrosis of the renal pelvis (Figure 61), sloughing off of the renal tubular epithelia and infiltration of renal pelvis with heterophils and macrophages.

A massive granulomatous lesion similar to those found during the prospective survey was observed in one (1/134) lesser flamingo (B36/97) sampled during the non-outbreak period in 1997. The lesion comprised homogenous eosinophilic masses surrounded by dead or dying cells, followed by a zone of fibroblasts and then a zone that was densely infiltrated with mononuclear cells, numerous giant cells and heterophils.

Sections of gastrointestinal helminth parasites were observed in 9/134 birds, 3 handled in 1997 and 6 in 2004. Sections of tissue parasites were found in the muscle of one bird (Figures 62).

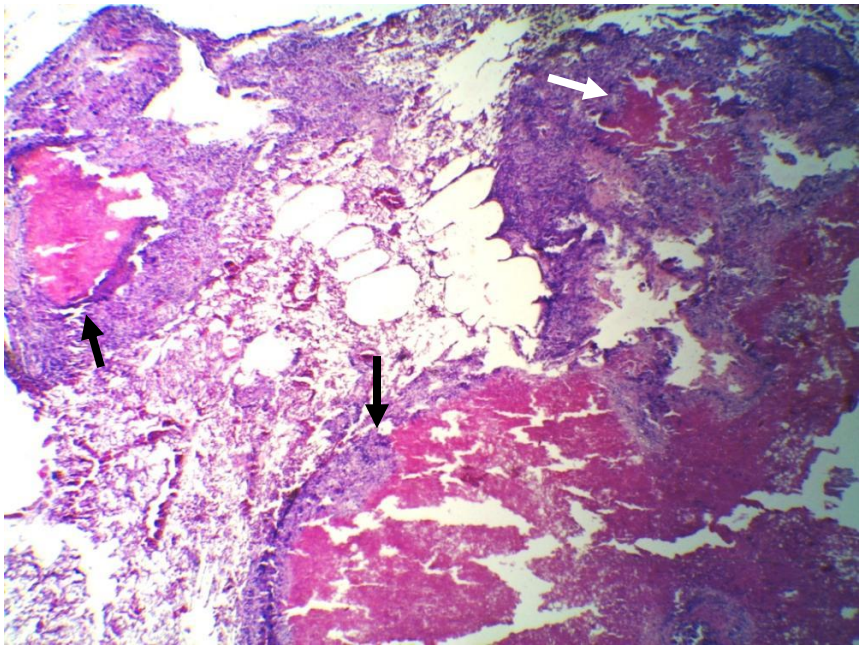


Figure 59: Multiple foci of necrosis (see arrows) in the lungs of a lesser flamingo sampled in 1997 in Lake Bogoria (B52/97)
Haematoxylin and eosin x 100

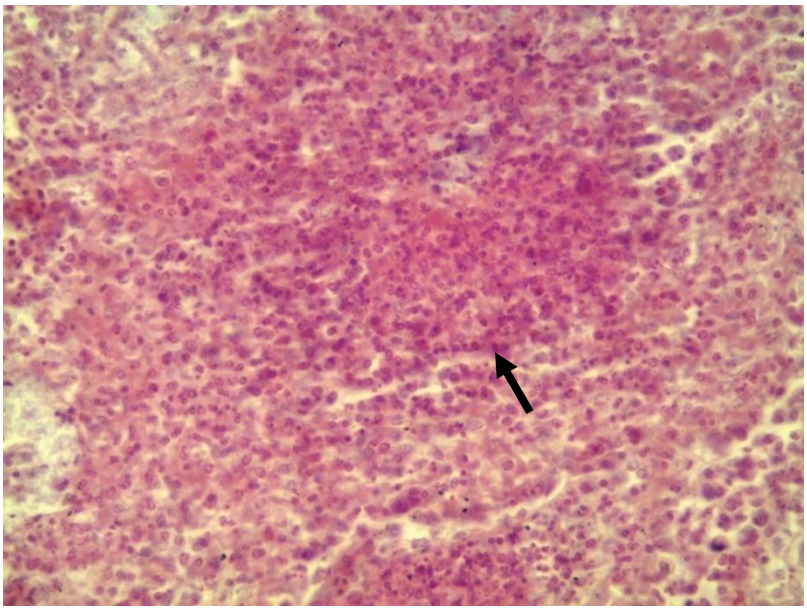


Figure 60: Extensive necrosis with massive nuclear debris (see arrow) in spleen of a lesser flamingo (LN40/2004) sampled from the 2004 die-off in Lake Nakuru.
Haematoxylin and eosin x 400

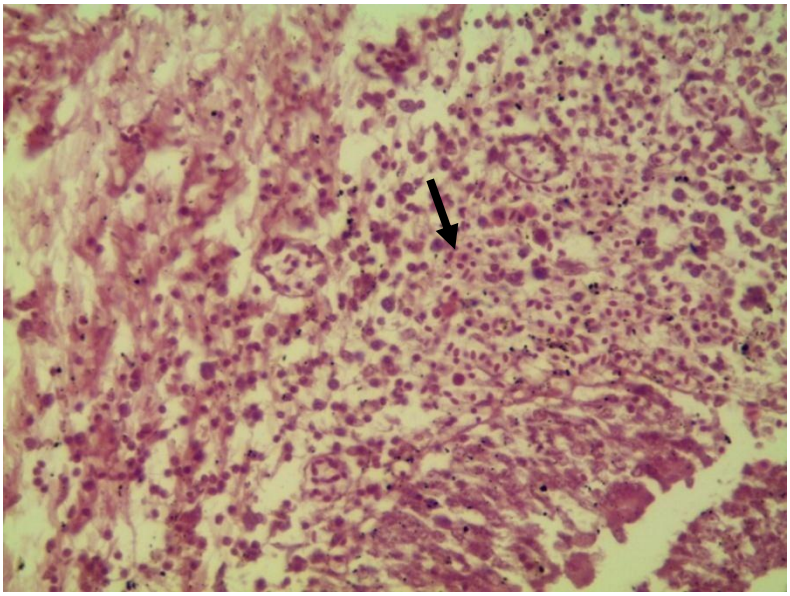


Figure 61: Necrosis (see arrow) in the renal pelvis of a lesser flamingo (LN4/2004) sampled from the 2004 die-off in Lake Nakuru.
Haematoxylin and eosin x 400

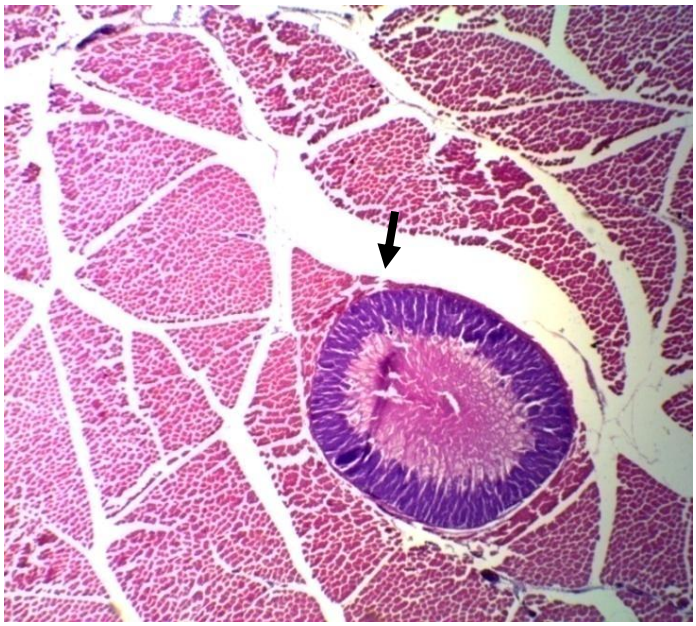


Figure 62: Section of parasite (see arrow) in the skeletal muscle of a lesser flamingo (B11/99) sampled in 2009 in Lake Bogoria.
Haematoxylin and eosin x 100

4.3.3 3 Comparison of severity of lesions

Mean scores of severity of histopathological lesions observed in retrospective tissue samples and in those collected during the 2009/2010 field study are summarized in Table 12.

The mean score of severity of congestion in tissues collected during the 2009/2010 monitoring phase was significantly lower than that of retrospective tissue samples collected during the 2004 mass mortality ($p<0.0001$). It, however, did not differ significantly from that of the retrospective tissue samples collected during the non-outbreak periods in 1997, 1998, 1999, 2000 and 2001 ($p=0.122$). The mean score of severity of haemorrhage in tissue samples collected during the monitoring phase was significantly lower than that of tissues collected during the 2004 mass mortality ($p<0.0001$). It was however, significantly higher than that of retrospective tissues samples collected during non-outbreak times ($p<0.0001$).

Mean score of severity of cellular infiltration in tissue samples collected during the monitoring phase was lower than that of samples collected during the 2004 mass mortality but higher than that of retrospective samples collected during non-outbreak times ($p=0.009$). The mean score of severity of necrosis in tissues collected during the study was significantly lower than that of the retrospective tissue samples collected during the 2004 mass mortality ($p<0.0001$) It was, however, significantly higher than that of the retrospective tissue samples collected during the non-outbreak period ($p=0.029$). The mean score of severity of granulomatous lesions in tissues

collected during the study did not differ significantly from that of the retrospective tissue samples collected during the outbreak (p=0.07) or non-outbreak periods (p=0.340)

Severity of histopathological lesions observed in the samples collected during the study was therefore lower than that observed in the retrospective samples collected during the 2004 mass mortality except for granulomas. It was however higher than that of retrospective samples collected during the non-outbreak period, except for congestion.

Table 12: Mean score of severity of lesions observed during the retrospective study and that observed during the monitoring period.

Lesion	**Mean score of severity**		
	1997-2001 **(n=81)**	2004 **(n=53)**	2009-2010 **(n=53)**
Congestion	2.1±1.6	3.8±0.4	2.5±1.2
Haemorrhage	1.0±1.6	3.9±0.3	2.1±1.2
Cellular infiltration	0.9±1.2	3.1±1.4	1.5±1.4
Necrosis	0.2±0.9	3.4±1.4	0.5±0.5
Granulomas	0.1±0.4	0	0.2±0.8

Key: 4-Severe 3-Marked 2-Moderate 1-Mild 0-Absent

X-Mean **δ** = standard deviation

n = Number of birds on which the mean was calculated

4.4 Frequency of pathogenic bacteria

The frequency of pathogenic bacteria in 46 lesser flamingos examined in the wet and dry seasons in Lakes Nakuru and Bogoria during the study is presented in Table 13. Three pathogenic bacteria, *Pasteurella multocida, Salmonella gallinarum and Mycobacterium spp.,* and a number of opportunistic pathogens were demonstrated in the tissues. The most common opportunistic pathogens isolated were *Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus.*

Table 13: Frequency of bacteria in lesser flamingos in lakes Nakuru and Bogoria during the dry and wet seasons

Species of bacteria	Frequency							
	Healthy	Sick	Dead	Dry season	Wet season	Lake Nakuru	Lake Bogoria	Overall
P. multocida	1/17	3/15	0/14	1/19	3/27	4/22	0/24	**4/46**
Mycobacterium spp	0/17	2/15	0/14	1/19	1/27	1/22	1/24	**2/46**
S.gallinarum	0/17	1/15	0/14	0/19	1/27	1/22	0/24	**1/46**
Streptococcus	5/17	3/15	3/14	5/19	6/27	6/22	5/24	**11/46**
E.coli	3/17	3/15	3/14	3/19	6/27	3/22	6/24	**9/46**
P. eroginosa	1/17	3/15	2/14	2/19	4/27	3/22	3/24	**6/46**
Staph. aureus	1/17	2/15	3/14	3/19	3/27	3/22	3/24	**6/46**

P.multocida was isolated from 4/46 (9%) lesser flamingos three of which were sick and one was healthy. All were from Lake Nakuru and three of them were sampled during the wet season and one during the dry season *Mycobacterium spp.*were demonstrated in livers and spleens of 2/46 (4%) sick birds through Ziehl-Nielsen (ZN) staining. One was from Lake Nakuru and the other

from Lake Bogoria. One was sampled during the wet season and the other during the dry season. *S.gallinarum* was isolated from 1/46 (2%) sick bird sampled in Lake Nakuru during the wet season.

E.coli was isolated from 9/46 (19%) birds; three of them were sick, three were found dead and three were healthy. Three were sampled in Lake Nakuru and 6 in Lake Bogoria. Three were sampled during the dry season and 6 during the wet season. *Pseudomonas spp.*were isolated from 6/46 (13%) birds; two of them were sick, three found dead and one healthy. Three were sampled in Lake Bogoria and the other 3 from Lake Nakuru. Two were sampled during the dry season and 4 during the wet season. *Staphylococcus aureus* was isolated from 6/46 (13%) birds three of which were sick, two found dead and one healthy. Three were sampled in Lake Nakuru and 3 in Lake Bogoria. Three were sampled during the dry season and 3 during the wet season.

Salmonella typhi, β-haemolytic *P. aeroginosa*, *E. coli and S. aureus* were isolated from lake water samples taken from various points in Lake Nakuru during the dry and wet seasons as illustrated in Table 14. *Salmonella typhi* and β-haemolytic *P. aeroginosa* were isolated from water taken from the sewage inlet near Baharini Springs in March 2009. β-haemolytic *P. aeroginosa* was also isolated from water taken during the same period from a point near the Park Headquarters with georeference: 37M0176314, UTM 99649840. *E.coli* and *P.aeroginosa* were isolated from water taken from the sewage inlet near Baharini Springs in July 2009. *E.coli* was isolated from water taken during the same period from Njoro river inlet. *S. aureus* was isolated from water taken from Hippo point in March 2010.

Table 14: Bacteria isolated from water in Lake Nakuru during the study

Location	**Season/period**	**Lake**	**Species of bacteria**
Baharini-treated sewage inlet	Dry /March 2009	Nakuru	β-haemolytic *P. aeroginosa*, S. Typhi,
	Dry / July 2009	Nakuru	*E. coli*, *Pseudomonas spp*
Near park HQ*	Dry / March 2009	Nakuru	β-haemolytic *P. aeroginosa*
Njoro river inlet	Dry /July 2009	Nakuru	*E. coli*
Hippo point	Wet / March 2010	Nakuru	*Staph. aureus*

Key
HQ* A point near Nakuru Park Headquarters with georeference (37M0176314, UTM 99649840)

4.5 Frequency of helminth parasites

The frequency of worms in 53 lesser flamingos examined for endoparasites during the study is illustrated in Table 15. Sixteen of the birds were sampled during the dry season and 37 during the wet season. Ninteen (19) were from Lake Nakuru and 34 from Lake Bogoria. Three endoparasites were found in the lesser flamingo, namely; the tapeworms *Cladogynia phoeniconiadis* and *Gynandrotaenia stammeri* and the nematode *Striatofilaria phoenicopteri.*

Table 15: Frequency of helminth parasites in lesser flamingos in Lakes nakuru and Bogoria during the dry and wet seasons

Species	**Frequency**							
	Healthy	Sick	Dead	Dry	Wet	Nakuru	Bogoria	Overall
Cladogynia phoeniconiadis	21/22	14/16	15/15	15/16	35/37	18/19	32/34	50/53
Gynandrotaenia stammeri	0/22	0/16	1/15	0/16	1/37	1/19	0/34	1/53
Striatofilaria phoenicopteri	1/22	0/16	1/15	0/16	2/37	0/19	2/34	2/53

Cladogynia phoeniconiadis was found in 50/53 (94%) birds of which 15/16 (93.7%) were sampled during the dry season and 35/37 (94.6%) during the wet season. It was present in 21/22 (95%) healthy birds, 14/16 (87%) sick birds and 15/15 (100%) birds that were found dead. It was present in 32/34 (94%) birds sampled in Lake Bogoria and 18/19 (94.7%) birds sampled in Lake Nakuru.

Gynandrotaenia stammeri was present in 1/53 (2%) bird found dead in Lake Nakuru during the wet season. *Striatofilaria phoenicopteri* was found in 2/53 (4%) birds sampled in Lake Bogoria during the wet season. One (1/22) bird was healthy and the other (1/15) was found dead

Helminth counts were determined for 37 birds all sampled during the wet season, majority (34) being from Lake Bogoria. Numbers of C. *phoeniconaiadis* were highly variable between individual birds, ranging from 0 to 345 with a mean of 44.4±76.0. The mean worm count of eight sick birds (83±141) was higher than that of 17 healthy birds (38±49) and higher still than that of the twelve dead birds (28±35). These differences were, however, not significant ($p = 0.250$). The lesser flamingo (LBF33) with the highest worm count (345) was the only one among the 37 that was in poor body condition (Appendix 7). In the group of birds that were healthy and in good body condition, the mean worm count was higher in immature birds (46±59, n=3) than in mature birds (14 ±5, n=10). This difference was, however, not statistically significant ($p = 0.387$). In the group of birds that were healthy and in good body condtion, the mean worm count was higher in female birds (58±82, n=5) than in male birds (26±23, n=8). This difference was, however not statistically significant ($p = 0.306$).

4.6 Environmental factors

4.6.1 Environmental factors during the field visits

4.6.1.1 Condition of lake and rivers

The first field visit was made to Lake Nakuru from 26th to 30th March 2009 during the dry season. All rivers supplying the lake were dry leaving the Baharini springs and the treated sewage from Nakuru Town as the only inlets. The water level in the lake was therefore low with a depth of 1.1m (long-term average depth is about 1.8m) and the shoreline had receded by over 200 m. The pH of lake water was 10.3 and conductivity was 36.2µS/cm. The water level in the lake was lower during subsequent visits, in July 2009 and March 2010, with a depth of 0.75m and 0.3m, respectively. The pH reduced marginally but conductivity increased to 59.9 µS/ cm in July 2009 and then reduced to 28.5µS/cm in March 2010. The condition of lakes Nakuru and Bogoria during the field visits in summarized below (Table 16).

4.6.1.2 Populations of lesser flamingos

The number of lesser flamingos in Lake Nakuru during the first visit in March 2009 was estimated at 30,000. Records at the KWS research station indicated that the number of birds had been about 8000 in mid March and had been increasing over the previous two weeks. Tweny other species of birds were recorded, the most abundant being the Great white pelican (*Pelecanus onocrotalus)* that was estimated at 7000 birds. One dead and two sick lesser flamingos and one dead Egyptian goose (*Alopochen aegyptiacus*) were recorded during the visit. The populations of

lesser flamingos and diversity of bird species recorded in the lakes during the visits are summarized in Table 17. Figures 63 and 64 demonstrate the relative abundance of the lesser flamingos observed during the visits in Lake Nakuru.

Table 16: Water quality in Lakes Nakuru and Bogoria during the study visits

Visit/Date	Lake visited	Season	Flow of rivers supplying lake	Lake centre Depth (m)	pH	Conductivity(µ S/cm)
Visit 1: March 09	Lake Nakuru	Dry	All rivers dry	1.1	10.3	36.2
Visit 2: July 09	Lake Nakuru	Dry	All rivers dry	0.75	10.2	59.9
Visit 3: November 09	Lake Bogoria	Wet	All rivers dry	-	-	72.7
Visit 4: March 10	Lake Nakuru	Wet	River Njoro flowing	0.3	10.1	28.5

Key

- = Parameter was not measured

The number of lesser flamingos in Lake Nakuru during the second visit in July 2009 was estimated at 500,000 birds, double the estimate of 250,000 made during the July census conducted earlier in the month by NMK. Twelve other species of birds were recorded, most of them in small numbers (less than twenty) except great white pelicans (*Pelecanus onocrotalus)* and marabou storks (*Leptoptilos crumeniferus*) which were unusually abundant, estimated at 30,000 and 5000, respectively. Five lesser flamingos, one Egyptian goose (*Alopochen aegyptiacus*) and one whiskered tern *(Chlidonias hybridus*) were found dead and 8 sick lesser flamingos were recorded. Marabou storks and hyenas were noted scavenging on dead flamingos (Figure 65).

Table 17: Population estimates of lesser flamingos and diversity of waterfowl species in the study sites.

Visit/Date	Lake visited	Season	N_{Lf}	N_{pel}	Number found dead or sick birds			Number of other species	Trend in lesser flamingos
					Lf_d	Lf_s	Eg_d		
Visit 1: March 09	Lake Nakuru	Dry	30,000	7000	1	2	1	>20	Increasing
Visit 2: July 09	Lake Nakuru	Dry	500,000	30000	5	8	1	10	Increasing
Visit 3: Nov 09	Lake Bogoria	Wet	734,000	0	100	5	0	15	Decreasing
Visit 4: March 10	Lake Nakuru	Wet	15,000	0	0	2	0	4	Stable for at least 3 months

Key:

N_{pel}=Estimated population of Great white pelicans N_{Lf}=Estimated population of Lesser flamingos L_d =Dead Lesser flamingos L_s =Sick Lesser flamingos
Eg_d= Dead Egyptian geese

The number of lesser flamingos in Lake Bogoria during the third visit was estimated at 734, 000. Fifteen other species of birds were recorded. About 100 carcasses of lesser flamingos, most of which were over a week old, were counted along the lake shore over a stretch of about 150 m in the Northern Basin of the lake and six sick lesser flamingos were also recorded. Twelve fresh carcasses and the 6 sick birds were further examined and sampled and the findings are described in section 4.2.

Figure 63: Relative abundance of lesser flamingo in Lake Nakuru during the March 2009 visit when population was about 30,000 (compare with Fig.11)

Figure 64: Relative abundance of lesser flamingo in Lake Nakuru during the July 2009 visit when population was about 500,000

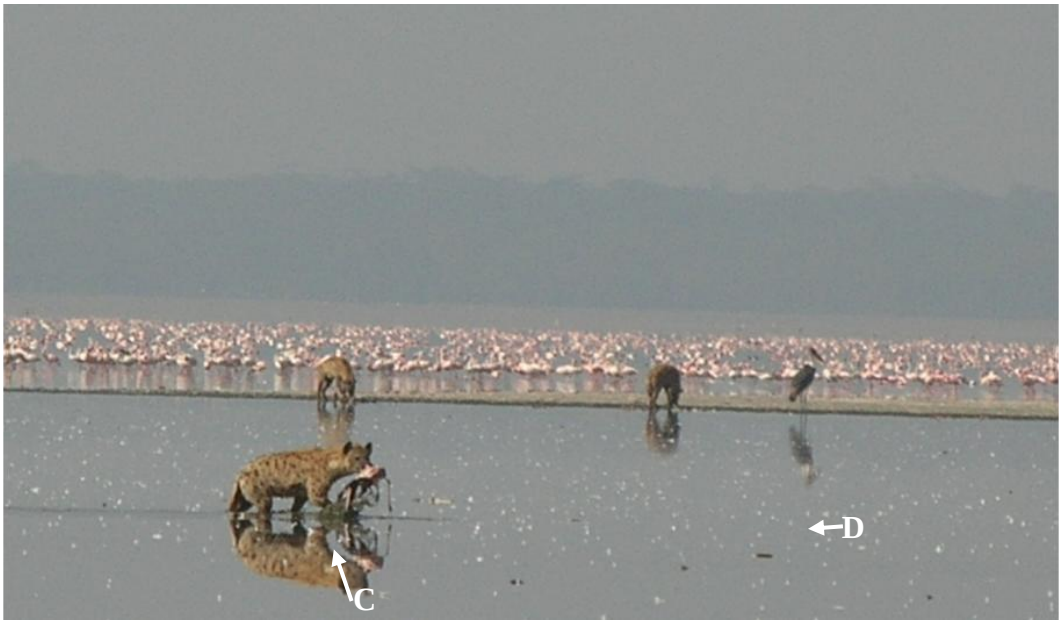


Figure 65: Hyenas (C) and marabou storks (D) scavenging on dead lesser flamingos in Lake Nakuru during July 2009 visit.

The number of lesser flamingos in Lake Nakuru during the fourth visit was estimated at 15,000. Four other species were recorded at the lake in small numbers (less than twenty) namely; marabou storks, black-winged stilts and Egyptian geese. All birds seemed healthy from a distance but the captured ones had signs of diarrhea. Deaths were, however, not recorded.

4.6.2 Trends in environmental factors based on secondary data

4.6.2.1 Rainfall and lake depth

The mean annual rainfall of Lake Nakuru in 2009 was 363.4 mm which was quite low compared to the normal annual mean for the lake of about 750mm. The year 2010 on the other hand was wetter than usual with a mean annual rainfall of 956.3mm.

The trend in mean monthly rainfall and depth of Lake Nakuru from 2008 to 2010 is illustrated in Figure 14. Coinciding with the drought in 2009, the water level in the lake decreased steadily from a depth of 1.67 m in December 2008 to a minimum level of 0.24 m in December 2009. The mean depth in 2009 was 0.77 m compared to 1.70 m in 2010 and 1.58 m in 2008. Following the onset of long rains in February 2010, the water level in the lake increased rapidly. There was no significance relationship between lake depth and amount of rainfall ($r = -0.065$, $p = 0.705$). This could be due to a time lag between rainfall and rise of water in the lake.

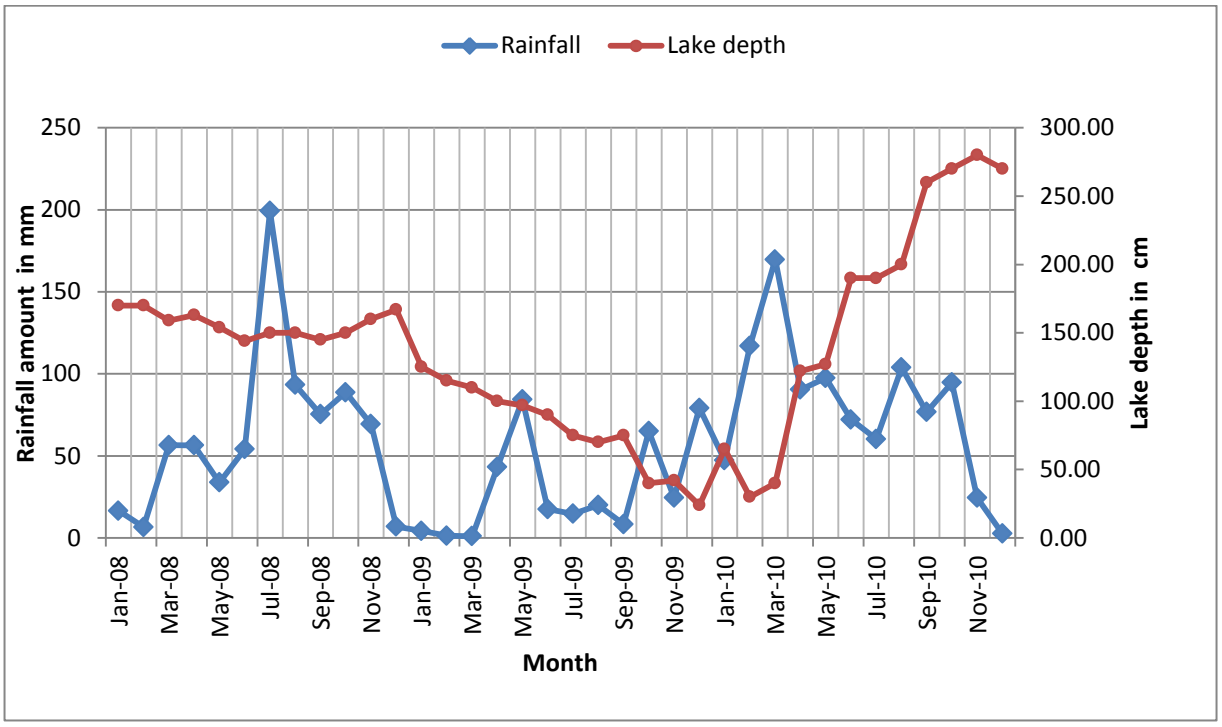


Figure 66: Trends in rainfall and depth of Lake Nakuru during the study period

4.6.2.2 Conductivity of lake water.

The conductivity of water in Lake Nakuru taken monthly from 2008 to 2010 had an inverse relationship with the depth of the lake ($r= -0.834$, $p<0.01$) (Figure 67). A regression analysis for the level of conductivity against depth of the lake indicated that the former is highly depend on the latter ($Y_{(conductivity)} = 79.95 - 313X_{(lake\ depth)}$). Conductivity increased steadily from the beginning to the end of 2009 coinciding with the drought period during which water level in the lake decreased steadily. It decreased following the onset of short rains in October and November 2009 and the long rains in February 2010, due to the dilution effect of water inflow into the lake.

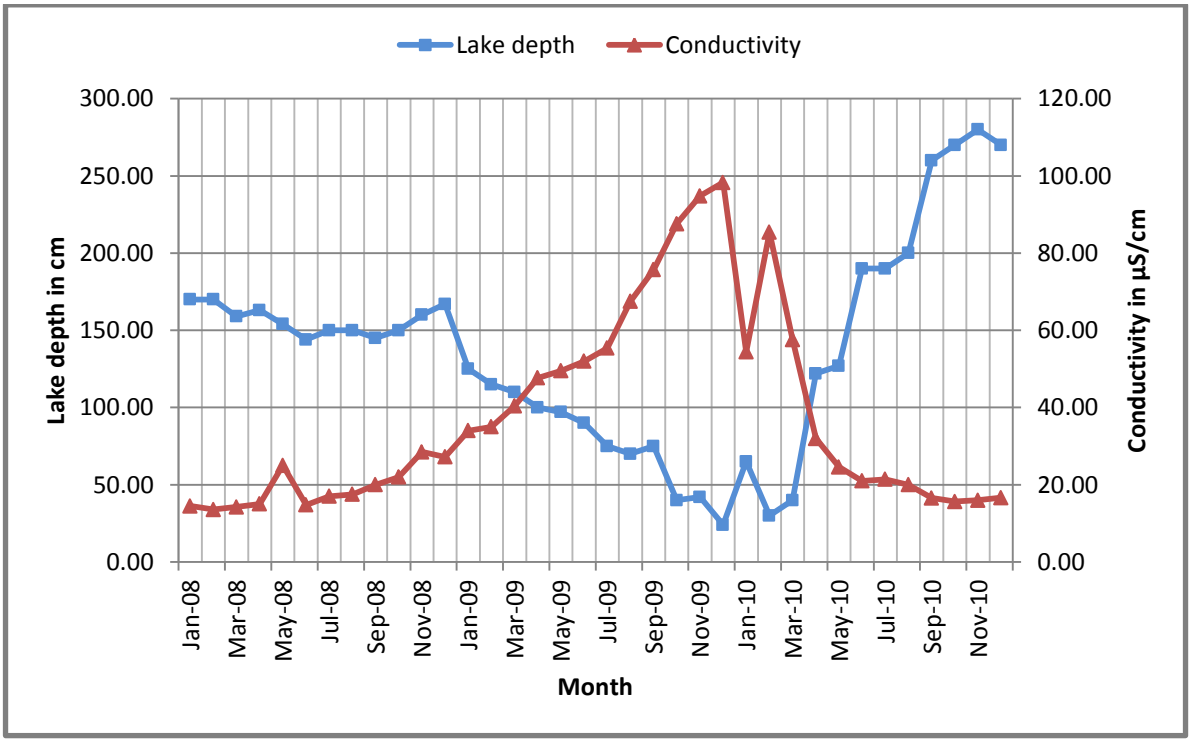


Figure 67: Conductivity and depth of water in Lake Nakuru during the study period

4.6.2.3 Concentration of nitrogen compounds in lake water

The concentrations of nitrates (NO3-N) and ammonium compounds (NH3-N) in the lake measured monthly from 2008 to 2010 were highly variable (Figure 68). Small peaks in concentration of nitrate compounds were recorded in May and November 2008, April 2009 and September 2009 all occurring about one to two months following the onset of the light rains. A major peak in the same compounds was recorded in August 2010 following prolonged heavy rains. Correlation between rainfall and nitrogen compounds was weak and insignificant. It was however stronger between ammonium compounds and rainfall (r=0.186, p=0.277) than between nitrates and rainfall (r=0.057, p=0.754).

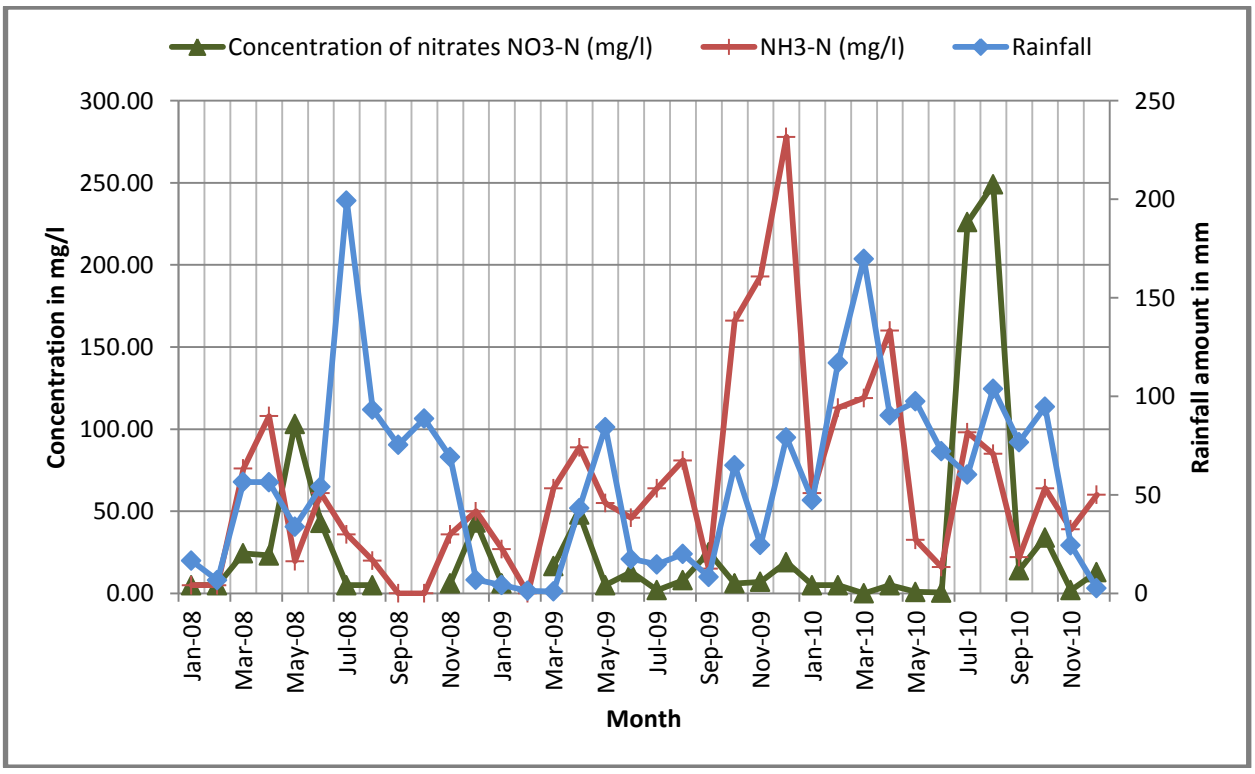


Figure 68: Concentration of nitrogen compounds in relation to rainfall in Lake Nakuru

4.6.2.4 Concentration of *Arthrospira spp*.in lake water

The concentration of *Arthrospira* in the lake measured monthly from 2009 to 2010 was highly variable (Figure 69). Peak concentrations in arthrospira were however reported in May 2009 and October 2010, one and two months respectively, after peak concentration of nitrates. There was no significant correlation between concentration of arthrospira and concentration of nitrates (r=-0.037, p=0.872).

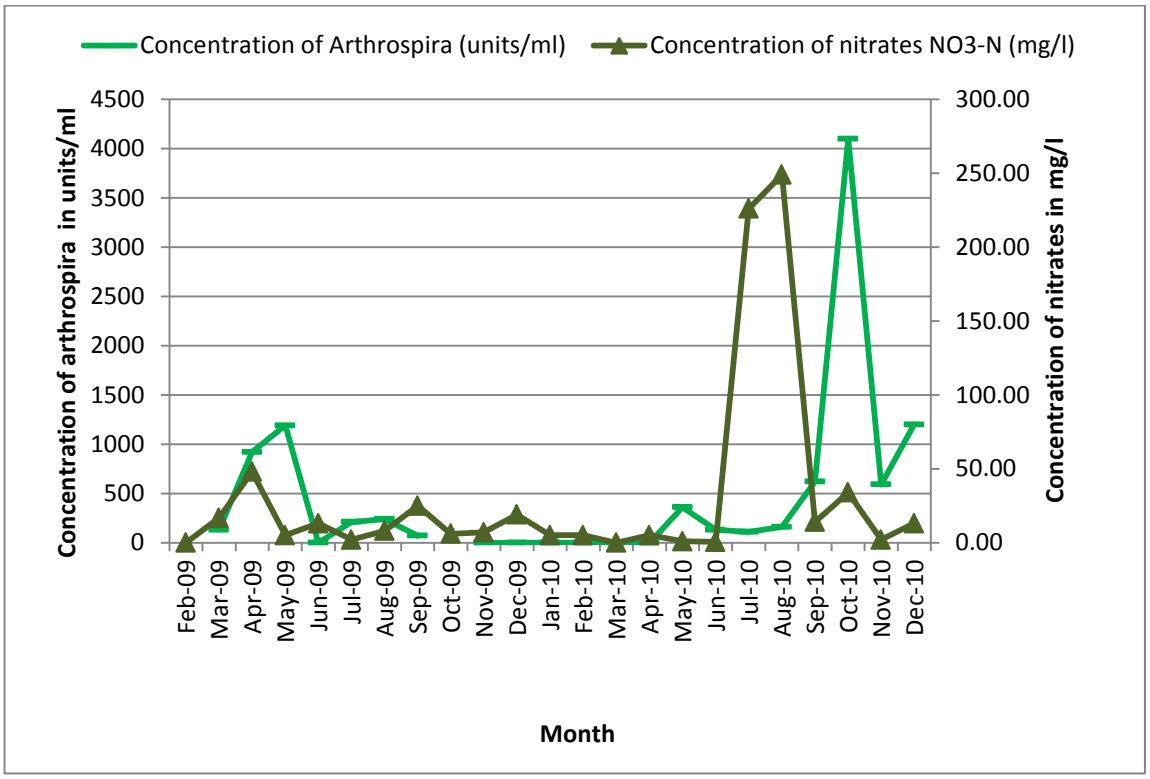


Figure 69: Concentration of *Arthrospira spp* in relation to nitrates in Lake Nakuru

4.6.2.5 Population of lesser flamingo in the two lakes.

The variation in population of lesser flamingo between Lakes Nakuru and Bogoria from 2009 to 2010, based on the biennial waterbird counts counducted by NMK and KWS, had a negative correlation (r=-0.503, p=0.497) (Figure 70). This was, however, not statistically significant. The biennial estimates were conducted at different times from the field visits in this study and therefore the July estimates in this study (Table 17) differ from the one from secondary data.

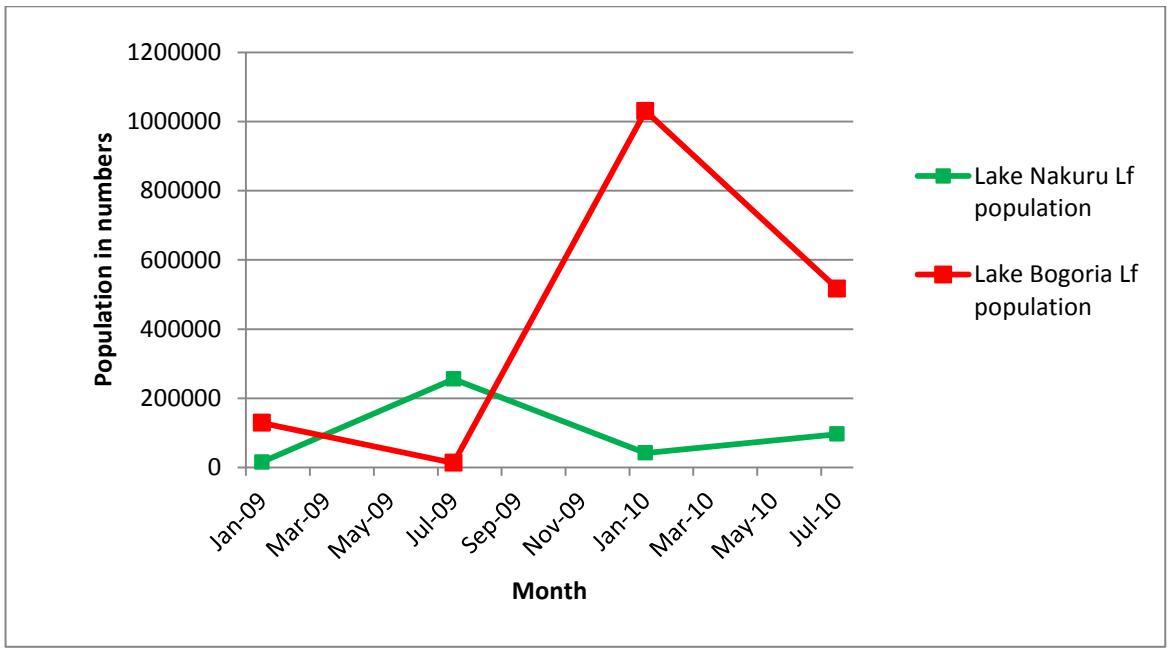


Figure 70: Patterns in lesser flamingo populations in the two lakes during the study

Key: Lf = Lesser flamingo

4.6.2.6 Populations of lesser flamingo in relation to rainfall.

The population of lesser flamingo in the lakes had a slight negative correlation with rainfall but this was not statistically significant (r = -0.154, p=0.846) (Figure 71). The population data available, however, was for January and July both of which are drier months though amounts of rainfall may differ between the months.

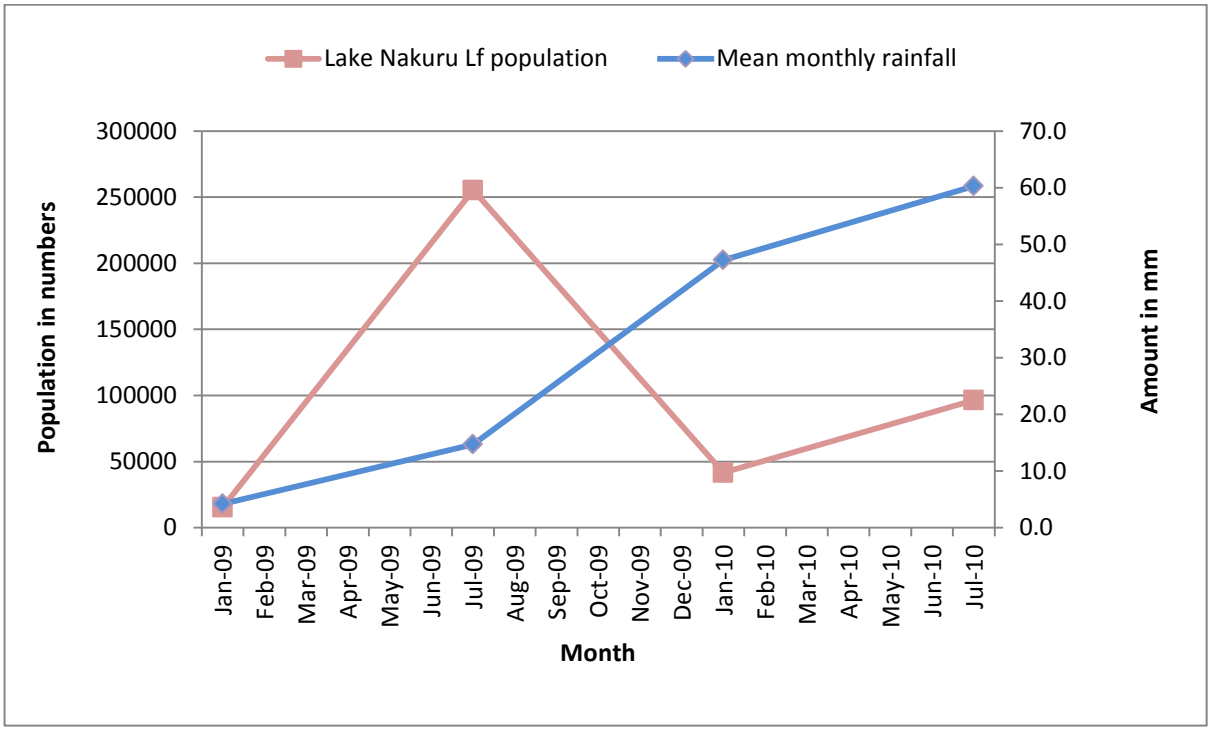


Figure 71: Population of lesser flamingo in relation to rainfall in Lake Nakuru

Key: Lf= Lesser flamingo

4.6.2.7 Populations of lesser flamingo in relation to *Arthrospira*

The population of lesser flamingo in Lake Nakuru during the study period had a strong and statistically significant positive corelation with the concentration of *Arthrospira* (r=0.968, p=0.032) (Figure 72). This was based on biennial waterbird counts conducted by NMK and water quality analyses done by KWS during the counting periods in January and July.

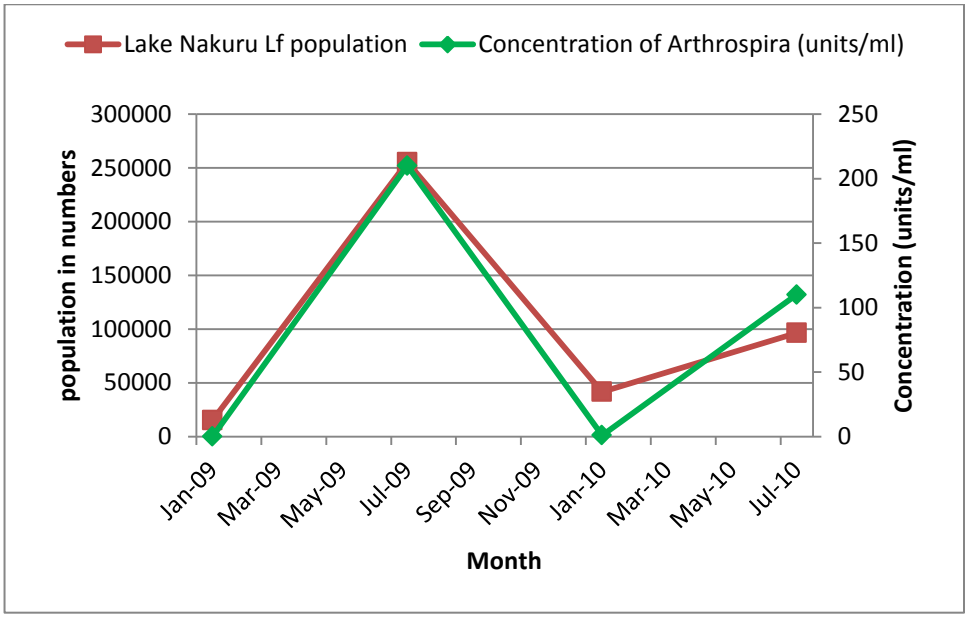


Figure 72: Population of lesser flamingos in Lake Nakuru in relation to *Arthrospira spp*

Key:
Lf=Lesser flamingos

4.6.2.8 Long-term trends in population of lesser flamingos.

The lesser flamingo population in Lakes Nakuru and Bogoria from 2000 to 2010 showed wide fluctuations and numbers recorded in either of the lakes ranged from below 15,000 to over a million birds (Figure 73).There was a negative correlation between the population of the birds in the two lakes though it was not statistically significant (r= -0.370, p=0.293). The population was higher in Lake Nakuru than Lake Bogoria during most of the censuses. A downward trend in the population was evident in Lake Bogoria between 2000 and 2009 but it rebounded in 2010.

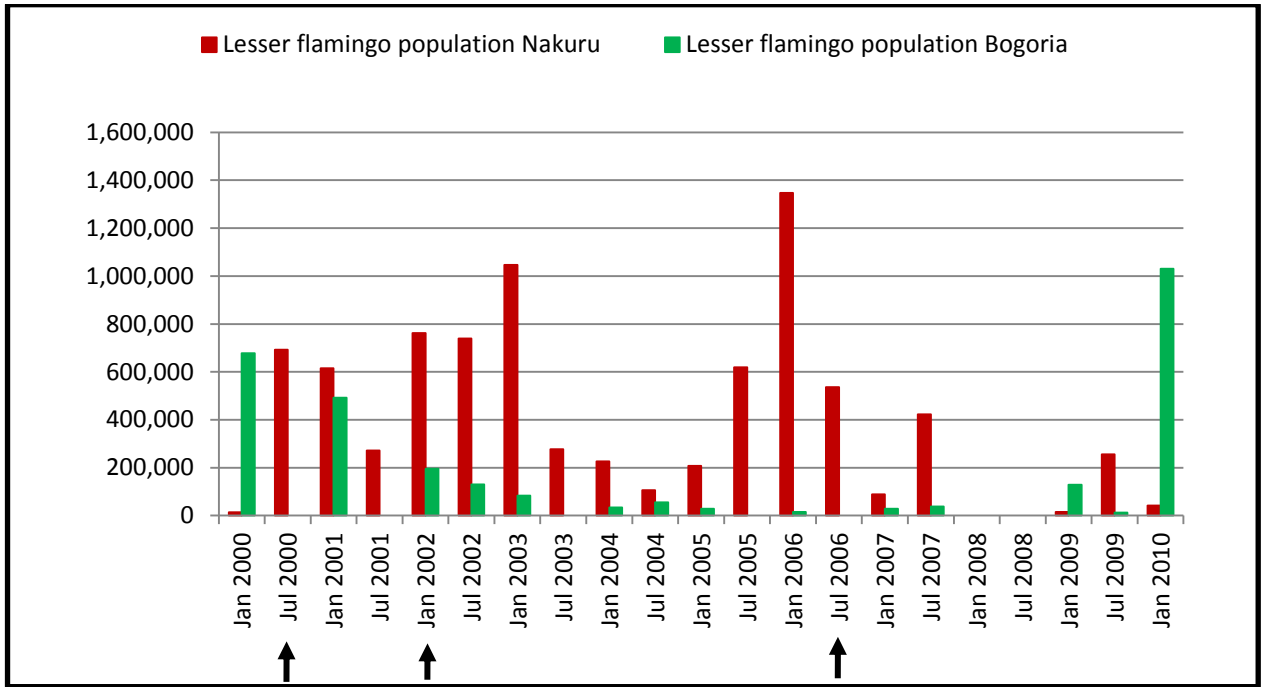


Figure 73: Ten-year counts of lesser flamingos in Lakes Nakuru and Bogoria for the months of Januay and July*

* July data for Lake Bogoria and 2008 data were unavailable

Mass mortalities in lesser flamingosreported in July 2000, July 2002, September 2004 and July 2006 corresponded to the times when there were high populations of the species (over 500,000) in Lake Nakuru. Numbers of dead flamingo recorded during bi-annual water bird censuses from 2000 to 2010 are shown in Table 18. The number counted in Lake Bogoria over the period ranged from none to 222 carcasses recorded in January 2009, with an average of 15 carcasses per census. The number recorded in Lake Nakuru ranged from zero to 1245 carcasses recorded in July 2006 during the mass mortality, with an average of 73 carcasses per census.

Table 18: Counts of dead lesser flamingos recorded during bi-annual water bird censuses

Year	Nakuru		Bogoria		Total
	January count	July count	January count	July count	
2003	0	19	0	0	19
2004	5	0	0	0	5
2005	0	7	0	0	7
2006	0	1245	0	0	1245
2007	124	1	4	2	131
2009	3	32	222	3	260
2010	22	0	3	0	25
Total	154	1304	229	5	1692

Various species of birds were recorded scavenging on lesser flamingo carcasses at various times during the biennual censuses. They included; Marabou Stork, Pied Crow, Tawny, Osprey and Steppe eagles, Sacred Ibis and Grey headed Gull.

4.6.2.9 Long-term trends in population of other birds.

The populations of Greater flamingos, Egyptian geese, Marabou storks and Great white pelicans were highly variable in the two lakes over the 10 years (2000-2010) but there was no overall increase or decline (Figures 74 and 75). The populations of the species, except the Greater flamingos, were much lower in Lake Bogoria than Lake Nakuru over the period. The variations in populations of resident species in both lakes did not show association to one another or to those of the lesser flamingos. The population patterns of the three resident species did not indicate any association with years when mass mortalities were reported.

The population of Palearctic migrants in Lake Nakuru showed a regular pattern of fluctuation between January (highs) and July (lows) with no overall increase or decline over the years (Figure 76). There was a sharp drop in population of these species to an all-time low in May 2003 followed by recovery to normal levels the following year. Fluctuations in the Afrotropical migrant species did not show a clear pattern. The population of these species declined from 2004 to 2007 followed by a rebound in 2007.

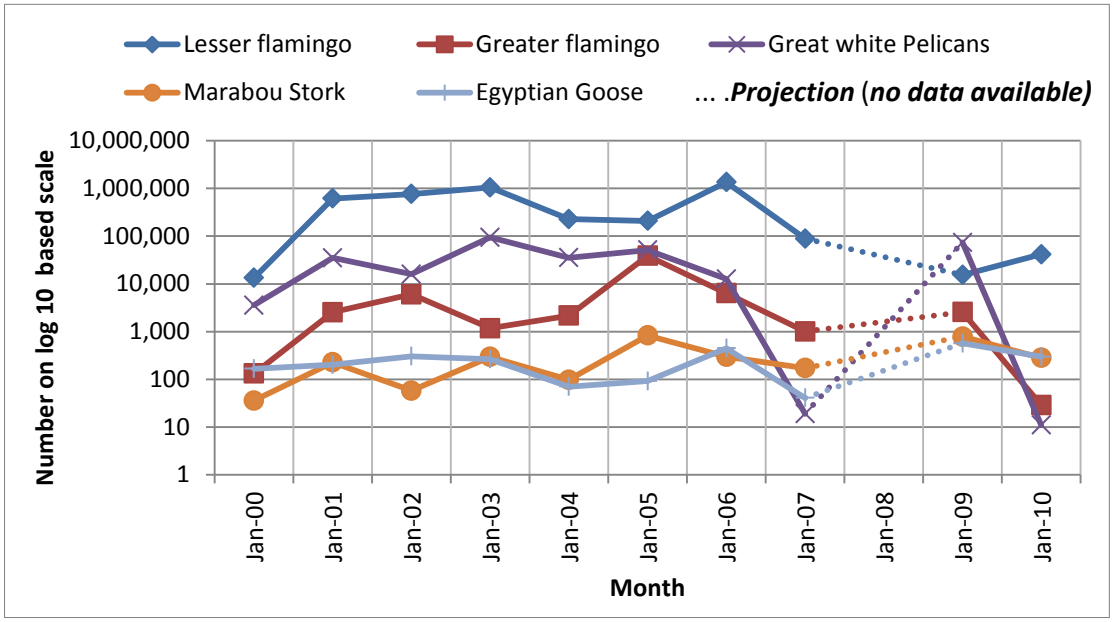


Figure 74: Population of lesser flamingo andlocalwaterfowl in Lake Nakuru

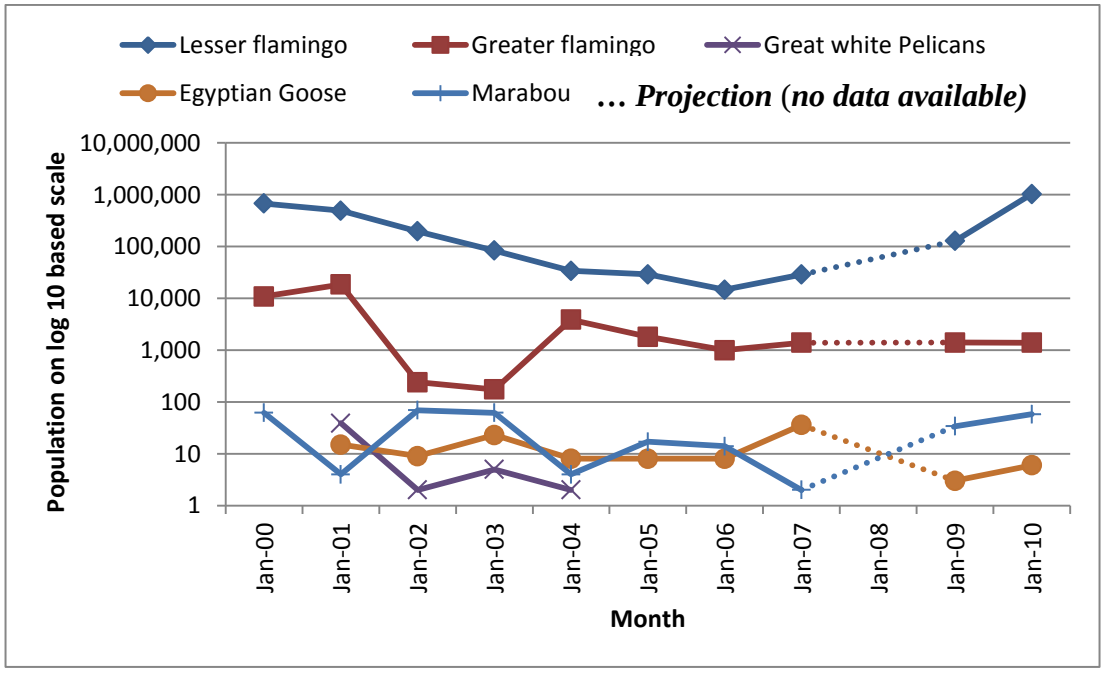


Figure 75: Population of lesser flamingo and local migrant waterfowl in Lake Bogoria

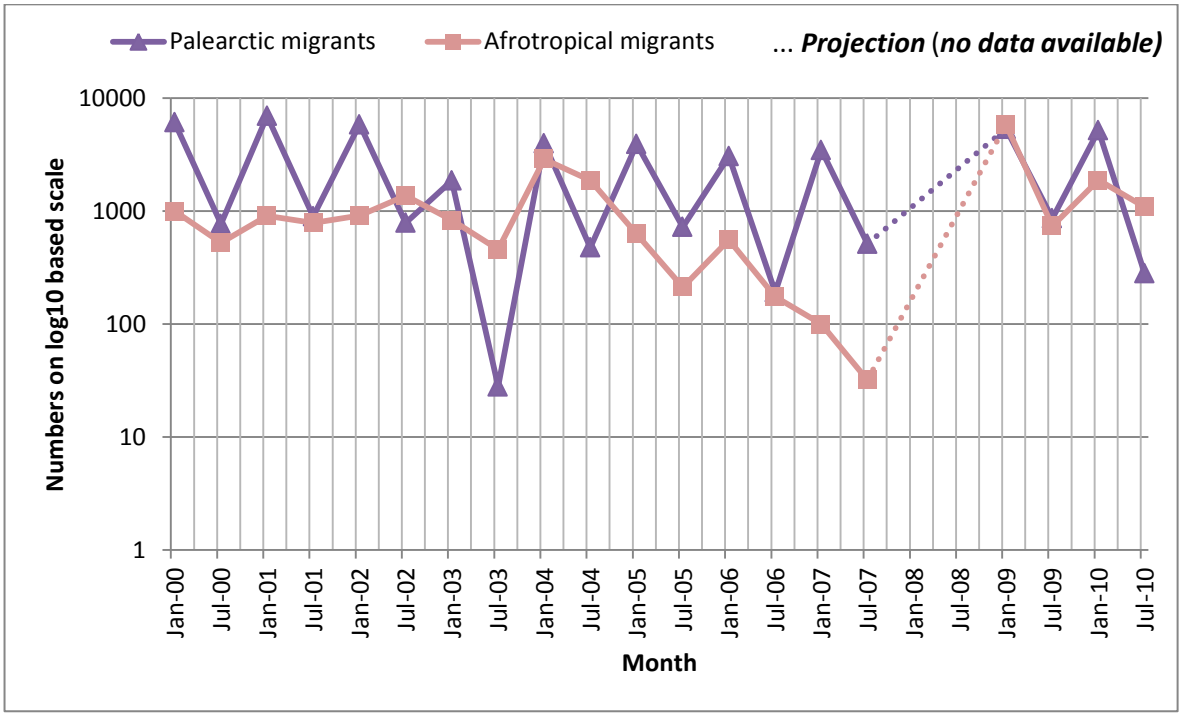


Figure 76: Population patterns of Palearctic and Afrotropical migrants in Lake Nakuru

CHAPTER 5

5. DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Health assessment

5.1.1 Clinical signs and gross postmortem lesions

Lesser flamingos with severe acute disease manifested weakness, impaired activity, coma and death in good body condition. These birds often had generalized congestion and haemorrhage of organs as the predominant lesions. Mean liver and spleen mass indices of sick and dead birds were higher than those of healthy birds and this provided quantitative support to observation of congestion in these organs. Haemorrhage was of petecchial and ecchymotic type and was severest in the livers, spleens, kidneys and lungs. These clinical signs and gross postmortem lesions have been reported in lesser flamingos during mass mortalities (Motelin *et al.,* 1995, Manyibe *et al.,* 2007). Such signs and gross lesions can be caused by a wide range of infectious or toxicological diseases.

Signs of diarrhea were observed in four lesser flamingos. It was greenish in one emaciated and weak bird that was examined in Lake Nakuru during the dry season. This bird had generalized granulomas in the visceral organs at necropsy.Whitish diarrhea was seen in two birds examined

in Lake Nakuru during the wet season. Both were captured randomly by trapping; one was in fair and the other in good body condition. They had moderate congestion of the intestinal mucosa and enlargement of the bursa of Fabricius at necropsy. Diarrhoea, manifested only by heavy matting of feathers around the cloaca, was seen in one bird in good body condition that was examined in Lake Bogoria during the wet season. This bird had a compound fracture of the left wing. .The signs of diarrhea and lesions observed in the two birds that were randomly trapped could indicate presence of a mild enteric disease in the lesser flamingo population that was difficult to notice as it did not visibly impair the activity of the birds. The signs of diarrhea observed in the two birds that had other obvious disease conditions, could be due to opportunistic infections by bacteria such as E.coli, Staphylococcus or other microbial agents.

Signs of chronic disease were observed in two lesser flamingos comprising poor body condition or emaciation accompanied by weakness and impaired activity. Granulomatous masses were present in the visceral organs of the two birds. These signs and lesions were similar to those reported by others during mass mortalities of lesser flamingos that were associated with mycobacteriosis (Cooper *et al.,* 1975; Kock *et al.,* 1999; Sileo *et al.,* 1979).

Lesions in the wings compring abscesses in the proximal joints in two birds and a compound fracture in one bird were observed in the lesser flamingos during the study. Such lesions have been reported previously in the species (Motelin et al., 1995) and have been attributed to physical trauma sustained by hitting objects while flying between lakes. The swelling of joints could also be caused by localized bacterial infections.

Enlargement of the bursae of Fabricius that was observed in 7/22 healthy and 5/35 sick birds during this study has not, to my knowledge, been reported previously in lesser flamingos. These lesion raised suspicion of Gumboro disease. However, rapid antigen detection tests conducted opportunistically on three birds for Gumboro, Newcastle Disease and Highly Pathogenic Avian Influenza were negative for all the diseases. The cause and significance of this lesion was therefore not established and should be investigated more comprehensively in the future.

5.1.2 Haematology

The mean PCV value (44.0 ±6.3) of 4 healthy flamingos in this study was comparable to that of 44.0±5.9 (n=55) reported for the species by the International Species Information System (ISIS) (ISIS, 2002). The mean of the same parameter was also comparable to that reported by Peinado *et al*. (1992) (45±0.03 (n=2)) and that reported by Hawky *et al.* (1985) (50±0.03 (n=10)). The mean PCV of the sick birds was lower than for the healthy birds but this difference was only significant for the 5 sick birds in poor body condition ($p = 0.029$). This could be attributed to the nutritional stress caused by chronic debilitating disease and possible heavy parasitism that was observed in the sampled lesser flamingos.

The mean absolute WBC count (22.8± 12.5 x 10^3 / µl) of the four healthy birds was higher than that of 11.9 ± 6.2 x 10^3 / µl (n=44) reported by ISIS (ISIS, 2002) but it was within the range of (4.6-40.4) x 10^3 / µl reported by the same author. The mean absolute WBC count was also higher than that reported by Peinado *et al.* (1992) ((11.9± 5.7) x 10^3 / µl (n=2)). It was also higher than

the mean reported by Hawky *et al.* (1985) ((6.0±2.0) x 10^3 / µl (n=10)) and the range reported by the same aithor (3.8 x 10^3 / µl -8.5 x 10^3 / µl). These differences could be due to the fact that the other reports are based on studies in captive lesser flamingos whereas the current study is done in free ranging birds: conditions in captivity differ significantly from free range. It could also be attributed to methodological variations between the different studies. This includes differences in method of handling of the birds which determines the level of haematological variation related handling-stress in the birds. The mean WBC of the 8 sick birds ((10.6± 7.8) x 10^3 / µl) was lower than that of the healthy group of birds. It was however comparable to the mean reported by ISIS for the parameter and to that reported by Peinado *et al.* (1992) for healthy lesser flamingos. These findings indicate that WBC values are highly variable and reference values are most useful diagnostically if they are determined for the specific group of birds under management. This is in agreement with observations by Vleck *et al*., 2000.

The mean absolute heterophil count ((2.7 ±1.0) x 10^3 / µl) of the 4 healthy lesser flamingos was lower than that reported by ISIS for the parameter ((7.0±5.3) x 10^3 / µl (n=42)) but within the range (0.75 x 10^3 / µl - 30.3 x 10^3 / µl) reported by the same author. The mean absolute heterophil count was also lower than that reported by Peinado *et al.* (1992) ((4.0±0.7) x 10^3 / µl (n=2)) and that reported by Hawky *et al.* (1985) ((4.6±1.8) x 10^3 / µl (n=10)) for the same parameter. These findings could reflect differences between lesser flamingos in captive situation and free ranging populations, as well as methodological variations between the studies. The wide reference range reported by ISIS for heterophil count indicates that the parameter is highly variable in individual birds and needs cautious interpretation for diagnostic purposes. The mean absolute heterophil

count of the 8 sick birds ((4.9±4.8) x 10^3 / µl) was higher than the mean of the healthy group of birds and higher than that reported by Peinado *et al.* (1992) and that reported by Hawk *et al.* (1985). It was however lower than the mean reported by ISIS. The relatively higher mean heterophil count of the sick birds compared to the healthy group could reflect haematological changes in the former due to immunological reaction due to infection.

The mean absolute lymphocyte count (18.0±10.6 x 10^3 / µl) of the 4 healthy lesser flamingos was higher than that reported by ISIS (ISIS, 2002) (4.4 ±2.7 x 10^3 / µl) and higher than the range reported by the same author (0.72 x 10^3 / µl -12.2 x 10^3 / µl). The mean of the parameter was also higher than that reported by Peinado *et al.* (1992) ((7.4±4.3) x 10^3 / µl (n=2)) and that reported by Hawky *et al.* (1985) ((1.21±0.56) x 10^3 / µl (n=10)). These differences could be attributable to differences between captive and free ranging conditions for lesser flamingos and methodological variations in the studies. The mean absolute lymphocyte count ((3.2±1.9) x 10^3 / µl) of the 8 sick lesser flamingos was significantly lower than that of the healthy group of birds ($p=0.002$). It was also lower than the mean for the parameter reported by ISIS (ISIS, 2002) and that reported by Peinado *et al.* (1992). However it was higher than the value reported Hawky *et al.* (1982). Based on the findings it is justified to conclude that the sick lesser flamingos had a notable lymphopaenia. This could be due to haematological changes in the sick birds associated with immunological reaction and the stress of disease. This is consistent with the observations of Vleck *et al.* (2000) in penguins.

The mean absolute monocyte count in the 4 healthy birds ((0.8 ±1.0) x 10^3 / µl) was higher than that of ((0.6±0.5) x 10^3 / µl (n=32)) reported by ISIS for the same parameter but was within the range of 0.08 x 10^3 / µl -2.8 x 10^3 / µl reported by the same author. The mean value of the same parameter was comparable to that reported by Peinado *et al*. (1992) ((0.08±0.1) x 10^3 / µl (n=2)) .It was however lower than that reported by Hawky *et al. (*1985) ((0.10±0.15) x 10^3 / µl (n=10). The differences could be explained by differences between captive and free ranging conditions for lesser flamingos as well as methodological variations between the studies. The mean absolute monocyte count in the 8 sick lesser flamingos ((0.4±0.5) x 10^3 / µl) was lower than that of the healthy birds, though the difference was not significant. It was also lower than the mean value for the same parameter reported by ISIS (ISIS, 2002) and Peinado *et al.* (1992) but higher than the mean value reported by Hawky *et al.* (1985).

The mean absolute eosinophil count ((0.48 ± 0.36) x 10^3 / µl) of 4 healthy lesser flamingos was comparable to that reported by ISIS (2002) ((0.4±0.6) x 10^3 / µl (n=22)) for the same parameter. It was higher than that reported by Peinado *et al. (*1992) ((0.16± 0.23) x 10^3 / µl (n=2)) and that reported by Hawky *et al.* (1985) ((0 / µl (n=10)). The mean absolute eosinophil count for the 8 sick birds ((0.4±0.5) x 10^3 / µl) was comparable to that of the healthy group of birds and to that reported by ISIS. It is notable that most of the birds had heavy worm burden and this may be suspected to have been the case in other studies since the birds are usually never dewormed. This may explain why eosinophil count, which often increases in parasitism, was uniform in all birds in the study and comparable to other studies.

The mean absolute basophil count (0) of the 4 healthy birds was lower than that reported by ISIS (2002) ((0.3±0.2) x 10^3 / μl (n=27)). It was also lower than the values reported by others (Peinado *et al.,* 1992; Hawky *et al.* 1982). The mean absolute basophil count of the 8 sick birds was also zero (0). It is notable that basophil counts rarely change significantly in infectious diseases.

The heterophil to lymphocyte ratio of the healthy group of birds was 1:5 while that of the sick group of birds was 4:5 reflecting a four-fold increase in this ratio in the sick birds. This indicates that heterophil counts were elevated while lymphocyte counts were lowered in the sick birds. It is therefore justified to conclude that the sick lesser flamingos examined in the study responded with a heterophilia and lymphopaenia.This could be explained by the immunological response and stress associated with disease which is consistent with observations made by others (Vleck *et al*., 2002).

5.1.4 Histopathology

Congestion, haemorrhage and extensive necrosis in the visceral organs associated with infiltration with heterophils and mononuclear inflammatory cells were major and consistent lesions in lesser flamingos that were examined during the field monitoring phase as well as during the retrospective study. The lesions were most frequent and severe in tissues from the flamingos that were sampled during the mass mortality in 2004. Infiltration of body tissues with heterophils, often accompanied by macrophages, was a predominant feature in 80% (44/53) of the birds sampled during the mass mortality and it was marked or severe in most of the birds.

Spleens and intestines were the most frequently affected by severe necrosis (recorded in 27/53 and 21/53 birds respectively), which was mostly accompanied by infiltration with inflammatory cells. Lungs were the most frequently affected by severe congestion and haemorrhage (recorded in 34/53 birds). These lesions were consistent with bacterial septicaemia. Similar infiltration of tissues with inflammatory cells was observed in 40% (10/22) of birds taken in 1997 and 70% (15/19) of those taken in 2001.

Focal and multifocal necrotic lesions were observed in tissues of 12/53 lesser flamingos that were sampled during the survey, and 6/134 that were examined retrospectively. They mostly affected livers, spleens and lungs and were characterized by infiltration with heterophils and mononuclear inflammatory cells, and fibrous encapsulation. Seven out of the 12 birds observed with these lesions during the survey were healthy. The lesions suggest sub-acute and asymptomatic bacterial infections in the birds or birds recovering from infection. Birds with such lesions could succumb to infection under situations of stress and depressed immunity.

Severe and widespread granulomatous lesions typical of mycobacteriosis occurred in tissues of 2/57 birds sampled during the 2009/2010 field study and those of 1/134 birds studied retrospectively over 6 years. Higher frequencies of such granulomatous lesions have been reported in previous mass mortalities that were associated with *Mycobacterium avium* Sileo *et al*.(1979) reported a frequency of 37% (19/57) of the lesions in the 1974 mortality and Kock *et al*.(1999).reported a frequency of over 40% (17/ 42) in the 1993 mortality.

Lymphocytic infiltration mostly into the periportal areas of the liver was present in 31/53 birds sampled during the survey affecting 13 that were healthy, 11 that were sick and 7 that were found dead. Infiltration was varied in intensity but there was no difference in degree of severity of the lesion between the three groups. Similar infiltration occurred less frequently into the renal pelvis (2/53) and the lung parenchyma (3/53). Lymphoid hypercellularity in Peyers patch, spleen and bursa of Fabricius was present in 10/53 birds of which 6 were healthy, two were dead and two were sick. Proliferation of reticuloendothelial tissues in the spleen was present in 6/53 birds sampled during the survey all of which were healthy. These features could suggest immunological responses of the lesser flamingos to constant challenges from infectious agents.

Sections of morphologically distinct protozoan parasites occurred in the serosal wall of the intestine of one (1/53) lesser flamingo sampled during the survey, and in skeletal muscle of one (1/134) that was studied retrospectively. Sections of tissue helminth parasites were present in the thymus of 2/53 birds sampled during the survey. Different types of tissue parasites have been documented previously in lesser flamingo including besnoitiosis, renal coccidiosis and sarcocystis (Kastard *et al.,* 1981). The significance of these parasites in disease is not documented but they could contribute to the overall stress that can cause a bird to succumb to other infections. To my knowledge, helminth parasites in thymus tissue have not been documented in lesser flamingos.

Sections of gastrointestinal worms occurred in tissues of 9/53 birds sampled during the survey as well as 7/134 studied retrospectively. Marked burrowing of the intestinal mucosa by the worms

and thickening of the wall was evident in tissues of one bird which was in poor body condition and had granulomatous lesions in the organs. This suggests that heavy infestation with gastrointestinal worms following chronic disease can cause significant lesions in lesser flamingos.

Mean scores of severity of congestion, haemorrhage, necrosis and cellular infiltration was significantly higher in tissues collected during the 2004 mass mortality compared to those collected during the 2009/2010 field study and the retrospective non-outbreak periods of 1997, 1998, 1999, 2000, and 2001 ($p<0.0001$). This indicates that the proportion of lesser flamingo with severe disease encountered during the years under study was highest during the 2004 mass mortality. Birds with severe disease tend to be sampled opportunistically during investigations when success of capture of healthy birds may be limited. Similar lesions were, however present in birds sampled during the other years. This suggests that diseases that cause mass mortality may be present in the population during non-outbreak years but at a low prevalence. Severity and frequency of mycobacterial granulomas did not vary significantly between the 2009/2010 study and the 2004 mass mortality period ($p=0.07$) or between the former and the non-outbreak periods ($p=0.340$). This suggests that the level of disease associated with *Mycobacterium spp* may not have changed drastically over recent years.

5.1.5 Bacteriology

Three pathogenic bacteria, *Mycobacterium spp., P. multocida and S. gallinarum*, were found in lesser flamingos during the 2009/2010 field study. *Mycobacterium spp* was also demonstrated in retrospective tissues samples of 1/22 of the birds sampled in 1997 through ZN staining. One human pathogen, *S. typhi*, was isolated from a water sample taken from the treated sewage inlet to Lake Nakuru.

Pasteurella multocida has been associated with mortalities of lesser flamingos in Kenya (Beasley *et al.*, 2004, Manyibe *et al.*, 2007). Beasley et al. (2004) demonstrated multifocal intrahistiocytic bacilli in liver of lesser flamingos sampled in 2000 and 2002 in Lake Bogoria and they isolated *P.multocida* type 3 in pure culture from 9 out of 10 liver samples. Manyibe *et al.* (2007) reported isolation of *P.multocida* from livers of 10/16 lesser flamingos during mass mortality of the species in 2004. Tissues from the birds sampled during the 2004 mortality were examined retrospectively during the current study and the histopathological lesions were consistent with bacterial septicaemia which matches with the the previous bacteriological findings and therefore suggests that *P.multocida* played an important role in the mass mortalities in 2004. During the 2009/2010 field study, which was within periods when there were no die-offs of lesser flamingos, *P.multocida* was isolated from the livers and spleens of 4/46 birds: three of these were sick and one was healthy. This suggests that *P.multocida* is present at low prevalence in the lesser flamingo population and will cause mass mortalities when certain trigger factors are present.

Mycobacterium avium has been associated with mass mortalities of lesser flamingos in Kenya (Cooper *et al.,* 1975; Sileo *et al.,* 1974; Kock *et al.,* 1999; Pessier *et al.,* 2004 and Oaks *et al.,* 2006). The prevalences of *M.avium* reported during the 1974 and 1993 mass mortality events were 19/51 (Sileo *et., al* 1979) and 17/42 (Kock *et al.*, 1999), respectively which are much higher than those of 1/22 and 2/57 found in the current study for 1997 and 2009, respectively. These observations suggest that *M.avium* is present within the lesser flamingo population in low prevalence and can cause epidemics when certain trigger factors are present.

Salmonella gallinarum, which was *isolated* from one sick lesser flamingo during the wet season in 2010, has not, to my knowledge, been reported as a cause of mortality in lesser flamingos. The bird, from which the pathogen was isolated was not visibly impaired but had signs of diarrhea, moderate congestion of the intestinal mucosa and enlargement of the bursa of Fabricius. Two other birds in the group of three had similar lesions, while the third bird had only an enlarged bursa of Fabricious. *Salmonella gallinarum* causes fowl typhoid in poultry and it has worldwide distribution (Pomeroy and Nagaraja, 1991). Natural outbreaks occur in chickens, turkeys, Guinea fowl, peafowl, ducklings and game birds such as quail, grouse, and pheasants. Wild birds are regarded as possible mechanical carriers of the disease (Pomeroy and Nagaraja, 1991). Presence of *S. gallinarum* in the lesser flamingos could suggest possible transmission of disease pathogens between poultry and the lesser flamingos. Future studies on the prevalences of infectious agents in the lesser flamingos will clarify the importance of *S. gallinarum* and other avian pathogens in the wild bird–domestic poultry interface in the wetland basins.

Salmonella typhi is a human pathogen that causes typhoid fever in man and is transmitted through any medium which can be contaminated with faecal matter (Merchant and Parker, 1983). Presence of the pathogen in the sewage can be expected since typhoid is a common and serious human disease that could be prevalent in the population living around Nakuru Muniscipality. Thus, a high load of this bacterium and other pathogenic bacteria in the water discharged into the lake poses a high risk of disease both to humans, lesser flamingos and other wildlife species.

Several opportunistic bacteria were also isolated from the livers and spleens of lesser flamingos during the study. The most frequent was *E.coli* isolated from 9/46 of the birds. However, it was equally frequent among the healthy, sick and dead birds. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were each isolated from 6/46 of the birds, mostly sick or dead ones and only in one case each from healthy birds. All the three bacteria were also isolated from the water taken at various points of Lake Nakuru. This shows that these opportunistic bacteria are present within the water at all times and only when birds are immuno-suppressed by other prevailing conditions do they become important. Opportunistic pathogens have been reported previously in association with mass mortalities (Kock *et al.,* 1999; Manyibe *et al.,* 2007). *Pseudomonas aeruginosa* has been reported more frequently and sometimes implicated in septicaemia in association with underlying debilitating disease (Kock *et al.,* 1999). Pseudomonas is described as an opportunist that causes localized disease or septicaemia when introduced into the tissues of susceptible birds, especially young ones as well as severely stressed or immunodeficient ones (Barness, 2003). Concurrent infections with viruses and other bacteria occur and may affect susceptibitity to Pseudomonas. Staphylococcus is described as a common opportunistic pathogen

that tends to cause localized infections in poultry but can cause fatal septicaemia that resembles that caused by *P.multocida* in severely immuno-suppressed birds.

Viral diseases such as infectious bursal disease are known as common triggers of staphylococcal infection. The isolation of opportunistic pathogens from the birds during this study points at the possible presence of other underlying predisposing factors such as severe stress, immuno-suppression and possibly viral diseases. The role of viruses in diseases of lesser flamingos was not addressed in this study and has not been adequately investigated previously. It is an important area for future investigations.

5.1.6 Parasitology

Two species of cestodes, *Cladogynia phoeniconiadis and Gynandrotaenia stammeri*, and one species of nematode, *Striatofilaria phoenicopteri* were found in the lesser flamingo during this study. The most prevalent was *C. phoeniconiadis*, which was present in 50/53 of the birds, while *G.stammeri* was found in one bird and *S.phoenicopteri* occurred in two birds. These helminth parasites were recovered by Jones and Khalil (1980) from lesser flamingos in Lake Nakuru. The two authors reported that both *C. phoeniconiadis* and *G. stammeri* were very common in the lesser flamingos examined in Lake Nakuru in 1974. Their observation is consistent with those made in the current study with regard to *C. phoeniconiadis.* However, the frequency of *G. stammeri* in the current study is very low.

In 37 birds in which worm counts were determined, there were no significant differences in mean counts of *C. phoeniconiadis* between different age categories or sex. This agrees with the findings of Jones and Khalil (1980). Inspite of the heavy presence of parasites in 37 lesser flamingos, only one bird was in poor body condition concurrent with a remarkably higher count of *C.phoeniconiadis* than the rest of the birds. These results of high parasitosis in the sampled birds suggest that helminthiasis is an important disease component of lesser flamingos.

5.2 Environmental factors

The physical and biotic characteristics of the Eastern African Rift Valley lakes that the lesser flamingos inhabit have been studied extensively (Vareschi 1978; Vareschi, 1982; Tuite 1979; Tuite, 2000; Githaiga, 1997). The high ambient temperatures, pH, conductivity and salinity of lake water observed during this study are normal features of these lakes (Githaiga, 1997).

The strong negative correlation between conductivity of lake water and lake depth ($r = -0.834$, $p = 0.01$) observed in the study reflects the concentration and dilution cycles of the lakes due to evaporation during dry seasons followed by recharge from river in-flows and water run-off during the wet seasons. These hydrological cycles have a profound effect on the water biota in the lakes (Githaiga, 1997).

The few aquatic species that are adapted to live in the highly alkaline water of the lakes attain very high levels of biomass that serves as food for primary feeders. The blue green algal species,

Arthrospira fusiformis is one such species and it is the main food for lesser flamingos. The high variability observed in the level of *Arthrosprira sp*.in this study has been documented previously: drastic changes in levels of the species have been observed to occur even between days (Tuite, 2000; Githaiga, 1997).

The increase in concentration of nitrogen compounds in the lake following the onset of rains suggests that agrochemical compounds are washed into the lakes from the catchment area. This is in agreement with previous observations (Thampy and Ndetei, 1995; Koyo and Owino, 2010). The sharp increase in concentration of *Arthrosprira sp*. observed in the lake in October 2010, two months after a similarly sharp increase in concentration of nitrate compounds, supports the proposition that these compounds have a potential to cause algal blooms and eutrophication (Thornton, 1986; Harper *et al*., 1993;). The lack of statistical correlation between rainfall and concentration of the nitrate compounds in the lake water ($r = 0.057$, $p = 0.754$) can be explained by the lag period between rainfall and water re-charge in the lakes. Similarly, the lack of statistical correlation between concentration of nitrate compounds and concentration of *Arthrosprira* sp. ($r = -0.017$, $p = 0.934$) can be explained by the time lag between the increase in concentration of the compounds and growth of the species.

Algal blooms are usually followed by death of the algae. Decomposing algae is postulated to cause proliferation of saprophytic bacteria and opportunistic pathogens such as *P.aerugenosa* that could infect immuno-suppressed individuals (Kock *et al.,* 1999). It has been observed that birds moving away from major lakes that are undergoing eutrophication tend to congregate in smaller

spaces in one or more of the other lakes (Ndetei and Muhandiki, 2005). Such congregation could lead to congestion, competition for limited food resources and increased levels of cross contamination. Such stresses could trigger latent or opportunistic infections.

Harmful algal blooms have been associated with deaths of water birds caused by cyanotoxins (Matsunaga *et al*., 1999; Carmichael, 1997). The role of cyanotoxicosis in the mass mortalities of lesser flamingos in the Rift Valley lakes has been studied (Ballot *et al*., 2004; Ballot *et al.*, 2005; Krienitz *et al*., 2003; Krienitz *et al*., 2005; Lugomela *et al*., 2006; Motelin *et al*., 2000). Demonstration of higher than normal levels of cyanotoxins in the tissues of dead or sick lesser flamingos during mortalities have led to the conclusion that these toxins may have an important role in the lesser flamingo deaths. The current study did not characterize algal species to determine their toxicity neither were the algal toxins assayed in the tissues of the birds. The definitive role of these toxins in causation of the mass mortalities, therefore, still remains a subject for further study.

The strong positive correlation between lesser flamingo population in Lake Nakuru and concentration of *Arthrospira* sp. ($r = 0.968$, $p = 0.032$) suggests that feed availability is a key determining factor of lesser flamingo distribution in the lakes. This is in agreement with previous findings that suggest that algal density could be a significant predictor of flamingo occurrence in Rift Valley lakes (Vareschi, 1978; Tuite, 2000).

Longer than usual droughts in Lake Nakuru, with rainfall of less than 700mm, are documented to have occurred cyclically with a time interval of 5-6 years (Melack, 1988). Shivoga et al (2007) suggest that their frequency may have increased due to human activities in the catchment area and in particular deforestation of the Mau forest (Shivoga *et al.*, 2007). Lower than usual recharge of the lakes due to over-extraction of water of the lake inlets up-stream for agricultural and other uses has been documented (Thampy and Ndetei, 1995; Koyo and Owino 2010). During the study the year 2009 was observed to be unusually dry with mean annual rainfall of about 363.4 mm and complete drying of rivers that flow into Lake Nakuru. It is therefore conceivable that the effects of human activities in the catchment areas are still persisting and contributing to droughts. These can influence physico-chemical properties of the lakes thus influencing population dynamics which could in turn trigger diseases that are associated with overcrowding or nutritional imbalances.

The negative correlation between the lesser flamingo population in the two lakes ($r = 0.503$, $p=0.497$) over the two years of field monitoring suggests movement of birds between the lakes. The population patterns observed during the two years suggest that there was an influx of birds from Lake Bogoria into Lake Nakuru and back between March and July 2009 and July 2009 and January 2010, respectively. Population change within Lake Nakuru was quite rapid in March 2009 during which there was an increase from 8000 to 30,000 birds within a period of two weeks. These observations are consistent with those of other investigators who observed that population of the species in a given lake can double or half in a period of just two weeks (Childress *et al*, 2004). This means therefore that censuses conducted within the lake during two

different days can be substantially different and that censuses in different lakes should be carried out within the same day to minimize double counts. These observations further suggest that movement of birds between lakes during a mass mortality may transfer the mortality event from the epicenter to other lakes as proposed by previous investigators (Kock *et al*., 1999). It may be postulated that such transfer of infection, in case of acute disease, may be caused by influx of susceptible birds that are incubating disease or contaminated birds that are rendered susceptible through the stress of flight.

The lesser flamingo populations in Lake Bogoria and Lake Nakuru over the period 2000-2010 were negatively correlated ($r = -0.370$, $p = 0.293$) suggesting movements of birds between the lakes over the period. However, simultaneous decrease in population of the species in both lakes occurred in some years. This concurrent decrease could suggest movement of the birds from both lakes and either dispersal to several lakes (within and outside Kenya) or congregation in a few major sites. Congregation in a major site can occur in numbers of over a million birds as was observed in Lake Bogoria in January 2010. The fact that the four years of mass mortality of the species in Lake Nakuru coincided with concentrations of birds above 500,000 suggests that population dynamics of the species is an important driver of disease occurrence. This could possibly be due to the stresse related to congestion.

The populations of other water bird species in the two lakes over the period 2000-2010 were highly variable. These observations are consistent with those of Owino *et al* (2002). Palearctic migrant species of birds originating from the Boreal and Temperate climatic regions of Euro-

Siberian region, Mediterranean basin, Sahara and Arabian basin deserts, Western and Central Asia, Eastern Asia and some rivers of Europe and Russia among others, migrate regularly into and out of Lakes Nakuru and Bogorias (Zimmerman *et al.,* 1999; Njoroge 2009 *Personal communication*.). Afro-tropical migrants on the other hand originate from Sub-Sahara Africa and Madagascar. Most Palearctic migrants are known to flock into the lakes during the August to November season and depart during the March to May season (Zimmerman *et al*., 1999; Njoroge 2009 *Personal communication*.). Afro-tropical migrants are unpredictable in their movements with some bird species moving in and out of the lakes in a single day while others spend some few days or months in the lake before departing (Njoroge 2009 *Personal communication.*). The movement patterns of both Palearctic and Afro-tropical migrants indicate the potential for transmission of avian pathogens between distant lands and the lakes. Lakes Bogoria and Nakuru also have species such as Egyptian geese and Marabou stork which move regularly between the lakes and grazing paddocks or farmland where they are capable of interacting with domestic species. Such species could act as 'bridges' for transmission of disease between the wetlands and domesticated birds, especially the backyard poultry (FAO, 2007).

5.3 Challenges and limitations

The key challenges faced during this study are briefly discussed below:

1. **Capture of birds and related logistics:**

Success of capture of lesser flamingo critically depends on the number of birds congregating in a site. The species being quite nomadic, its population at the study sites was unpredictable during this study. In addition, during the wet season, bird trapping sites in Lake Nakuru were rendered inaccessible by deep mud along the shore. Feasibility of capture was therefore variable over the study period.

Field visits were planned based on the feasibility of capture, as explained above, and the availability of capture personnel from the istitutions.that the researcher was collaborating with. Sometimes capture was feasible but personnel were unavailable and other times personnel were available but capture was not feasible.

As a result of the above constraints an inadequate number of visits were made to the study sites and fewer birds were captured per visit than anticipated. This had a negative impact on the required sample size, thereby precluding the determination of prevalences of factors.

2. **Laboratory related logistics:**

Due to limited proximity of the laboratories from the field sites, laboratory tests that require prompt analysis of fresh samples within hours of collection were done with varied success. In particular, only a limited number of blood samples were analysed for haematology and a few of these were analysed for differential blood counts only. Likewise, blood biochemistry could not be done in the field necessitating storage of blood plasma in liquid nitrogen for about a week prior to analysis. This contributed to un-reliability of biochemistry results. Similarly, testing of water

samples, taken during visits, for *Arthrospira spp* was delayed due to logistics and the results produced were un-reliable. Both the biochemistry and Arthrospira results were therefore excluded from the analysis

The challenges discussed above affected the sample sizes for the various factors that were studied. As a result, presence or absence of these factors was determined rather than the respective prevalences as initially intended.

These challenges, in my experience, are in-herent in working with free-ranging wildlife species. The number of samples obtained during the field monitoring phase was augemented with retrospective tissues samples from 6 previous years to obtain a total sample size of 134 birds for histopathological analysis. This number of lesser flamigos samples is, to my knowledge, the largest that has been analysed so far for histopathology in any single study in Kenya.

5.4 Conclusions

Disease conditions are present in the lesser flamingo population at low level in periods when there is no mass mortality This is supported by the range of pathological lesions observed in tissues that were collected during the study period and in previous years when there were no significant mortality of lesser flamingos.

Acute lesions consistent with bacterial septicaemia were predominant in tissues that were sampled during the mass mortality of lesser flamingo in 2004. Such lesions occurred to a lesser extent in 1997, 2001 and, during the field study, in 2009 and 2010. Bacteriological tests conducted during the mass mortality in 2004 confirmed the presence of *P. multocida* and this has been colloborated by the current histopathological findings to suggest that this was the primary cause of mass mortality in 2004. The present study also confirms that *P.multocida* occurs in lesser flamingo population with sub-clinical or no disease, causing a low prevalence of acute septicaemic disease occasionally. This low prevalence of sub-clinical *P. multocida* may explode to an overt disease when predisposing factors such as stress and immunosupression develop.

The present study shows that *Mycobacterium sp.* is present in the lesser flamingo population and is responsible for a low prevalence of chronic debilitating disease that could flare up, as in 1973 and 1993, when conditions are right (Sileo *et.al.,* 1979; Kock *et. al.,* 1999).

Isolation of *S.gallinarum,* a consistent poultry pathogen, from one lesser flamingo that had a mild enteric syndrome points to the possibility of transmission of disease between domestic poultry and lesser flamingos. This is the first time, to my knowledge, that this pathogen is being reported from the species. Disease dynamics at areas of interfase between poultry and lesser flamingos in the wetlands could therefore be important.

Gastrointestinal and tissue parasites are prevalent within the population of lesser flamingos. The study demonstrates that these parasites can cause severe pathology in the lesser flamingos. The

study suggests that a link between severity of gastrointestinal parasites in lesser flamingos and other chronic debilitating diseases occurs in such an interaction. Future studies would verify the interrelationships between parasitic infection and other diseases.

The lesser flamingo ecosystem is subject to great environmental variations. This is demonstrated by the variability observed in the physico-chemichal and biological qualities of lake water as well as the population dynamics of lesser flamingos and other bird species in the lakes. The study suggests that population dynamics of lesser flamingos may be influenced by availability of their main food, *Arthrospira spp*, which may in turn be influenced by physico-chemical properties of water. Altered hydrology of the lakes and frequent overcrowding of the birds in smaller feeding grounds, in changing environmental conditions, may alter the normal rhythm of these variations and constitute stress factors that could suppress the immunity of the lesser flamingos causing them to succumb to infection by the various pathogenic and opportunistic bacteria within their environment.

The study underscores that causes of mass mortality in lesser flamingos remain as sub-clinical infections in the host and flare up to acute severe disease with high mortalities when suitable conditions for the pathogen prevail. The causes of such mortalities have a multifactorial perspective.

5.5 Recommendations

1. Continuous health monitoring of lesser flamingo should form an integral part of conservation efforts for the species as it is important in determining the causes of mortality and designing appropriate control measures.

2. Contingency plans for the management of lesser flamingo mortality should include biosecurity measures that are necessary to minimize contamination with bacterial pathogens among birds especially for *P. multocida* and from birds to humans especially for *Mycobacterium spp*.

3. Ecosystem management in the watersheds of the lesser flamingo lakes should be an integral part of conservation of the species as it will ensure stability in patterns of variation of the physico-chemical and biological qualities of the lakes and therefore minimize environmental stressors that contribute to mass mortalities

4. Future research should look into the role of viruses in the lesser flamingo mortality as well as the disease dynamics of the domestic poultry-lesser flamingo interfase.

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APPENDICES

Appendix 1: Lesser flamingo health monitoring field form
(developed for the study in Lakes Nakuru and Bogoria 2009-2010)

Wetland name: _____ ***Date:*** _____ ***Section:*** _____

Recorder: _____ ***Start:*** _____ ***Stop:*** _____

1. Historical data

Historical data will be gathered during every visit to each of the lakes through key informant interviews. The key informants will include Research Officers, Game Wardens or other staff involved in monitoring the lakes. The following questions will be asked:

a. Has there been any recent changes in weather pattern in the area (e.g rainfall, temperature,wind)?

b. What has been the recent trend in water level in the lake?

c. What has been the recent trend in flow of rivers that feed into the lake?

d. Have there been recent algae changes in the water (e.g algae bloom, algae crush)?

e. What have been the recent population trends and movement patterns of lesser flamingo in the lake?

f. What have been the population trends of other water bird species within the lakes?

g. Have any cases of dead or sick birds been observed recently

2. Environmental information:

Weather Condition: Record: **a)** whether it is sunny or cloudy; windy or calm; rainy, drizzling or dry; chilly, cool, warm or hot, at the time of the monitoring. **b)** Indicate the most predominant weather pattern over the past 2-3 months (e.g hot and dry, rainy, chilly and dry).

a)

b)

Habitat condition: Indicate: **a)** the state of flow of water at the inlets to the lake and any unusual observation on the physical quality of the water, **b)** the water level in the lake (general description of how far the water has receded from the shores and, where possible, the most recent standard measurement of lake depth), **c)** indicate the visible state of algae in the lake (whether appearing normal or showing signs of a bloom or a crush), any other unusual observations in the habitat **d)** indicate other changes in the habitat e.g unusual increase in number of insects (possible disease vectors) etc

a)

b)

c)

d)

Estimated flamingo population: a) Indicate the estimated number of flamingos at the time of the visit (the NMK ornithologist will assist in making a rough estimate based on previous experience). b) Also record the most recent census (from NMK or KWS research station) c) indicate the age structure of the population (proportion of young vs adult birds)

a)

b)

c)

Abundance of other bird species Record: **a)** Any unusual increase in abundance of the common species of birds **b)** any uncommon species observed in the table below:

a) Unusual increase in abundance of common species			
Common name	**Scientific name**	**Estimated number**	**Usual range**

b) Uncommon species observed		
Common name	**Scientific name**	**Number**

If nothing unusual in abundance of other species of birds indicate below:

☐Nothing unusual

Note: Any extra species of birds under this category may listed at the back of the page

3. Detailed mortality (deaths) and morbidity (sicknesses) information

a) Deaths

Location (GPS) of the carcass (es)

_____

Species of bird

_____

If there is only one carcass in the specific location, please record the following details:

Age of the bird: ☐ Unknown ☐Adult ☐ Immature ☐Juvenile

Sex: ☐ Unknown ☐ Female ☐ Male

State of the carcass: ☐ Old ☐Recent ☐Fresh. ☐ Scavenged on

Body condition: ☐ Emaciated ☐ Fair ☐Good-Excellent

Visible abnormalities: (record any visible abnormalities such as swollen legs/joints, fractured wings etc**)**

_____
_____
_____

If there is more than one carcass in the specific location, please indicate the following

a) Number of carcasses by age group:

Adults_____ Immature_____ Juveniles_____
Unknown_____

b) Number of carcasses by state of decomposition:

a) Old_____ Recent_____ Fresh_____
Scavenged_____

c) The number of birds found
dead_____

b) Sicknesses

Location (GPS) of sick bird (s)
_____
Species affected;
_____
Signs of sickness observed (see the list below)
_____
_____
_____
_____
_____

If the signs recorded above are observed in only one bird, please record the following details:

Age of the bird: ☐ Unknown ☐Adult ☐ Immature
☐Juvenile

Sex: ☐ Unknown ☐Adult ☐ Immature
☐Juvenile

If the signs are observed in more that one bird in the same area, please indicate:

a) The number of sick birds_____

b) The number of apparently normal birds_____

Notes for reference

Signs of sickness (Rose K. et al., 2006)

The following are some of the obvious signs of sickness in birds:

1. Sudden death-you see several dead birds but no sick or weak ones
2. Locomotion/ movement abnormalities e.g. unable to stand or flap wings properly when approached droopy wings (sagging wings).
3. Behaviour abnormalities e.g. falling over, unusual neck twisting, circling (moving round in circles), paralysis
4. Abnormal feathers e.g. rough feathers.
5. Swelling of body parts e.g. leg joints, tissues of the head
6. Unusual discharges e.g. bloody discharge from the nose, mouth, or vent
7. Unexplained emaciation or poor body condition.
8. Diarrhoea
9. Open wounds

State of carcasses

State		**stage of post-mortem change (see the description below)**
Fresh	=	1
Recent	=	2, 3&4
Old	=	5&6
Scavenged on	=	Carcass so mutilated by scavengers that it is not easy to asses its state

Stages of post-mortem change (Adopted from Cooper and Deacon, 2008 (*in review)*)

1. The carcass is intact, including eyes. The eyes appear clear and normal. The body is fleshy (not contracted or desiccated). The colour of both the skin and feathers is normal. The skin is still elastic and the feathers are still soft and glossy. The carcass may be flaccid (the neck and the legs-at the joints- are not stiff) or may be showing early signs of stiffening-known as *rigor mortis*. Lice (external parasites) may still be present. The carcass has no smell of decomposition.
2. The carcass is still fleshy but beginning to decompose. The eyes are dull/ cloudy and convex. The skin is showing early signs of change of colour (discolouration) and it is beginning to lose its elasticity (the normal stretching when pinched/ pulled). The feathers are still soft. Lice are sometimes present. The carcass has a slight smell of decomposition (depends on hydration of (amount of water in) the carcase)and may have spongy or gassy feeling when pressed
3. The beak is friable (breaks off easily). The body shape is contracting and the skin is drying. The feathers are losing softness. The carcass has a moderate smell of decomposition (depending on hydration of the carcase).
4. Maggots are visible. The skin from the legs is peeling. The eyes not readily visible. The abdomen is becoming obviously sunken (concave). The carcass has a marked smell (depends on hydration of the carcase).
5. Internal organs are missing. Maggots are mature, and are on the ground as well as on the body. Other invertebrates, such as beetles, often present. Some parts of the skeleton are visible. Feathers are missing. Eyes are missing and the orbit is apparent.
6. Bones and dry skin only seen, often scattered.

Scoring of body condition (Adopted from Gregory and Robins, 1998)

Body condition		**Score (see description below)**	
Emaciated	=	0	(see description below)
Fair	=	1	(see description below)

Good-Excellent = 2-3 (see description below)

0) Prominent ridge on the breast (due to prominence of the keel/ breast-bone) with very little breast muscle forming a concavity alongside the breast-bone
1) Greater development of breast muscle which is not concave and feels more or less flat. The keel is still prominent
2) Moderately developed convex breast muscle. The keel is less prominent
3) Well developed relatively plumb breast. Smooth over the keel

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Appendix 2: Flamingo necropsy protocol

1. **On site at time of capture**: Collect blood sample from LIVE birds into 4 ml heparinized for clinical pathology. Make a blood smear using the last drop in the syringe. If handling the birds, wear protective clothing and wash up afterwards.
2. Euthanize with 0.2 ml of 20% pentobarbiturate via the occipital sinus on if necropsy is being conducted on-site or in the lab if necropsy is conducted in the lag .
3. Do oropharyngeal and cloacal swabs both for bacteriology and virology using using asceptic techniques
4. Take pictures of the lesser flamingo
5. Don gloves and protective clothing when conducting necropsy .
6. Wet down bird/feathers with disinfectant, part feathers along ventral midline
7. Open coelomic cavity with sterile blade and sterile forceps–careful not to contaminate internal organs prior to bacteriology (spleen and liver) sampling!
8. Using aseptic technique, incise the liver and spleen swab the parenchyma. Place the swabs in bacteriological transport media and keep in cool box.
9. Incise skin over keel, reflect. Incise lateral extent of pectorals or reflect pectorals from midline laterally.
10. Using heavier scissors, cut both sides of thorax where keel meets ribs and remove keel, breaking corocoid as reflect toward head.
11. Observe organs grossly *in situ* and record any abnormalities
12. Systematically examine all systems, note any abnormalities, collect all listed samples for histopathology (Check off list as collected and placed in formalin)
13. Cut 3cm long portions of the duodenum, ileum and caecum, flush the contents with a little water into a plastic container with 70% alcohol and place the pieces in 10% buffered formalin. Put the remaining portions of the gut in the 70% alcohol container for assay of gut parasites (after taking representative pieces for histopathology). Label with bird ID#, date and location.
14. Remove brain–slice along mid-sagittal plane. Put half in 10% buffered formalin for histopathology and freeze the rest for toxicology.
15. Collect other tissues for histopathology , toxicology and bacteriology according to the checklists
16. Make sure your name and all information are recorded on gross pathology report form.

Courtesy of Envirovet summer institute training 18th June to 20th July 2000, Florida USA

Appendix 3: Flamingo tissue collection checklist

CLINICAL PATHOLOGY:

- Whole blood for haematology
- Plasma for biochemistry-Freeze
- Serum-Freeze

(Require heparinized tubes, plain tubes, syringes, needles, sterile gauze swabs, field centrifuge, and liquid nitrogen)

MICROBIOLOGY: use aseptic technique, place in separate labeled containers.

Bacteriology: keep the samples in transport media transport to lab in cool box

- Internal organ swabs - Liver, spleen
- Oropharyngeal and cloacal swabs
- Swabs of any lesion found-if the lesion is granulomatous record in the submission form so that precautions for avian tuberculosis can be taken. At the same time, take a piece of tissue about 5g and store it frozen for bacteriology-submit to VIL Kabete for tuberculosis testing.

Virology

- Oropharyngeal and cloacal swabs in virological media-freeze submit to VIL Kabete for screening

TOXICOLOGY: Place in plastic containers to be frozen

- Liver–20 g
- Kidney–two halves
- Brain- Half
- Blood in plain tube-4ml

PARASITOLOGY

- Collect ectoparasites and place in 70% alcohol.
- Collect most of the intestine with contents in 70% alcohol.

FIXED TISSUE COLLECTION FOR HISTOPATHOLOGY

Sections of tissues should be no more than 1 cm in width and should be placed in 10% buffered formalin at a ratio of one part tissue to ten parts formalin.

Sample normal appearing as well as any lesions or abnormalities within these or other organs:

- Thymus

- Thyroids and parathyroids
- Trachea including syrinx
- Lungs–one entire
- Heart–collect entire heart, open ventricles prior for proper fixation
- Tongue–cross section.
- Esophagus–3 cm in length, opened longitudinally
- Crop–3 cm in length, opened longitudinally
- Proventriculus–3 cm long sections
- Ventriculus–3 cm long sections
- Small intestine–multiple sections, each 3 cm in length. Inject formalin into lumen or flush through with formalin.
- Ceca and large intestine–multiple sections, each 3 cm in length
- Liver
- Pancreas
- Spleen
- Adrenals
- Testis or Ovary
- Oviduct
- Kidney
- Brain
- Skeletal muscle–longitudinal section of thigh and pectoral muscle
- Bone/bone marrow–cut off small piece of proximal tibiatarsus and fix it whole, don't attempt to remove marrow

Appendix 4. Flamingo necropsy data collection form

PRINT LEGIBLY

Bird Identification No:		Ring No if any:	
Date of capture/collection:		Date of necropsy:	
Time of capture/collection:		Time at start of necropsy:	
Time of death:		Time at end of necropsy:	

Gross Necropsy Performed By: _____

Assistants_____

Digital Photography/Videography Recorder_____

Age, sex and morphological characteristics:

Age (Juvenile /Immature/Adult):	Sex :
Mass (g):	Flattened wing length (mm):
Culmen length (mm):	Skull (mm):
Total tarsus length (mm):	Head-and-bill length (mm):
Important morphological characteristics	
Bill: (□Dark grey; □Light red ; □Dark red, black tipped)	Plumage: (□Greyish; □Whitish with no red in wings; □Deep pink, deeper reddish pink on breasts)
Iris: (□Brown; □Brown/Orange; □Yellow to orange)	Back:(□Grey/White; □White/Pink; □Pink)
Head/Neck:□Grey; □White/Pink ; □ Pink	Legs/Skin:(□Grey legs and feet; □Whitish with no red in wings; □Bright red legs and feet)
Weight of spleen: Weight of bursa of fabricius:	Weight of liver:

History: _____

_____

_____

Clinical Signs /Antemortem observations:

_____

_____

_____

_____

Circumstances of Death (euthanasia method, found dead, etc.)

□ Euthanasia

Method _____
□ Found dead

GROSS PATHOLOGY

1. Describe any lesions or abnormalities seen grossly
   Remember: **DDD TAP** =
   Degree-slightly, minimal, moderately, mild, marked, severe
   Distribution-focal, multifocal, diffuse, locally extensive, cranial, circumferential, ¼, ½, 90% etc.
   Duration -- acute, subacute, chronic
   Tissue
   Adjective -- describe surface, consistency, shape, color, size, smell
   Process -- inflammation, fibrosis, granuloma, abcess…..
2. Obtain samples for bacteriology before contaminating carcass with intestinal contents, etc.
3. Cut samples of tissue for histopathology that are no more than 1 cm in thickness, take extra samples of any lesions

General Condition: (Nutritional status,description of keel muscle and body score**)**

_____
_____

Integumentary System: (Skin/feathers, note lice or mites and collect)

_____
_____

Musculoskeletal System: (Skeletal muscle, bone, joints)

_____
_____
_____

Coelomic Cavity: (Fat stores, abnormal fluid, exudates, fibrin, etc.)

_____
_____
_____
_____

Hemolymphatic System: (Spleen, thymus, bursa of Fabricius)

_____
_____
_____

Respiratory System: (Nares, sinuses, choanae, syrinx, trachea, lungs, air sacs)

_____
_____
_____

Cardiovascular System: (Heart, pericardium, great vessels)

_____
_____
_____

Digestive System: (Beak, oral cavity, tongue, esophagus, proventriculus, ventriculus, intestines, cloaca)

_____

_____
_____
_____
_____

Glandular Organs: (Liver, pancreas)

_____
_____
_____
_____

Urinary System: (Kidneys, ureters)

_____
_____
_____

Reproductive System: (Testis/ovary, oviduct)

_____
_____
_____

Endocrine System: (Adrenals, thyroid, parathyroids, pituitary)

_____
_____
_____

Nervous and Sensory Systems: (Brain, spinal cord, peripheral nerves, eyes, ears)

_____
_____
_____

Gross Diagnoses: (List each lesion separately. Include organ, type of lesion, severity, etc.)

_____
_____
_____
_____
_____
_____

Most Significant Finding(s)/Lesion(s): (Based on gross examination)

_____
_____
_____
_____
_____
_____
_____

Digital Photography/Videography Performed? (Carotenoids/list organs/lesions photographed etc./List Picture ID of digital pictures)

_____
_____
_____
_____

Miscellaneous comments/remarks

_____
_____
_____
_____
_____
_____

Verified completed: _____(signature of recorder)

Appendix 5: Haematological values for individual birds

Haematological parameters of individual lesser flamingos sampled in Lake Nakuru national park during the dry season

	LNF F2	LNF F4	LNF F5	LNF F6	LNF F7	LNF F8	LNF 10	LNF 11	LNF 13	LNF 14	LNF 16	LNF 18	LNF 19	LNF 20
PCV	nd	42	49	36	55	49	36	45	46	29	34	26	45	55
Total soilds (g/dl)	nd	2.6	4.8	4.8	3.2	5.2	nd	4.9	2	3.6	4.4	4.8	5	5.5
Plasma proteins (g/dl)	nd	81	14	42	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
TotalWBCx10^3 (cells/µl)	nd	36	26	21	nd	23	6	5	1	8	8	6	13	23
Differential heterophil count (%)	21.0	7	14	71	16	14	21	17	24	38	93	40	38	14
Heterophils x10^3 (cells/µl)	nd	2	3	15	nd	3	1	0	0	3	8	2	5	3
Diff. lymphocyte count (%)	71.0	86	67	24	69	79	75	72	52	57	6	56	38	76
Abs. lymphocyte count x10^3 (cells/µl)	nd	31	17	5	nd	18	5	3	0	4	0	3	5	1
Diff. granulated lymphocytes	1.0	6	6	0	nd	nd	nd	nd	nd	nd	0	nd	nd	nd
Abs. granulated lymphocyte count x10^3 (cells/µl)	nd	2	1	0	nd	nd	nd	nd	nd	nd	0	nd	nd	nd
Diff. monocyte count (%)	5.0	0	8	5	5	6	0	6	10	1	0	1	8	8
Abs. monocyte count x10^3 (cells/µl)	nd	0	2	1	nd	1	0	0	0	0	0	0	1	1
Diff. eosinophil count (%)	1.0	1	4	0	10	1	4	5	14	4	1	3	14	2
Abs. eosinophil count x10^3 (cells/µl)	nd	0	1	0	nd	0	0	0	0	0	0	0	1	0
Diff. basophil count (%)	1.0	0	1	0	0	0	0	0	0	0	0	0	2	0
Abs. basophil count x10^3 (cells/µl)	nd	0	0	0	nd	0	0	0	0	0	0	0	0	0

Appendix 6: Organ weight indices of individual birds

No.	Bird Id Number	Location	Season	Age	Sex	Health	body score	Liver mass index	spleen mass index	Bursa mass index
1.	LNF1	Nakuru	Dry	Mature	Male	Sick	good	5.1	0.2	
2.	LNF2	Nakuru	Dry	Mature	Female	Apparently healthy	good	2.5	0.1	0.2
3.	LNF3	Nakuru	Dry	Mature	Male	Dead	fair	7.5	0.2	
4.	LNF4	Nakuru	Dry	Mature	Male	Apparently healthy	good	3.9	0.1	0.6
5.	LNF5	Nakuru	Dry	Mature	Male	Apparently healthy	good	2.8	0.1	0.0
6.	LNF6	Nakuru	Dry	Mature	Male	Sick	poor	5.7	0.2	
7.	LNF07	Nakuru	Dry	Mature	Female	Sick	ND	5.2	0.2	
8.	LNF08	Nakuru	Dry	Mature	Male	Apparently healthy	good	2.8	0.1	0.8
9.	LNF09	Nakuru	Dry	Juvenile	Male	Dead	poor	8.2	0.3	
10.	LNF10	Nakuru	Dry	Mature	Male	Apparently healthy	good	2.9	0.1	
11.	LNF11	Nakuru	Dry	Mature	Male	Sick	good	0.0	0.0	
12.	LNF12	Nakuru	Dry	Immature	Female	Dead	good	3.4	0.1	
13.	LNF13	Nakuru	Dry	Juvenile	Female	Sick	good	5.0	0.2	
14.	LNF14	Nakuru	Dry	Immature	Male	Sick	poor	0.0	0.0	
15.	LNF15	Nakuru	Dry	Juvenile	Female	Dead	fair	6.0	0.1	
16.	LNF16	Nakuru	Dry	Juvenile	Female	Sick	poor	7.1	0.1	
17.	LNF17	Nakuru	Dry	Mature	Male	Dead	poor	3.3	0.2	
18.	LNF18	Nakuru	Dry	Immature	Male	Sick	poor	4.0	0.2	
19.	LNF19	Nakuru	Dry	Juvenile	Female	Sick	poor	10.1	0.4	
20.	LNF20	Nakuru	Dry	Immature	Male	Sick	good	4.2	0.2	

21.	LBF1.20	Bogoria	Wet	Juvenile	Male	Dead	good	2.9	0.2	0.1
22.	LBF2	Bogoria	Wet	Immature	Male	Dead	good	4.0	0.2	0.2
23.	LBF3.20	Bogoria	Wet	Mature	Female	Sick	fair	4.5	0.4	0.1
24.	LBF4	Bogoria	Wet	Immature	Female	Dead	good	4.6	0.2	0.2
25.	LBF6	Bogoria	Wet	Juvenile	Male	Dead	good	5.5	0.2	0.2
26.	LBF7	Bogoria	Wet	Mature	Female	Apparently healthy	good	4.5	0.1	0.1
27.	LBF8	Bogoria	Wet	Mature	Male	Apparently healthy	good	2.5	0.1	0.2
28.	LBF9	Bogoria	Wet	Immature	Female	Apparently healthy	good	3.3	0.1	0.1
29.	LBF10	Bogoria	Wet	Immature	Male	Apparently healthy	fair	3.3	0.2	0.4
30.	LBF11	Bogoria	Wet	Immature	Male	Apparently healthy	good	3.0	0.1	0.8
31.	LBF12	Bogoria	Wet	Mature	Male	Apparently healthy	good	3.4	0.1	0.3
32.	LBF13	Bogoria	Wet	Mature	Male	Apparently healthy	good	2.3	0.1	0.1
33.	LBF14	Bogoria	Wet	Immature	Male	Apparently healthy	good	1.9	0.1	0.2
34.	LBF19	Bogoria	Wet	Mature	Female	Apparently healthy	good	2.9	0.1	0.1
35.	LBF20	Bogoria	Wet	Juvenile	Male	Apparently healthy	fair	3.0	0.1	0.3
36.	LBF21	Bogoria	Wet	Mature	Female	Apparently healthy	good	3.3	0.1	0.1
37.	LBF22	Bogoria	Wet	Immature	Male	Apparently healthy	fair	2.5	0.1	0.4
38.	LBF23	Bogoria	Wet	Mature	Male	Apparently healthy	good	2.7	0.2	0.2
39.	LBF24	Bogoria	Wet	Mature	Male	Apparently healthy	good	2.3	0.1	0.2
40.	LBF25	Bogoria	Wet	Mature	Male	Apparently	good	3.9	0.1	0.2

						healthy				
41.	LBF26	Bogoria	Wet	Mature	Female	Sick	good	3.1	0.1	0.1
42.	LBF27	Bogoria	Wet	Immature	Female	Dead	fair	4.0	0.1	0.2
43.	LBF28	Bogoria	Wet	Mature	Male	Sick	good	3.7	0.1	
44.	LBF29	Bogoria	Wet	Immature	Male	Dead	fair	5.1	0.2	0.3
45.	LBF30	Bogoria	Wet	Mature	Female	Dead	good	4.7	0.1	0.2
46.	LBF31	Bogoria	Wet	Mature	Male	Dead	fair	4.2	0.1	
47.	LBF32	Bogoria	Wet	Mature	Male	Sick	good	5.5	0.1	0.1
48.	LBF33	Bogoria	Wet	Immature	Male	Sick	poor	5.0	0.2	
49.	LBF34	Bogoria	Wet	Mature	Female	Sick	fair	2.7	0.0	0.0

Appendix 7: Endoparasite counts in individual birds

Bird Id Number	Season	Age	Sex	Status	body score	Cladogynia phoeniconaiadis	Gynandrotaenia stammeri	Stratofilaria phoenicopteri
LBF1	Wet	Juvenile	Male	Dead	good	5	0	0
LBF2	Wet	Immature	Male	Dead	good	23	0	0
LBF3	Wet	Mature	Female	Sick	fair	16	0	0
LBF4	Wet	Immature	Female	Dead	good	8	0	ND
LBF5	Wet	Juvenile	Male	Healthy	fair	17	0	1
LBF6	Wet	Juvenile	Male	Dead	good	64	0	1
LBF7	Wet	Mature	Female	Healthy	good	18	0	0
LBF8	Wet	Mature	Male	Healthy	good	72	0	0
LBF9	Wet	Immature	Female	Healthy	good	10	0	0
LBF10	Wet	Immature	Male	Healthy	fair	11	0	0
LBF11	Wet	Immature	Male	Healthy	good	11	0	0
LBF12	Wet	Mature	Male	Healthy	good	15	0	0
LBF13	Wet	Mature	Male	Healthy	good	20	0	0
LBF14	Wet	Immature	Male	Healthy	good	20	0	0
LBF15	Wet	Mature	Male	Dead	good	21	0	0
LBF16	Wet	Mature	Female	Dead	good	49	0	0
LBF17	Wet	Mature	Female	Dead	good	121	0	0
LBF18	Wet	Juvenile	Male	Dead	good	5	0	0
LBF19	Wet	Mature	Female	Healthy	good	51	0	0
LBF20	Wet	Juvenile	Male	Healthy	fair	96	0	0
LBF21	Wet	Mature	Female	Healthy	good	10	0	0
LBF22	Wet	Immature	Male	Healthy	fair	21	0	0

LBF23	Wet	Mature	Male	Healthy	good	52	0	0
LBF24	Wet	Mature	Male	Healthy	good	0	0	0
LBF25	Wet	Mature	Male	Healthy	good	16	0	0
LBF26	Wet	Mature	Female	Sick	good	16	0	0
LBF27	Wet	Immature	Female	Dead	fair	5	0	0
LBF28	Wet	Mature	Male	Sick	good	0	0	0
LBF29	Wet	Immature	Male	Dead	fair	7	0	0
LBF30	Wet	Mature	Female	Dead	good	23	0	0
LBF31	Wet	Mature	Male	Dead	fair	2	0	0
LBF32	Wet	Mature	Male	Sick	good	1	0	0
LBF33	Wet	Immature	Male	Sick	poor	345	0	0
LBF34	Wet	Mature	Female	Sick	fair	1	0	0
LNF21	Wet	Mature	Female	Sick	good	15	0	0
LNF22	Wet	Mature	Female	Sick	fair	274	10	0
LNF23	Wet	Mature	Female	Healthy	good	202	0	0

Appendix 8: Monthly rainfall for Lake Nakuru from 2007 to 2010

(KWS data)

Sum of Rainfall		Station							Grand Total
Year	Month	Lanet	M/Gate	Naishi	Nderit	Nganyoi	Pwani	Zakaria	
Jan 2007	Jan	76	56	53.5	37.2	32	70	77	401.7
	Feb	69	44	28.5	22	54	48	43	308.5
	Mar	50	24	73.8	31.9	78	35	37	329.7
	Apr	123	114	63.5	76.5	79.2	93	107	656.2
	May	88	78	142	48.7	149	59	89.5	654.2
	Jun	74	126	137	66.1	182	104	186.5	875.6
	Jul	83	99	50	72.3	145.2	117	126	692.5
	Aug	117	71	0	82.4	174.3	167	179	790.7
	Sep	98	66	17	96.1	174	153	175	779.1
	Oct	130.7	69	17.9	38.4	57	46	46	405
	Nov	46	35	41.1	25	51	35	54	287.1
	Dec	32	2	11.9	9	29	15	18	116.9
Jan 2007 Total		986.7	784	636.2	605.6	1204.7	942	1138	6297.2
2008	January	30	22.5	9	3.2	23	14	14	115.7
	Feb	5	7	3.2	7.2	3	6	15	46.4
	Mar	61	49	54.65	44.8	7	94	85	395.45
	Apr	118	46	15.9	17.9	60	67	70	394.8
	May	24	24	2.4	46.8	70.2	42.5	27	236.9
	Jun	98	30	45.9	22	16.7	69	97	378.6
	Jul	147	175	188.4	159.2	153	224	348	1394.6
	Aug	98	62	167.4	68.7	76.9	108	72	653
	Sep	75	29	259.5	16.5	36.4	45	66	527.4
	Oct	103.5	79	55	63.8	53.7	155	110.9	620.9
	Nov	92.1	69	53.7	56.4	45.6	99	69	484.8
	Dec	9	4	3.2	2	5	8	17	48.2
	Apr	2	0	0	0	0	4	0	6
2008 Total		862.6	596.5	858.25	508.5	550.5	935.5	990.9	5302.75

Sum of Rainfall	month	Lanet	M/Gate	Naishi	Nderit	Nganyoi	Pwani	Zakaria	Grand Total
2009	Jan	4	6	1.3	5	2.2	8	3	29.5
	Feb	0	0	0	0	2	3	4	9
	Mar	0	2	0	0	0	5	0	7
	Apr	45	33	44.5	40.2	30.6	65.5	44	302.8
	May	106	73.7	71	47	74.3	93.5	125	590.5
	Jun	39	11.3	12.3	15	11.3	7	26.5	122.4
	Jul	11.5	15	3	2.3	9.7	38.5	23	103
	Aug	29.2	4.5	11.9	18.4	22.9	10	43	139.9
	Sep	21	4	12	0	5	3.5	13	58.5
	Oct	124	28.5	19.8	133	34.4	65	50.5	455.2
	Nov	43	28	1.5	25	14.9	45	14	171.4
	Dec	94	41.9	54.4	76.2	88.3	125.5	74	554.3
2009 Total		516.7	247.9	231.7	362.1	295.6	469.5	420	2543.5
2010	Jan	79	42.1	15.8	29.2	54.1	56.5	54	330.7
	Feb	142	82.9	77.8	134	122.6	144.7	114.6	818.6
	Mar	237	127	88.9	196	104.7	223	211.2	1187.8
	Apr	151	45	58.5	122.6	82.4	84.7	88.2	632.4
	May	131	83.1	87.5	113.7	67.4	130	69.4	682.1
	Jun	24	17.5	100.7	92.5	80.2	121	69	504.9
	Jul	65	55.9	71.5	38.5	40.5	66	84.5	421.9
	Aug	117	71.6	74	105	102.8	123	133.5	726.9
	Sep	90	66	29.3	115	31.2	107	98.8	537.3
	Oct	138	56.8	47.7	107.5	77	121	114.5	662.5
	Nov	20.9	9.8	25.7	34.1	28	23.5	29	171
	Dec	5	1.7	0	0	1.3	7	3	18
2010 Total		1199.9	659.4	677.4	1088.1	792.2	1207.4	1069.7	6694.1

Appendix 9: Monthly water quality parameters for Lake Nakuru from 2008 to 2010
(KWS data)

Year	Spirulina counts units/ml	NH_3-N ($x10^3$)	NO_3-N ($x10^3$)	Conductivity.	Total depth (Cm)
Jan-08	640.00	50.00	50	14.50	170.00
Feb-08	640.00	50.00	50	13.60	170
Mar-08		760.00	244	14.25	159
Apr-08		1080.00	231	15.03	163
May-08		196.00	1032	25.00	154
Jun-08		610.00	430	14.80	144
Jul-08	0.00	360.00	50	17.00	150
Aug-08	0.00	200.00	50	17.50	150
Nov-08		360.00	60	28.50	160
Dec-08		500.00	431.2	27.20	167
Jan-09	0.00	270.00	60	34.00	125
Mar-09	130.00	640.00	167	40.40	110
Apr-09	920.00	890.00	480	47.70	100
May-09	1190.00	550.00	50	49.50	97
Jun-09	0	460.00	130	52.00	90
Jul-09	210.00	640.00	20	55.40	75
Aug-09	240.00	810.00	80	67.50	70
Sep-09	70.00	150.00	250	75.70	75
Oct-09		1660.00	60	87.60	40
Nov-09	0.00	1930.00	70	94.80	42
Dec-09	0.00	2780.00	190	98.30	24
Jan-10	1.00	610.00	50	54.40	65
Feb-10	0.00	1130.00	50	85.40	30
Mar-10	1.00	1190.00	0	57.60	40
Apr-10	0.00	1600.00	50	32.00	122
May-10	360.00	325.00	9	24.60	127
Jun-10	130.00	160.00	5	21.00	190
Jul-10	110.00	980.00	2260	21.40	190
Aug-10	160.00	850.00	2490	20.00	200
Sep-10	620.00	220.00	140	16.58	260
Oct-10	4100.00	640.00	340	15.65	270
Nov-10	590.00	390.00	20	15.98	280
Dec-10	1200.00	600.00	130	16.64	270

Appendix 10: Population of waterbirds in Lakes Bogoria and Nakuru, 2000 to 2010

(NMK data)

Site	BIRD SPECIES	Jan-00	Jul-00	Jan-01	Jul-01	Jan-02	Jul-02	Jan-03	Jul-03
Nakuru	Lesser flamingo	13,407	692,325	614,512	272,046	761,679	739,177	1,046,988	277,236
Nakuru	Greater flamingo	132	798	2,540	644	6,043	4,050	1,179	4,933
Nakuru	Dead flamingos	0	0	0	0	0	0	0	19
Nakuru	Great white Pelicans	3,565	1,751	35,160	4,576	15,996	13,930	94,311	1,372
Nakuru	Pink-backed Pelicans	356	86	116	23	477	5,167	402	75
Nakuru	Marabou Stork	36	72	227	54	58	133	295	74
Nakuru	Egyptian Goose	167	134	202	208	304	216	265	124
Bogoria	Lesser flamingo	678,140	#	491,972	#	195,879	130,248	83,616	#
Bogoria	Greater flamingo	10,875	#	18,540	#	240	2,067	175	#
Bogoria	Dead flamingos	0	#	0	#	0	0	0	#
Bogoria	Great white Pelicans	0	#	39	#	2	1	5	#
Bogoria	Pink-backed Pelicans	0	#	8	#	0	1	0	#
Bogoria	Egyptian Goose	0	#	15	#	9	18	23	#
Bogoria	Marabou Stork	62	#	4	#	69	31	62	#

Appendix 10 continuation

Site	BIRD SPECIES	Jan-04	Jul-04	Jan-05	Jul-05	Jan-06	Jul-06	Jan-07	Jul-07
Nakuru	Lesser flamingo	226,265	105,933	208,217	619,296	1,346,984	536,211	88,666	422,341
Nakuru	Greater flamingo	2,160	28	38,754	5,130	6,346	105	1,009	2,217
Nakuru	Dead flamingos	5	0	0	7	0	1245	124	1
Nakuru	Great white Pelicans	35,204	51,500	51,194	5,358	12,676	1132	19	821
Nakuru	Pink-backed Pelicans	10,068	4,619	416	500	14	290	4	535
Nakuru	Marabou Stork	98	195	831	233	297	388	172	14
Nakuru	Egyptian Goose	70	369	92	86	449	54	41	89
Bogoria	Lesser flamingo	33,875	54,835	29,085	#	14,645	#	28736	38,218
Bogoria	Greater flamingo	3,875	590	1,815	#	995	#	1396	3,563
Bogoria	Dead flamingos	1	0	0	#	0	#	4	2
Bogoria	Great white Pelicans	2	27	0	#	0	#	0	2
Bogoria	Pink-backed Pelicans	0	0	4	#	0	#	0	0
Bogoria	Egyptian Goose	8	37	8	#	8	#	36	62
Bogoria	Marabou Stork	4	30	17	#	14	#	2	7

Appendix 10 continuation

Site	BIRD SPECIES	Jan-09	Jul-09	Jan-10	Jul-10
Nakuru	Lesser flamingo	15340	255294	41592	96489
Nakuru	Greater flamingo	2556	1694	29	10906
Nakuru	Dead flamingos	3	32	22	0
Nakuru	Great white Pelicans	73387	23237	11	318
Nakuru	Pink-backed Pelicans	3372	2315	0	8
Nakuru	Marabou Stork	784	216	281	179
Nakuru	Egyptian Goose	570	166	298	273
Bogoria	Lesser flamingo	128515	12929	1030511	516979
Bogoria	Greater flamingo	1402	823	1394	6796
Bogoria	Dead flamingos	222	3	3	0
Bogoria	Great white Pelicans	0	174	0	13
Bogoria	Pink-backed Pelicans	0	0	0	0
Bogoria	Egyptian Goose	3	10	6	4
Bogoria	Marabou Stork	34	12	58	13

BIRD SPECIES	Jan-00	Jul-00	Jan-01	Jul-01	Jan-02	Jul-02	Jan-03	Jul-03	Jan-04	Jul-04	Jan-05	Jul-05
Total Palleactic migrant counts	6105	769	6967	899	5851	788	1861	28	3980	477	3920	724
Total afrotropical mirant counts	2000	1048	1810	1570	1830	2734	1668	918	5764	3742	1256	424
BIRD SPECIES	Jan-06	Jul-06	Jan-07	Jul-07	Jan-08	Jul-08	Jan-09	Jul-09	Jan-10	Jul-10		
Total Palleactic migrant counts	3063	191	3472	514	0	0	5346	863	5196	282		
Total afrotropical mirant counts	1112	348	200	62	0	0	11620	1478	3766	2180		