Microcystin in Ugandan lakes: Production dynamics, accumulation in fish, and risk evaluation

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.
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Abstract

Eutrophication of freshwater lakes has led to an increase in the occurrence of harmful cyanobacterial blooms, and it is expected that a warming climate will further exacerbate the frequency and duration of such blooms. Microcystin is a cyanobacterial hepatotoxin that is found worldwide, and poses a serious threat to the ecological communities in which it is found as well as to those who use these waters for drinking, recreation, or as a food source. Although microcystin is known to accumulate in fish and other aquatic biota, the prevalence of microcystin in fish tissue and the human health risks posed by microcystin exposure through fish consumption remain poorly resolved. Very few studies have quantified microcystin (a broadly present cyanotoxin) in water from East African lakes, despite the large human and animal populations that rely on these lakes for both water and food, and to date there is very little information available on the accumulation of microcystin in fish from these lakes.

A comprehensive set of water and fish samples was collected on a monthly basis between September 2008 and February 2009 from several lakes in Uganda. The study sites included two embayments in northern Lake Victoria (Murchison Bay and Napoleon Gulf), Lake Edward, Lake George, Lake Mburo, and the crater lakes Saka and Nkuruba. The large lakes sampled all support substantial commercially important fisheries, while the smaller lakes support subsistence fisheries that provide a critically important source of protein and income for riparian communities.

Microcystin concentrations in water were determined in addition to chlorophyll and nutrient concentrations, phytoplankton community composition, mixing dynamics and light conditions. At all study sites except Lake Nkuruba, microcystin concentrations in water regularly exceeded the WHO guideline for microcystin in drinking water of 1.0 µg/L. *Microcystis* spp. emerged as the cyanobacterial taxa that is primarily responsible for microcystin production in these lakes, and as such, microcystin concentrations were closely linked to environmental factors that favour the development of high *Microcystis* biomass, including high nutrient concentrations, as well as shallow mixing depth which acts to increase mean mixed layer light intensity.

Because of the importance of understanding the underlying food web when considering the accumulation and trophic transfer of a compound, stable carbon and nitrogen isotope analysis was used to characterize the food webs at the previously mentioned Ugandan study sites as well as in the East African great lake Albert. Omnivory was found to be common at all study sites, and based on $\delta^{13}C$ values, the food webs in these lakes were strongly based on pelagic primary production, with no strong evidence of

substantial benthic contribution to these food webs, likely as a result of reduced benthic primary productivity in these generally low-transparency eutrophic lakes.

The distribution and trophic transfer of mercury was also characterized in the Ugandan study lakes (including Lake Albert) in order to provide a contrast for the trophic transfer of microcystin in the same lakes. Furthermore, relatively little is known about the behaviour of mercury in tropical hypereutrophic lakes, and the study sites included in the current study provided an opportunity for the exploration of this topic. Consistent biomagnification of mercury was observed at all study sites; however, mercury concentrations in fish were generally low, and would not be expected to pose a risk to consumers. Mercury dynamics were strongly linked to lake trophic status, with biomagnification rates significantly lower at the hypereutrophic study sites than at the mesotrophic and eutrophic study sites. I found evidence that growth and possibly biomass dilution can reduce mercury concentrations at the base of the food web, while growth dilution of mercury at consumer trophic levels might effectively reduce the biomagnification rate of mercury in these hypereutrophic lakes.

Microcystin was prevalent in fish muscle tissue from all study sites and at all trophic levels. In contrast to mercury, for which consistent biomagnification was observed, neither biomagnification nor biodilution was observed for microcystin; and concentrations were relatively consistent throughout the fish food web, including in top predators, indicating that efficient trophic transfer of microcystin is occurring in these lakes. Microcystin concentrations in fish from several study sites followed seasonal trends that were similar to those observed for microcystin concentrations in water at these sites, suggesting that fish can rapidly respond to changes in microcystin concentrations in water through accumulation and depuration of this toxin.

Microcystin concentrations in water and fish from all Ugandan study sites (including Lake Albert) in addition to data from two temperate eutrophic embayments (Maumee Bay in Lake Erie, and the Bay of Quinte in Lake Ontario) were compiled and used to estimate potential microcystin exposure to human consumers of both water and fish from these study sites. Microcystin was pervasive in water and fish from both the tropical and temperate study sites. Also, these results establish that fish consumption can be an important and even dominant source of microcystin to humans, and can cause consumers to exceed recommended total daily intake guidelines for microcystin. These results highlight the need to consider potential exposure to microcystin through fish consumption in addition to water consumption in order to adequately assess human exposure and risk.

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Chapter 1
General Introduction

1.1 General context of study

Uganda, in equatorial East Africa, has abundant freshwater resources, with lakes, wetlands and rivers covering approximately 16 % of Uganda's surface area (World Water Assessment Programme, UNESCO 2006). Uganda is home to some of the largest freshwater lakes in the world, including Lake Victoria, the world's largest tropical freshwater lake, which also sustains the world's largest freshwater fishery (Kolding *et al.* 2008). These lakes provide both water (for drinking, sanitation, and agriculture) and fish for tens of millions of people. However, both in Uganda and globally, lakes are being threatened by anthropogenic activities, raising many ecological and public health concerns, including the potential for harmful blooms of toxic cyanobacteria.

The primary objective of this thesis is to characterize the distribution and trophic transfer of microcystin in water and food webs of Ugandan lakes. This research also seeks to provide a quantitative assessment of the risks posed by microcystin in these lakes, and in particular explores the potential for microcystin exposure through fish consumption. To assess the trophic transfer of microcystin as well as the effect of fish diet on microcystin accumulation, stable carbon and nitrogen isotope analysis is used to examine food-web structure in the study lakes. Additionally, by measuring mercury concentrations and quantifying the biomagnification of this contaminant using the same fish, I am able to use the better-understood process of mercury biomagnification as a "standard" to which microcystin trophodynamics can be compared in order to gain insight into the movement of this cyanotoxin through aquatic food webs.

1.2 Microcystin

Globally, cyanobacterial dominance of freshwater systems is increasing, as is the occurrence of hazardous blooms of cyanobacteria (de Figueiredo 2004). These cyanobacterial blooms can have several deleterious effects on freshwater ecosystems and those who rely on them including beach fouling, oxygen depletion, food web alteration, taste and odour problems and toxin production (Oliver and Ganf 2000, de Figueiredo *et al.* 2004). The toxins produced by cyanobacteria include hepatotoxins (such as microcystin, nodularin and cylindrospermopsin), neurotoxins (such as anatoxin and saxitoxin), dermatotoxins and cytotoxins (such as lyngbyatoxin and aplysiatoxin), and endotoxins (lipopolysaccharides) (Codd *et al.* 2005). The most common cyanotoxins produced in fresh waters belong to the hepatotoxic microcystin family, while the closely related nodularins are commonly produced in brackish waters (Sivonen and Jones 1999).

Cyanobacterial taxa known to be capable of microcystin production include *Microcystis*, *Anabaena*, *Anabaenopsis*, *Planktothrix (Oscillatoria)*, and *Nostoc* (Sivonen and Jones 1999). There are more than 80 known congeners of microcystin, with varying levels of toxicity (Dietrich and Hoeger 2005). For the purpose of this study, "microcystin" will refer to all congeners as a whole. Microcystin is a monocyclic heptapeptide, with a general structure of:

$$\text{cyclo-(D-alanine}^{1}\text{-X}^{2}\text{-D-MeAsp}^{3}\text{-Z}^{4}\text{-Adda}^{5}\text{-D-glutamate}^{6}\text{-Mdha}^{7}\text{)}$$

(Sivonen and Jones 1999, Babica *et al.* 2006) where X and Z represent two variable L-amino acids, which, along with degree of methylation, distinguish the different microcystin congeners (Williams *et al.* 1997c, Sivonen and Jones 1999). The Adda-glutamate region, found in both microcystin and nodularin, interacts with protein phosphatases, and it is through this interaction that these cyanotoxins exert their toxic effects (Sivonen and Jones 1999). Meanwhile, the Mdha region (N-methyldehydroalanine), which is unique to microcystin, has been shown to bind covalently to protein phosphatase enzymes (Williams *et al.* 1997a, Sivonen and Jones 1999).

Microcystin is a powerful inhibitor of protein phosphatases 1 and 2A in both animals and plants (MacKintosh *et al.* 1990). These are key regulatory enzymes, and their inhibition can cause a range of negative effects, including cell death (Blom and Jüttner 2005, Codd *et al.* 2005, Babica *et al.* 2006). Additionally, microcystin is a potential tumour promoter, indicating that chronic exposure to this toxin may pose serious health risks (Falconer and Humpage 1996, de Figueiredo 2004, Chen *et al.* 2009). The World Health Organization has set a tolerable daily intake (TDI) guideline for chronic exposure to microcystin of 0.04 µg/kg body weight of consumer (WHO 1998, Falconer *et al.* 1999). Based on this value, and the assumption that 80 % of human exposure to microcystin will be via drinking water, the WHO recommended guideline for microcystin in drinking water is 1.0 µg/L (WHO 1998, Falconer *et al.* 1999). Additionally, microcystin is a heat stable compound, and neither boiling water nor cooking fish prior to consumption is expected to reduce the potential for exposure (Harada 1996, Zhang *et al.* 2010).

1.2.1 The function of microcystin

Microcystin is generally considered to be a cyanobacterial secondary metabolite (Carmichael 1992, Wiegand and Pflugmacher 2005, but see Orr and Jones 1998), and although there has been considerable debate surrounding the endogenous function of microcystin, several possible explanations for the production of this toxin have been suggested. There is some evidence that microcystin can offer protection against grazing by zooplankton, which have been observed to distinguish and selectively reject

toxic cyanobacteria (Babica *et al.* 2006). Alternatively, microcystin has a high affinity for iron and binds Fe^{2+}, and it has been proposed that microcystin could be involved in either collecting iron where limiting (Lukac *et al.* 1993), or chelating intracellular iron to reduce the production of oxygen free radicals (Utkilen and Gjolme 1995). Also, several studies have shown evidence of allelopathic effects of microcystin on both phytoplankton and macrophytes (Pflugmacher 2002, Hu *et al.* 2004, LeBlanc *et al.* 2005, Babica *et al.* 2006). However, given the genetic evidence that cyanobacterial ability to produce microcystin preceded the evolution of eukaryotic photoautotrophs or metazoans (Rantala *et al.* 2004), it is unlikely that the primary role of microcystin is grazer defence or allelopathy against photoautotrophs (Babica *et al.* 2006, Schatz *et al.* 2007). The diversity of these theories highlights the current lack of consensus on the cellular role of microcystin.

1.2.2 Factors affecting microcystin production

The presence and concentration of microcystin in an aquatic system depends on two essential requirements: the presence of cyanobacteria capable of microcystin production and the active synthesis of microcystin by these cyanobacteria (Sivonen and Jones 1999). There is a well-documented relationship between the global increase in cultural eutrophication and a corresponding increase in the occurrence of microcystin producing blooms, especially blooms of *Microcystis aeruginosa* (de Figueiredo 2004). However, microcystin production has also been observed in oligotrophic systems, where highly toxic dense metalimnetic blooms of *Planktothrix* have been observed (Mur *et al.* 1999, Fastner *et al.* 1999). Furthermore, microcystin production by benthic cyanobacterial taxa such as *Oscillatoria* or *Phormidium* has been observed in lakes with high water clarity (Mez *et al.* 1997, Mur *et al.* 1999). Within a cyanobacterial species, there exist a wide range of genotypic strains, some of which are capable of toxin production and some of which are not. The ability of cyanobacterial strains to produce microcystin is related to the presence of the *mcy* gene cluster, while expression of this gene cluster is known to vary greatly (Meisner *et al.* 1996).

Toxin production has been related to several environmental factors including light, temperature, nutrient concentrations, nutrient ratios, and pH; and how these factors affect microcystin production is known to differ between cyanobacterial strains (Sivonen and Jones 1999, Giani *et al.* 2005, Kardinaal and Visser 2005, Billam *et al.* 2006). In a review of culture studies, Sivonen and Jones (1998) found that microcystin production within a strain can vary by a factor of 3 to 4, while differences between strains can be much larger, suggesting that the high variability observed in field microcystin concentrations is primarily attributable to differences in the relative abundance of toxic strains (Giani *et al.* 2005, Sivonen

and Jones 1998). The factors driving cellular microcystin production within a strain are complex and often appear to be contradictory, however, much evidence supports the theory that microcystin synthesis tends to be highest where conditions are favourable for cell growth (Orr & Jones 1998, Briand 2005).

1.2.3 Accumulation and Trophic Transfer of Microcystin

Accumulation of microcystin has been observed in organisms such as zooplankton, gastropods, fish, macrophytes and even terrestrial crops irrigated with contaminated water (Kotak *et al.* 1996, Prepas *et al.* 1997, Zurawell *et al.* 1999, Magalhaes *et al.* 2003, de Figueiredo 2004, Ibelings and Chorus 2007). Due to its high molecular weight (from approximately 900 to 1100 Da), microcystin cannot easily cross cell membranes, however, some types of cells (especially mammalian hepatocytes) have membrane transporters facilitating toxin uptake (Fischer *et al.* 2005, Amado and Monserrat 2010).

Data suggest that, in fish, the majority of microcystin uptake occurs in the digestive tract, followed by the rapid distribution of microcystin throughout the fish body via the blood-stream, with highly-vascularized organs experiencing the highest exposure to and accumulation of microcystin (Cazenave *et al.* 2005, Xie *et al.* 2005, Smith *et al.* 2008, Wang *et al*. 2008, Martins and Vasconcelos 2009). Microcystin has been demonstrated to have both acute and chronic effects on fish liver function, and may act to decrease fish growth efficiency (Andersen *et al.* 1993, Malbrouk and Kestemont 2006). Net Pen Liver Disease (NPLD) in pen-reared Atlantic salmon (in which severe liver lesions and mortality occur) has been directly attributed to microcystin (Andersen *et al.* 1993). There is also a growing body of evidence that microcystin can have detrimental effects on the early development of fish, leading to deformations and mortality (Oberemm *et al.* 1997, Malbrouk and Kestemont 2006).

Microcystin has been detected in higher food web organisms even when microcystin concentrations in the water were below the WHO recommended guideline of 1 µg/L (Prepas *et al.* 1997, Zurawell *et al.* 1999, Magalhaes *et al.* 2003). There is little evidence of biomagnification (increasing concentrations of a compound at successively higher levels in a food chain) of microcystin in aquatic food webs (Kotak *et al.* 1996, Ibelings *et al.* 2005, Zhang *et al.* 2009), although Xie *et al.* (2005) did observe higher microcystin concentrations in the muscle tissue of carnivorous fish relative to phytoplanktivorous fish. Several studies have suggested that microcystin may instead undergo biodilution, with decreasing concentrations due to metabolism and excretion of microcystin at each successive trophic level (Ibelings *et al.* 2005, Karjalainen *et al.* 2005, Ibelings and Havens 2008).

1.2.4 Detection of microcystin

In this study I will measure microcystin in water and methanol-extracted fish tissue using an Enzyme-Linked Immunosorbent Assay (ELISA) that is specific for the Adda side-chain found in all microcystin and nodularin congeners (this type of ELISA is considered a congener-independent detection method; Fischer *et al.* 2001). It is important to note that methanol extraction of fish followed by ELISA is not expected to measure microcystin that is covalently bound to protein phosphatases in the fish tissue; and as a result, the microcystin concentrations reported in this study will represent unbound extractable microcystin rather than the total microcystin burden of the organism (Williams *et al.* 1997b). Although there is some evidence of toxicity of potential break-down products of the protein phosphatase-microcystin complex (Smith *et al.* 2010), little remains known about the risks posed by this covalently-bound microcystin pool as compared to the well-studied toxic effects of unbound microcystin. ELISA is known to be a sensitive and robust method for detection of microcystin concentrations in both water and fish, and the simplicity, rapidity and affordability of this method makes ELISA particularly appropriate for rapid assessment of microcystin concentrations in water or fish, as well as for large-scale studies such as the one described in this thesis.

1.3 Mercury

The global release of mercury to the environment has increased greatly as a result of human activities such as coal combustion, mining, smelting, industrial processes and biomass burning (Pacyna *et al.* 2006). Most developed countries have implemented regulations to reduce the use and emission of mercury with the aim of lessening human and wildlife exposure to this contaminant. However, many countries in the developing world have not yet taken these measures. Although emissions of mercury in Europe and North America have decreased since the early 1980s when industrial controls were implemented, on a global scale, total mercury emissions are increasing due to an ongoing increase in emissions from Asia, Africa and South America (Pacyna *et al.* 2006).

In Africa, biomass burning, coal combustion, artisanal and small-scale gold mining and metal processing are the primary sources of mercury emissions (UNEP 2002, AMAP/UNEP 2008). However, emissions from biomass burning are difficult to quantify and are often excluded from global and regional emissions estimates. Estimated release of mercury through biomass burning in Africa for 1997–2006 was approximately 140 tonnes per year (Friedli *et al.* 2009). Population continues to increase rapidly throughout Africa, and mercury emissions from fuel combustion and biomass burning are likely to increase. In addition to local deposition, mercury is subject to long distance atmospheric transport,

therefore, its continued use and emission poses risks to humans and animals both at the point of use and in remote locations (Jackson 1997). As a result, regional emissions estimates may not provide a comprehensive picture of the subsequent regional deposition rates or exposure risks.

Mercury exists in several different forms in the environment. However, not all forms are equally bioavailable—and therefore capable of accumulating and biomagnifying in the food web. The bioaccumulative potential of mercury is increased greatly when transformed into methyl mercury through the methylation of inorganic mercury by bacteria in anoxic conditions (Morel *et al.* 1998). Methyl mercury exhibits toxicity through covalent binding to sulfhydryl groups on proteins (including enzymes), which play an important role in the formation of disulphide bridges as well as in other conformational changes in proteins (Clarkson 1997). Because methyl mercury is able to pass through cell membranes, has a diffuse distribution throughout the soluble fraction of the cell, and is covalently bound to essential sulfhydryl bearing amino acids, it is readily assimilated rather than excreted by the consumer organism. This facilitates bioaccumulation and subsequent food web transmission of this compound (Morel *et al.* 1998).

Of the total mercury (THg) present in freshwater fish muscle tissue, 85–95% is in the form of methyl mercury (Bloom 1992). Methyl mercury is a potent neurotoxin that is known to have many detrimental effects on humans, with children and fetuses *in utero* being particularly vulnerable to methyl mercury exposure (Clarkson 1997, WHO 1990). Human exposure to mercury is predominantly through the consumption of fish (WHO 1990). For fish tissue, the World Health Organization's total mercury guideline value for at-risk groups (including children, pregnant women and frequent fish consumers) is 200 ng g^{-1} wet weight (WHO 1990).

There are many factors that influence mercury concentrations in fish: the concentration of mercury in the water, mercury methylation rates, the length and structure of the food web (Cabana and Rasmussen 1994), fish age and length (MacCrimmon *et al.* 1983), as well as the productivity of the system. When algal biomass is high, the accumulation of algal biomass may outstrip the production of methyl Hg and result in "biomass dilution" (Pickhardt *et al.* 2002). Alternatively, where growth rates are high at the base of the food web, rapidly dividing cells may not fully equilibrate with available methyl Hg concentrations and "growth dilution" may occur (Herendeen and Hill 2004). Effectively either high growth rates or high algal biomass can serve to dilute the mercury concentration in the phytoplankton, and lower concentrations at the point of entry into the food web can translate into lower concentrations throughout the food web (Meili 1991, Pickhardt *et al.* 2002). Similarly, lifespan and growth rate can influence

mercury concentrations in fish; with longer-lived and slower growing fish (common in temperate systems) tending to have higher mercury concentrations (Stafford and Haines 2001, Kidd *et al.* 2003, Simoneau *et al.* 2005) than faster growing fish (common in tropical systems) at similar age and size (Kidd *et al.* 2003).

1.4 Study Sites

This thesis includes data from 8 Ugandan study sites and two eutrophic embayments in the Laurentian Great Lakes. Lakes Edward, George, Mburo, Murchison Bay (Lake Victoria), Napoleon Gulf (Lake Victoria), Lake Nkuruba and Lake Saka are included as study sites in all data chapters, Lake Albert is included Chapters 3, 4 and 6; and Maumee Bay (Lake Erie) and the Bay of Quinte (Lake Ontario) are included as study sites in Chapter 6. A summary of general site characteristics for the Ugandan study lakes is found in Table 1.1, and maps showing the location of these sites are found in Figures 1.1–1.3.

1.4.1 Lakes Edward and George

Lake George (Figure 1.2) is a highly productive shallow lake with a mean depth of 2.4 m (Lehman *et al.* 1998). This lake is known to have persistently high cyanobacterial biomass, with year-round presence of potentially toxigenic cyanobacteria (generally *Microcystis* spp.) (Ganf 1974, Lehman *et al.* 1998). There is a substantial (180 km^2) papyrus-dominated wetland that surrounds Lake George, which was designated a Ramsar site in 1988 by the Ugandan Ministry of Environmental Protection (Denny *et al.* 1995, Lwanga *et al.* 2003). Lake George is located within Queen Elizabeth National Park, however, a history of nearby copper mining has led to elevated heavy metal concentrations in this relatively undisturbed lake (Denny *et al.* 1995, Lwanga *et al.* 2003).

Water from Lake George flows into the larger, deeper and less productive Lake Edward via the narrow Kazinga channel (Figure 1.2). Lake Edward has a maximum depth of 112 m, and is one of the least studied of the African great lakes (Lehman *et al.* 1998). Much of Lake Edward lies within protected areas of both Uganda and the Democratic Republic of Congo (Lehman *et al.* 1998).

1.4.2 Lake Mburo

Lake Mburo is located in Lake Mburo National Park (Bwanika *et al.* 2004) and is an important source of drinking water for wild game (Mbabazi *et al.* 2004). The lake also supports a large population of hippopotamuses, which are important contributors of dissolved nutrients to the lake, supporting growth of phytoplankton and bacteria (Mbabazi *et al.* 2004). Lake Mburo is highly productive, with a phytoplankton

community dominated by *Microcystis* spp. (Okello *et al.* 2009, Nyakoojo and Byarujali 2010). Although Lakes Edward, George and Mburo are all within protected areas, fishing communities exist within these national parks, and controlled fishing provides both food and a source of income for these villages.

1.4.3 Lake Victoria (Murchison Bay and Napoleon Gulf)

Lake Victoria is the world's largest tropical lake by area (66 368 km^2), with a maximum depth of 75m (Silsbe 2004). This lake provides drinking water for more than ten million people and sustains a large population of domestic and wild animals (Mugidde *et al.* 2003). Lake Victoria is also home to the world's most productive freshwater fishery, with annual catches exceeding 500 000 metric tonnes since the late 1980s and recent catches reaching one million tonnes (Kolding *et al.* 2008).

However, over the past several decades, Lake Victoria has experienced rapid change that is largely attributable to anthropogenic influences driven by factors such as population increase, agriculture, deforestation, overfishing and climate warming (reviewed in Hecky *et al.* 2010). Lake Victoria's food web structure and species assemblages have been largely restructured by both eutrophication and the deliberate introduction of exotic species including the Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*) (Ogutu-Ohwayo 1990). Another exotic species that has strongly impacted the lake ecosystem is water hyacinth (*Eichhornia crassipes*); however, this macrophyte has declined greatly in abundance due to a successful biological control programme (Williams *et al.* 2005). Nutrient enrichment of the lake has supported increasingly eutrophic conditions (Hecky 1993), leading to increased hypoxia in deep waters (Hecky *et al.* 1994). Eutrophication and the resulting depletion of silica in the lake are thought to have contributed to a shift in phytoplankton community composition from dominance by chlorophytes and large diatoms to dominance by cyanobacteria (Kling *et al.* 2001, Hecky *et al.* 2010). Lake Victoria is now dominated by *Cylindrospermopsis* spp, *Anabaena* spp. (Mugidde *et al.* 2003), as well as by *Microcystis* spp (Kling *et al.* 2001); these are all taxa that are known to include toxin-producing species. Furthermore, microcystin has been observed in the water of Lake Victoria on several occasions (Sekadende *et al.* 2005, Haande 2008, Okello *et al.* 2009).

The current study includes two embayments in Northern Lake Victoria: Murchison Bay and Napoleon Gulf (Figure 1.3). Shallow Murchison Bay is located in a densely population urban area and provides water to and receives waste from Kampala, Uganda's largest city. Meanwhile, Napoleon Gulf is located at the outflow of the lake to the Victoria Nile, and is situated in a mixed urban and agricultural region.

1.4.4 Crater lakes Nkuruba and Saka

Western Uganda is home to more than 80 small volcanic crater lakes (Bwanika *et al.* 2004). These lakes are limnologically diverse, with a wide range of depth, salinity, phosphorus and silica concentrations (Melack 1978), and they are an important source of water and fish for the surrounding communities. This study includes two of these crater lakes: Lake Saka and Lake Nkuruba, which, although geographically very close to one another, exhibit very different ecological conditions.

Lake Saka is a shallow crater lake (mean depth of 3.6 m) that is surrounded by extensive wetlands (Crisman *et al.* 2001, Campbell *et al.* 2006, Melack 1978). Lake Saka's catchment has been highly impacted by deforestation and agriculture (Crisman *et al.* 2001, Campbell *et al.* 2006), and the lake is now hypereutrophic, with previously recorded chlorophyll *a* values as high as 134 μg L^{-1} (Campbell *et al.* 2006). Lake Saka was stocked with *O. niloticus* and *L. niloticus* in the early 1970s, and there are several other species of indigenous haplochromine cichlids also present in the lake (Binning *et al.* 2009). Lake Saka is cyanobacterially dominated, and microcystin concentrations exceeding 3 μg/L have previously been observed in this lake (Campbell *et al.* 2006).

Comparatively, Lake Nkuruba has been much less impacted by recent anthropogenic activities. It is a small mesotrophic lake surrounded by an intact rainforest ecosystem, with a maximum depth of 38 m and a permanently anoxic hypolimnion (Chapman *et al.* 1998). The phytoplankton community in this lake has been observed to be dominated by small cyanobacteria and chlorophytes (Chapman *et al.* 1998). Lake Nkuruba is home to three species of fish, all of which were introduced, and none of which is known to be piscivorous (Campbell *et al.* 2006).

1.4.5 Lake Albert

Lake Albert lies between Uganda and the Democratic Republic of the Congo and has an area of 5 600 km^2, a mean depth of 25 m, and a maximum depth of 58 m (Talling 1963). The primary inflow to Lake Albert is the Semliki River, which drains Lake Edward. A diel stratification regime is observed in the inshore regions, and increasingly, persistent thermal stratification is seen offshore (Mugidde *et al.* 2007). This differs from the well mixed "constant temperature bath" described by Talling (1963). In the 1960's, diatoms from the genus *Stephanodiscus* dominated Lake Albert's phytoplankton, while only localized cyanobacteria were seasonally present (Evans 1997). However, primary productivity in the lake nearly doubled throughout the 1990's, leading to a shift toward more consistent cyanobacterial dominance (Mugidde *et al.* 2007), as diatom growth in Lake Albert was likely already limited by low concentrations

of silica in the euphotic zone (Talling 1963). Meanwhile low concentrations of available nitrogen promote nitrogen-fixing cyanobacteria. Currently, northern Lake Albert exhibits repetitive blooms of *Anabaena* spp. and *Microcystis* spp. (Mugidde *et al.* 2007), both known to be capable of toxin production.

1.4.6 Maumee Bay, Lake Erie

Maumee Bay is a shallow eutrophic embayment in the western basin of Lake Erie (mean depth < 2m). The Maumee River flows into the bay at the city of Toledo, a heavily industrialized and busy port. The western basin of Lake Erie is known to exhibit regular blooms of *Microcystis* spp., and microcystin has been detected on several occasions (Rinta-Kanto *et al.* 2005, Ouellette *et al.* 2006, Yakobowski 2008). This lake has been greatly affected by invasive dreissenid mussels, which may favour the dominance of phytoplankton communities by toxin producing cyanobacteria through selective rejection of toxic cells during feeding (Vanderploeg *et al.* 2001).

1.4.7 Bay of Quinte, Lake Ontario

The Bay of Quinte is also a shallow eutrophic embayment; it is located on the north shore of Lake Ontario and is strongly influenced by the inflow of the Trent and Napanee Rivers. The upper Bay of Quinte has little contact with open Lake Ontario. As in Western Lake Erie, invasive dreissenid mussels have strongly affected the food web of the Bay of Quinte, and may have led to an increase in *Microcystis aeruginosa* (Nicholls *et al.* 2002). Blooms of *M. aeruginosa* are now a common occurrence in the Bay of Quinte, and microcystin is detected regularly throughout the summer months (Watson *et al.* 2008, Yakobowski 2008).

1.5 General thesis structure

This thesis is divided into five related but independent data chapters (Chapters 2–6). Data chapters were generally prepared as manuscripts for submission to peer-reviewed journals, and as such, some repetition of information is unavoidable; however, I attempted to minimize repetition where possible.

Chapter 2: Physicochemical drivers of microcystin production in several Ugandan lakes.

In this chapter, I characterize mixing dynamics, nutrient concentrations, phytoplankton community composition and microcystin concentrations in water from several Ugandan lakes over a six month period, including Lakes Edward, George, Mburo, Napoleon Gulf (Lake Victoria), Murchison Bay (Lake Victoria), Saka and Nkuruba. I explore the factors that predict both the biomass of microcystin producing cyanobacteria, as well as microcystin concentrations in these lakes.

Chapter 3: Characterization and comparison of food web structure in several Ugandan lakes using stable isotope analysis.

In this chapter, I use stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis to characterize and compare food-web structure and fish diet in several Ugandan lakes, including Lakes Albert, George, Mburo, Napoleon Gulf (Lake Victoria), Murchison Bay (Lake Victoria), Saka and Nkuruba.

Chapter 4: Bioaccumulation and biomagnification of mercury in several Ugandan lakes: the importance of lake trophic status.

In this chapter, I characterize mercury concentrations in the water and fish at 8 Ugandan study sites (those included in Chapter 3), and explore the accumulation and trophic transfer of mercury in the context of food-web structure. In particular, biomagnification of mercury is quantified based on stable nitrogen isotopic ratios (as indicators of trophic level), and is compared among sites. By examining mercury, a compound whose movement through aquatic food webs has been well studied, I will have a "standard" to which I can compare the accumulation and trophic transfer of microcystin at the same study lakes (and in the same fish).

Chapter 5: Accumulation, trophic transfer, and seasonality of microcystin in fish from several Ugandan lakes.

In this chapter, I examine the accumulation and trophic transfer of microcystin in fish from several Ugandan study sites (those included in Chapter 2). These processes are explored in the context of fish diet and food web structure, as characterized using stable isotope analysis. I also explore seasonal patterns in microcystin concentrations in fish in the context of seasonality of microcystin in water at these study sites.

Chapter 6: Evaluation of microcystin exposure risk through fish consumption

In this chapter, I summarize and integrate data on microcystin concentrations in water as well as fish from all study sites, including all 8 Ugandan sites as well as two temperate eutrophic embayments on the Laurentian Great Lakes. Potential human exposure to microcystin through both drinking water and fish is estimated, and in particular, the microcystin exposure risk posed by fish consumption at all study sites is evaluated.

Table 1.1 General characteristics of Ugandan study lakes and sampling stations. The abbreviation z_{max} refers to maximum lake depth.

Lake	Lat.	Lon.	z_{max} (m)	Mean Depth (m)	Site Depth (m)	Area (km^2)	Volume (km^3)	Reference
Albert	1º34'N	30º58'E	58	25	12.0	5600	140	Talling 1963
Edward	~	~	120	33	~	2325	76.7	Lehman *et al.* 2004
Nearshore	0º13'S	29º53'E	~	~	3.5	~	~	~
Offshore	0º13'S	29º52'E	~	~	7.3	~	~	~
George	0º00'S	30º11'E	7	2.4	2.8	250	0.5	Viner and Smith 1973
Mburo	0º39'S	30º56'E	4	2	3.2	13	0.325	Nyakoojo & Byarujali 2010
Victoria	~	~	75	39	~	66368	2598	Silsbe 2004
Inner Murchison Bay	0º15'N	32º39'E	7	3.2	5.2	18	0.113	Haande *et al.* 2010
Napoleon Gulf	0º24'N	33º14'E	20.5	7.9	17.5	26.5	0.22	Jackson 2004
Nkuruba	0º31'N	30º18'E	38	16	33.4	0.03	0.000481	Chapman *et al.* 1998
Saka	0º41'N	30º15'E	8.5	3.6	3.2	0.15	0.000054	Melack 1978

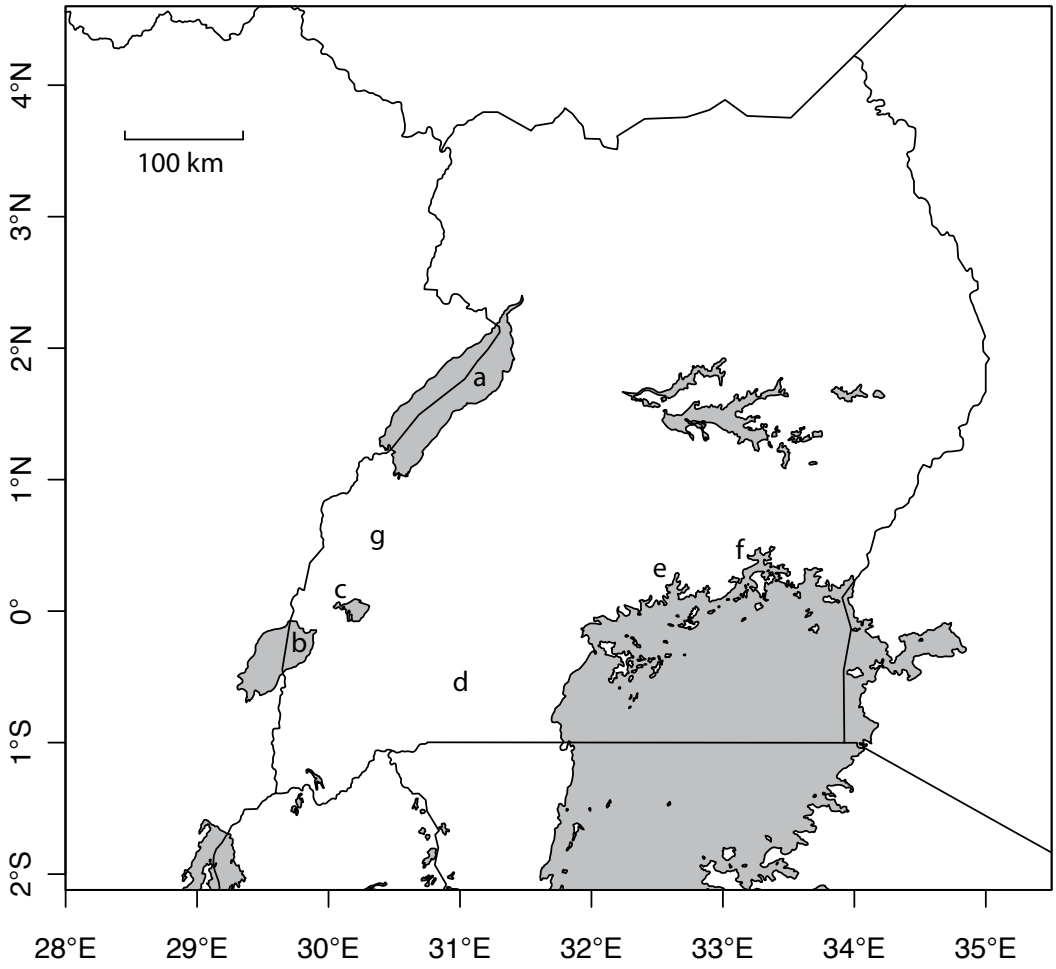

Figure 1.1 Map of Uganda showing all study sites: a) Lake Albert, b) Lake Edward, c) Lake George, d) Lake Mburo, e) Murchison Bay, f) Napoleon Gulf, g) Lakes Nkuruba and Saka (small crater lakes that are geographically close to one another). A more detailed map showing Lakes Edward and George is found in Figure 1.2, and a more detailed map showing Murchison Bay and Napoleon Gulf is found in Figure 1.3.

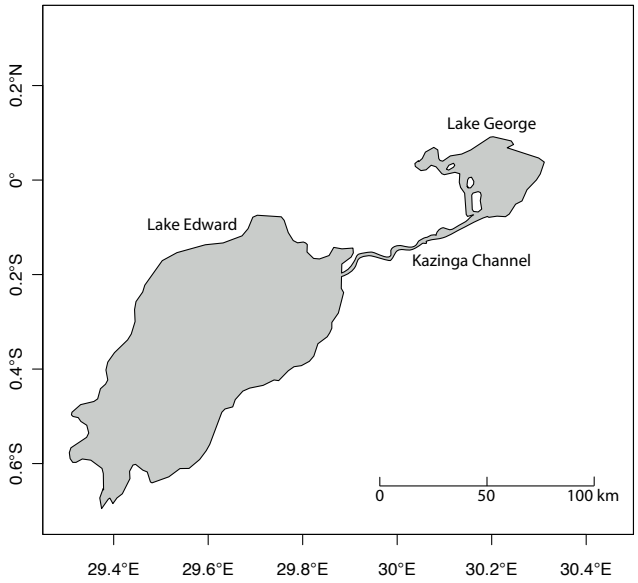

Figure 1.2 Map of Lake Edward and Lake George. Water from Lake George enters Lake Edward via the Kazinga Channel. The lake George study site was located near the centre of the open water, while the nearshore Lake Edward study site (depth of 3.5 m) was located near the mouth of the Kazinga Channel, and the offshore site (depth of 7.3 m) was located 2.5 km to the southwest.

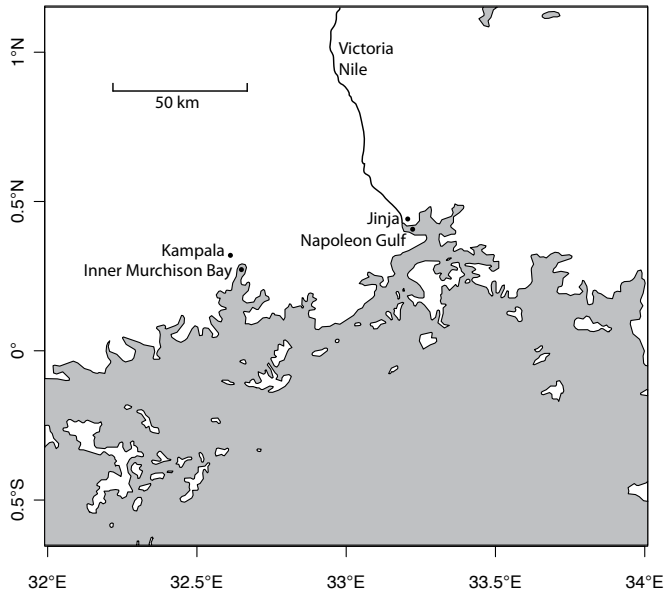

Figure 1.3 Map of northern Lake Victoria showing the Napoleon Gulf and Inner Murchison Bay sampling sites. The cities of Kampala and Jinja are also shown. Note that the Victoria Nile (indicated on map) exits Lake Victoria via Napoleon Gulf.

Chapter 2
Physicochemical drivers of microcystin production in several Ugandan lakes

2.1 Introduction

Globally, harmful cyanobacterial blooms are increasing both in frequency and magnitude (de Figueiredo *et al.* 2004), often as a result of anthropogenic nutrient input (Fogg 1969, Paerl and Fulton 2006). Climate warming can also cause shifts in phytoplankton community composition that favour bloom-forming cyanobacteria that are often capable of toxin production (Paerl and Huisman 2009). The most common cyanobacterial toxins produced in fresh and brackish water belong to the microcystin and nodularin families—which are hepatotoxic cyclic peptides (Sivonen and Jones 1999). Aquatic microcystin producing taxa include *Microcystis*, *Anabaena*, *Anabaenopsis*, *Planktothrix (Oscillatoria)*, and *Nostoc* (Sivonen and Jones 1999), and at least 80 congeners of microcystin have been identified, with varying levels of toxicity (Dietrich and Hoeger 2005).

Microcystin production in a lake depends on both the presence of cyanobacteria capable of microcystin production and the active synthesis of microcystin by these cyanobacteria (Sivonen and Jones 1999). Toxin production has been related to a number of environmental factors including: light, temperature, nutrient concentrations, nutrient ratios, and pH (Sivonen and Jones 1999). The factors driving cellular microcystin production are complex and often appear to be contradictory, however, most evidence supports the theory that microcystin synthesis tends to be highest where conditions are favourable for cell growth (Orr and Jones 1998, Briand 2005).

To date, most studies of microcystin and the factors that encourage microcystin production have focused on temperate systems (Sivonen and Jones 1999). As a result, considerably less is known about microcystin dynamics in tropical lakes. Unlike in temperate lakes, where strong seasonal changes in temperature, light and mixing allow for cyanobacterial dominance only in the summer and early fall (Munawar and Munawar 1986), in tropical lakes, the conditions are such that there is the potential for year-round dominance of potentially toxic cyanobacteria, often at high biomasses (Oliver and Ganf 2000, Kling *et al.* 2001).

Uganda, East Africa, is home to many freshwater lakes, including some of Africa's great lakes. Large human populations rely on these lakes for drinking water, water for human domestic use, and fish for

food (Mugidde *et al.* 2003). These lakes also provide drinking water for domestic and wild animals (Mugidde *et al.* 2003). As such, water quality in these lakes is of critical importance for the tens of millions of people who rely on them. Microcystin concentrations in water have been reported for some East African lakes, particularly for Lake Victoria, where studies have detected microcystin in three embayments: Mwanza Gulf (Tanzania; Sekadende *et al.* 2005), Napoleon Gulf (Uganda; Okello *et al.* 2009, 2010) and Murchison Bay (Uganda; Okello *et al.* 2009, 2010; Haande *et al.* 2010). Microcystin concentrations have also been reported for Lake Edward, and several smaller Ugandan lakes (Okello *et al.* 2009, 2010), many of which are included in the current study. Also both microcystin and anatoxin were detected in two Kenyan alkaline crater lakes (Lakes Sonachi and Simbi; Ballot *et al.* 2005). However, few of these studies have sought to elucidate broad patterns in microcystin concentrations and the factors that encourage microcystin production in these lakes. Also, given the spatial and temporal variability inherent in microcystin concentrations (at both seasonal and inter-annual scales), this study will increase our understanding of the range of microcystin concentrations that can occur in these lakes.

The current study includes six East African lakes, including the tropical great lakes Victoria and Edward, as well as four smaller Ugandan lakes: Lake George, Lake Mburo, and the crater lakes Saka and Nkuruba (see Figure 1.1 for a map indicating study site locations). By characterizing microcystin concentrations, phytoplankton community composition, and physicochemical variables in a broad range of lakes over a six-month period (during a time of year known to be conducive for cyanobacterial growth in these lakes), insight will be gained into the cyanobacterial taxa responsible for toxin production, the factors that determine the presence of these taxa, as well as the factors that encourage the active production of microcystin. Also, I seek to determine whether microcystin concentrations in these lakes represent a human health risk.

2.2 Methods

2.2.1 Study Sites

Detailed site descriptions as well as a map showing the location of the study sites can be found in Chapter 1 (Figure 1.1), and some general characteristics of the study lakes are outlined in Table 2.1. In Lake Victoria, I collected samples from two embayments in the northern part of the lake, Murchison Bay and Napoleon Gulf (Figure 1.3), which are the respective main water sources for Kampala and Jinja, Uganda's two largest cities. These embayments also receive wastewater from these cities, however, Napoleon Gulf is well flushed with water from offshore Lake Victoria due to the outflow of the Victoria

Nile from this embayment. In Napoleon Gulf, samples were collected from one site (18 m depth), and in Inner Murchison Bay, three stations were sampled, however, there were no significant differences in any variables among these three stations, and data from these stations were pooled for both statistical analysis and interpretation of results.

Lake George is a highly productive shallow lake (mean depth of 2.4 m) that is dominated by cyanobacteria year-round (Ganf 1974, Lehman *et al.* 1998). Some water from Lake George flows into the larger, deeper and less productive Lake Edward via the Kazinga channel. Samples were collected from central Lake George, nearshore Lake Edward (at the opening of the Kazinga Channel), and offshore Lake Edward (approximately 2.5 km offshore; Figure 1.2). Meanwhile, Lake Mburo is a shallow and productive lake that lies within Lake Mburo National Park (Bwanika *et al.* 2004). The lake is an important source of drinking water for wild game and supports a large population of hippopotamuses (Mbabazi *et al.* 2004).

Lakes Saka and Nkuruba are volcanic crater lakes in Western Uganda. Lake Saka's catchment has been highly impacted by deforestation and agriculture (Crisman *et al.* 2001, Campbell *et al.* 2006), and the lake has very high phytoplankton biomass (Campbell *et al.* 2006). Meanwhile, Lake Nkuruba is a small lake surrounded by an intact rainforest ecosystem; it has a maximum depth of 38 m and a permanently anoxic hypolimnion (Chapman *et al.* 1998).

2.2.2 Sample Collection and Physical Observations

Samples were collected on a monthly basis between September 2008 and February 2009 (except for Murchison Bay and Napoleon Gulf, where samples were collected every two weeks). Secchi depth was measured and vertical profiles of the water column were carried out with a spectral fluorometer (bbe Fluoroprobe^{TM}) which measured temperature and the vertical distribution of algal classes as estimated by fluorescence induced by excitation of different diagnostic pigments (Gregor and Maršálek 2004). Integrated euphotic zone water samples were collected using a Niskin (water from just under the surface, at Secchi depth and at twice the Secchi Depth was sampled and pooled). In Murchison Bay and Napoleon Gulf, samples were always collected in the morning, while at the other sites samples were collected in the morning where possible, but occasionally were collected later in the day.

Although I did not measure light attenuation using a light meter, I estimated this parameter based on both chlorophyll *a* concentrations (using the relationship of Silsbe *et al.* 2006; $k_{PAR} = 0.20(\text{Chl } a)^{0.52}$). I then estimated mean water column irradiance in the mixed layer as a proportion of surface light

(Guildford *et al.* 2000) using estimated k_{PAR} and mixed depth (based on temperature profiles). Absolute mean mixed layer irradiance was calculated assuming a surface irradiance of 50 000 mmol photons/m^2/day (from Guildford *et al.* 2000; representing the amount of light reaching the earth's surface at Lake Victoria's latitude assuming a cloudless atmosphere).

2.2.3 Nutrient and Chlorophyll *a* Analysis

Water samples were processed through filtration, preservation and/or freezing as soon as possible on the same day as sampling. Whole water samples were preserved with 0.0075 v/v H_2SO_4 for analysis of total phosphorus (TP) and total nitrogen (TN). TP samples were analyzed as in Stainton *et al.* (1977), while TN samples were analyzed using a Lachat chemical analyzer (Lachat QuikChem® FIA+ Series 8000; QuikChem® Method 31-107-04-1-C). For analysis of particulate silica (PartSi), whole water was filtered through a Millipore 0.2 µm polycarbonate filter, and the filter was frozen until analysis. The filtrate was also frozen until analysis (within 30 days of sample collection) of ammonium (NH_4-N, using the indophenol method), soluble reactive phosphorus (SRP), and soluble reactive silica (SRSi). PartSi and all dissolved nutrient concentrations were determined using the methods outlined in Stainton *et al.* (1977).

Whole water was filtered through Whatman glass fibre filters (nominal pore size of 0.7 µm) and filters were frozen and kept until analysis of chlorophyll *a* (which was carried out through acetone extraction and measured fluorometrically; Stainton *et al.* 1977) and particulate phosphorus (PartP; analyzed as in Stainton *et al.* 1977). For analysis of particulate carbon and nitrogen, water was filtered through a precombusted (at 450 ºC for 4 hrs) Whatman glass fibre filter, and filters were stored frozen until drying (at 65 ºC for 24 hours), then were kept in a desiccator until analysis. Analysis was carried out at the University of Waterloo (ON, Canada) using an elemental analyzer (Exeter CEC-440 CHN/O/S Elemental Analyzer). Molar ratios of particulate (C:N, C:P, N:P) and total nutrient concentrations (TN:TP) were calculated in order to assess potential nutrient deficiencies (I used the criteria for deficiency from Healey 1975, as outlined in Guildford and Hecky 2000).

2.2.4 Phytoplankton Community Composition

Whole water samples were preserved with Lugol's iodine shortly after collection. Samples were settled in an Ütermohl chamber and phytoplankton were enumerated using an inverted microscope. Cell volume was calculated using linear measurements of cells and established geometrical formulas (Wetzel and Likens 1991), and biomass was calculated assuming a cell specific gravity of 1 g/cm^3 (Nauwerck 1963).

2.2.5 Microcystin Analysis

Microcystin in water was measured using indirect competitive ELISA (enzyme-linked immunosorbent assay; Abraxis LLC, Microcystins-ADDA ELISA kits, PN 520011). This is a congener-independent ELISA based on the detection of the Adda side-chain found in microcystins and nodularins (Fischer *et al.* 2001). Total microcystin (cell-bound and dissolved) was measured in whole water, and dissolved microcystin was measured in filtrate (filtered through Whatman glass fibre filter with a nominal pore size of 0.7 μm). Whole water samples were prepared for use in ELISA assays through chemical lysis (using the Abraxis LLC QuikLyse method, Loftin *et al.* 2008). Cell specific microcystin concentrations (cell quotas) were calculated as microcystin concentration divided by number of *Microcystis* spp. cells (as determined through microscopy), and are expressed in units of fg MC/cell *Microcystis*.

2.2.6 Statistical Analyses

Data for all variables with the exception of mean water column temperature and station depth (which were both normally distributed) were log-transformed prior to inclusion in statistical analyses. I used analysis of variance to determine whether there were differences in mean values for physicochemical variables between sampling sites, as well as across all sampling sites between months. Additionally I used linear regression and correlation (where appropriate) to examine relationships between the variables included in this study. All statistical analyses were carried out using R, version 2.11.1 (R Development Core Team 2010).

2.3 Results

Table 2.2 includes a summary of the physicochemical observations for the study lakes, while results for ANOVAs comparing each variable between sites (in order to determine general differences between the study sites) are found in Table 2.3.

2.3.1 Physical Observations

Mean water column temperatures over the study period (Figure 2.1) were between 24.7 ºC and 26.6 ºC for all sites except for the two crater lakes where mean water column temperatures were significantly (ANOVA, $P<0.001$) lower than at the other sites (22.1 ± 0.5 ºC in Lake Saka and 22.8 ± 0.4 ºC in Lake Nkuruba). These crater lakes are located in the foothills of the Rwenzori Mountains, where high elevation and local conditions lead to lower air temperatures than other regions of Uganda (Chapman *et al.* 1998). At all sites, water column temperatures remained stable throughout the study period (Figure 2.2) with the

differences between the minimum and maximum mean water column temperatures observed ranging from 1.1 to 2.1 ºC, except for in Lakes George (2.6 ºC) and Edward nearshore, where there was a difference of 5.5 ºC between the minimum and maximum values throughout the study period. Most sites had fully mixed water columns when sampled (Figure 2.2). I defined stable stratification as the presence of a thermocline where the temperature changes by more than 1ºC; however, when such changes in temperature were observed in the top 1 m of the water column, this was not considered to be stable stratification and was instead attributed to diurnal stratification. Stable stratification was observed in Napoleon Gulf on several occasions between late September and November, as well as in Lake Nkuruba, where a persistent and well-defined thermocline was observed throughout the whole study period at a depth ranging from 6.0–9.1 m (Figure 2.2). At many sites, although stable stratification was not observed, the upper portion of the water column was often warmer than the underlying waters suggesting the early stages of diurnal stratification.

Mean Secchi depth over the study period (Figures 2.1, 2.3) was deepest in Lake Nkuruba (1.8 ± 0.4 m), Napoleon Gulf (1.4 ± 0.2 m) and offshore Lake Edward (1.1 ± 0.3 m), and ranged from 0.4 m to 0.7 m at the remaining sites.

Mean mixed layer irradiances based on attenuation calculated from chlorophyll *a* (as in Silsbe *et al.* 2006) were between 0.8 and 13.7 mmol photons/m^2/day (2–39 % of surface irradiance) (Table 2.4). Mean mixed layer irradiances were significantly lower in Napoleon Gulf and Murchison Bay than in either Lake Nkuruba or nearshore Lake Edward ($P<0.01$). In Lake Nkuruba, mean mixed layer irradiance decreased steadily over the study period, while in Lake Edward and Napoleon Gulf mean mixed layer irradiance exhibited higher variability over the sampling season than at other sites (Figure 2.4).

2.3.2 Nutrient and Chlorophyll *a* Concentrations

Across all lakes, mean total phosphorus (TP) concentrations (Figures 2.5, 2.6) ranged from 35.6 to 186.5 µg/L. Mean TP exceeded 100 µg/L at the Lake Edward nearshore station, Lake George, Lake Mburo, Murchison Bay and Lake Saka; while mean TP was just under 60 µg/L at both Lake Edward offshore and Napoleon Gulf, and was lowest in Lake Nkuruba. Mean total nitrogen (TN) concentrations (Figure 2.5) ranged from 1013 µg/L to 2440 µg/L, and were highest (>1900 µg/L) in Lake Saka, Murchison Bay and Lake Mburo; intermediate (1500–1700 µg/L) in Lake Edward nearshore and Napoleon Gulf, and between 1000–1500 µg/L in offshore Lake Edward, Lake Nkuruba and Lake George. Total nitrogen concentrations tended to exhibit a great deal of variability (much more than was observed in TP

concentrations, Figure 2.6) and there were no statistically significant differences in TN concentrations between study sites (Table 2.3).

Soluble reactive silica concentrations (Figures 2.5, 2.8) were significantly lower (ANOVA, $P<0.001$) in Murchison Bay and Napoleon Gulf (usually under 1000 µg/L) than at any of the other study sites (where concentrations were generally between 5000 and 10 000 µg/L). There were no significant differences in concentrations between sites for ammonium (NH_4-N) or soluble reactive phosphorus (SRP), although NH_4-N concentrations were generally higher in Murchison Bay, Napoleon Gulf and Lake Saka than at other sites (Figure 2.5, Table 2.3). Across all sites, NH_4-N concentrations (Figure 2.9) in September were significantly lower than concentrations observed throughout the rest of the study period except for February ($P<0.01$). Meanwhile, across all study sites SRP concentrations (Figure 2.10) were significantly lower in October than in all other months but November ($P<0.001$). Unlike SRSi, which remained fairly stable throughout the study period (Figure 2.8), NH_4-N and SRP concentrations tended to be highly variable, experiencing large peaks and troughs throughout the study period (Figures 2.9, 2.10), and were the only variables for which analysis of variance revealed significant differences between sampling months. Several of the total and dissolved nutrient concentrations were strongly correlated with one another, and these relationships (as well as others) are summarized in Table 2.5.

Mean chlorophyll *a* concentrations were significantly ($P<0.05$) lower in Lake Nkuruba than at any other site, and were significantly higher in Lakes George, Saka and Murchison Bay than in offshore Lake Edward, Napoleon Gulf, and Lake Nkuruba (Figures 2.5, 2.11). In Lake Mburo and nearshore Lake Edward, chlorophyll *a* concentrations were significantly higher ($P<0.05$) than those observed in offshore Lake Edward (and Lake Nkuruba), but were not significantly different from those observed in Napoleon Gulf (Table 2.3).

Across all lakes (including all data; $n = 55$), significant positive relationships were observed between chlorophyll *a* and TP, PartP, PartSi, TN, PN:PP, total algal biomass, and Cyanophyta biomass (Table 2.5). Strong negative relationships were observed between chlorophyll *a* and Secchi depth, station depth, mean mixed layer irradiance, and TN:TP ratio. At the within lake level, very few of these relationships were statistically significant over the period of observation.

Station depth was negatively related to phosphorus, chlorophyll *a*, total phytoplankton biomass and positively related to Secchi depth. All of these relationships were significant at the $P<0.001$ level, and had r^2_{adj} values ranging from 0.48–0.66.

Based on particulate and total nutrient ratios, both nitrogen deficiency and phosphorus deficiency (using the deficiency indicators outlined by Guildford and Hecky 2000) were detected on several occasions in the study lakes (Figures 2.12–2.16). Particulate C:N molar ratios suggested consistent moderate nitrogen deficiency (C:N ratios from 8.3–14.6) in Lakes George and Edward (nearshore and offshore). In Murchison Bay, Napoleon Gulf and Lake Saka, no N-deficiency was ever observed based on C:N ratios, while in one sample from Lake Nkuruba, and in half of the samples from Lake Mburo, moderate nitrogen deficiency was observed (Figure 2.13). Throughout the study period, all sites usually had particulate C:P ratios (Figure 2.14) consistent with moderate P-deficiency; with ratios indicating extreme P-deficiency (PP:PC > 258) or no P-deficiency (PP:PC < 159) observed only occasionally.

Based on particulate N:P ratios (Figure 2.15), phosphorus deficiency would not be expected in Lake Edward (both nearshore and offshore). At the remainder of the study sites, PN:PP ratios indicate occasional P-deficiency, and appear to follow seasonal patterns. With the exception of Lakes Edward and George, P-deficiency appears to be rare at the beginning and the end of the sampling period, with a peak in potential P-deficiency occurring in between. Meanwhile, based on TN:TP ratios and diagnostic values from the literature (Figure 2.16), in Lake Nkuruba, the potential for phosphorus deficiency (TN:TP > 50) was observed throughout much of the study period. On several occasions TN:TP ratios in Lake George were below 20, indicating possible nitrogen deficiency. However, in most study lakes, intermediate values (between 20–50) were generally observed, indicating that either nitrogen or phosphorus (or some other factor, such as light) could be limiting.

2.3.3 Phytoplankton Community Composition

Cyanobacteria dominated the phytoplankton biomass throughout the whole study period in most lakes (Figure 2.17). The mean percentage of total biomass made up by cyanobacteria ranged from 39 % in offshore Lake Edward to 94 % in Lake George (and the overall range was from 17–99 %). All sites experienced occasions when cyanobacteria made up more than 80 % of total biomass, and all sites except nearshore Lake Edward and Napoleon Gulf experienced occasions where cyanobacterial biomass exceeded 90 % of total biomass. Diatoms were also important contributors to the phytoplankton biomass, making up between 0–78 % of total biomass. Diatoms were of particular importance in offshore Lake Edward where they made up on average 50 % of total biomass. At Lake Edward offshore, diatoms were dominant from mid-November until February, while in Murchison Bay and Lake Mburo diatoms were dominant in mid-October. In Lake Saka, diatom biomass was similar to that of cyanobacteria in both December and February. I also observed a strong positive relationship between diatom biomass and

PartSi ($n = 55$, $r^2_{adj} = 0.46$, $P<0.001$). Chlorophyta, Cryptophyta, Dinophyta and Euglenophyta were also present at several of the study sites, however, generally at low biomasses.

The primary contributors to the cyanobacterial biomass at the study sites were *Microcystis* spp., *Planktolyngbya* spp., *Anabaena* spp., and occasionally *Cylindrospermopsis* spp. (Figure 2.18). In Lake Saka, *Planktothrix* spp. was also an important component of the cyanobacteria. *Planktolyngbya* spp. was, on average, the largest contributor to the cyanobacterial biomass in Lake George (mean ± s.d.: 50 ± 31 %), Lake Mburo (28 ± 21 %), Napoleon Gulf (42 ± 29 %), Lake Nkuruba (79 ± 40 %), nearshore Lake Edward (61 ± 13 %), and offshore Lake Edward (69 ± 20 %). In Murchison Bay, *Microcystis* spp. was the most important contributor to the cyanobacteria (67 ± 14 %), and at all sites but Lake Nkuruba *Microcystis* spp. made up an appreciable portion of the total biomass. In Lake Saka, *Planktothrix* spp. was the dominant cyanobacterial taxon. *Cylindrospermopsis* spp. was an important contributor to the cyanobacteria in nearshore Lake Edward (11 ± 13 %), offshore Lake Edward (23 ± 15 %), and Lake Nkuruba (21 ± 40 %). Meanwhile, *Anabaena* spp. was of importance in Lake Mburo (22 ± 21 %), Murchison Bay (9 %), and Napoleon Gulf (42 ± 29 %).

2.3.4 Microcystin

Throughout the study period, total microcystin concentrations ranged from a low of 0.1 µg/L in Lake Nkuruba to a high of 166 µg/L in Lake Saka (Figure 2.19, Table 2.6), and analysis of variance revealed significant differences in microcystin concentrations between many of the sampling sites. Microcystin concentrations in Lake Nkuruba were significantly lower than at all other sites except for offshore Lake Edward. Meanwhile, microcystin concentrations in Lake Saka significantly exceeded those observed at all sites but Lake George and Murchison Bay. Also, in offshore Lake Edward, microcystin concentrations were significantly lower than in Lake George and Murchison Bay.

While there was no significant influence of sampling month on microcystin concentrations across all sites, concentrations exhibited fairly similar seasonal patterns within all sites with lower concentrations in September through November, then higher concentrations in December through February (Figure 2.20). However, in Lake George and nearshore Lake Edward, higher concentrations were observed beginning in November; and in Murchison Bay, microcystin concentrations were already high in September, coincident with a *Microcystis flos-aquae* bloom, but fell rapidly in October and then rose throughout the remainder of the sampling period.

At all sites, dissolved microcystin was present at low concentrations (mean concentrations ranged from 0.1 µg/L in Lake Nkuruba to 0.8 µg/L in Lake Saka). The mean proportion of the total microcystin that was cell-bound ranged from 55 % in Lake Nkuruba to 96 % in Lake Saka. On average, cell-bound microcystin accounted for more than 90 % of total microcystin at all sites except Lake Edward offshore, Napoleon Gulf and Lake Nkuruba.

Cell quotas of microcystin in *Microcystis* spp. (fg microcystin/cell *Microcystis*) exhibited a wide range over the study period (from 0.8–517 fg/cell). Cell quotas were not calculated for Lake Nkuruba as *Microcystis* spp. was not present in this lake. Mean microcystin cell quotas were below 10 fg/cell in Lake George and Murchison Bay; between 15 and 25 fg/cell in nearshore Lake Edward and Lake Mburo; and between 100 and 200 in offshore Lake Edward, Napoleon Gulf and Lake Saka (Table 2.6, Figure 2.19, 2.21). Microcystin cell quotas were significantly higher (ANOVA, $P<0.05$) in Napoleon Gulf than in Murchison Bay, Lake George and Lake Mburo; cell quotas in Lake Mburo were also significantly lower than in Lake Saka and offshore Lake Edward. Across all study lakes, microcystin cell quotas were significantly negatively related to *Microcystis* biomass ($r^2_{adj} = 0.22$, $P<0.001$; Figure 2.21).

There were several environmental variables that were significantly related to microcystin concentrations. These relationships are summarized in Table 2.7 for both among lake and within lake levels, and the correlation coefficients for these relationships are found in Table 2.5. Across all lakes there were significant positive relationships between microcystin and chlorophyll *a*, TP, PartP, PartSi, and particulate N:P ratios. Meanwhile, site depth and Secchi depth were negatively related to microcystin concentrations. At the within lake level, several variables were significant ($P<0.05$) predictors of microcystin concentrations including chlorophyll *a* concentrations, nutrient concentrations and temperature; however, no relationships were consistenly present within multiple study lakes (Table 2.7).

One of the strongest predictors for microcystin concentrations in the study lakes was *Microcystis* spp. biomass (Table 2.5, Figure 2.22). Across all lakes, this relationship was highly significant ($r^2_{adj} = 0.69$, $P<0.001$), and this relationship was also observed to be significant (at $P<0.05$) at the within site level for both offshore Lake Edward and Napoleon Gulf (Table 2.7). Furthermore, at all sites, seasonal changes in *Microcystis* biomass were generally similar to the seasonal patterns observed for microcystin concentrations (Figures 2.18 and 2.20).

The variables that were significantly related to *Microcystis* spp. biomass across all lakes are outlined in Table 2.7. Significant positive relationships were observed with chlorophyll *a* (Figure 2.22), TP, and PartP; while a significant negative relationship with *Microcystis* biomass was observed for Secchi depth

and sampling site depth. Within lakes, no single variable was consistently a predictor of *Microcystis* biomass in every lake (Table 2.7).

Across all lakes, significant negative relationships were observed between cell quota of microcystin and *Microcystis* spp. biomass, chlorophyll *a*, total phytoplankton biomass, and Cyanophyta biomass. Meanwhile significant positive relationships were observed with site depth and Secchi depth. As observed for both microcystin concentrations and *Microcystis* biomass, there were no variables that were consistent predictors of microcystin cell quota within all study lakes. However it is of interest to note that in Napoleon Gulf mean water column irradiance was positively related ($P<0.05$) to microcystin cell quota.

2.4 Discussion

2.4.1 Physical Observations

Unlike in temperate lakes, where large seasonal changes in both temperature and solar irradiance strongly affect the total biomass and taxonomic composition of phytoplankton communities, tropical lakes (especially those that are shallow) experience much more stable conditions throughout the year (Oliver and Ganf 2000). In Ugandan lakes, seasonality in conditions is largely driven by the oscillation of the Inter-Tropical Convergence Zone (ITCZ) (Asnani 1993, Stager *et al.* 2005). Annual movement of the ITCZ drives a bimodal rainfall pattern for Uganda, with increased rainfall during approximately March–May and August–November (Stager *et al.* 2005). In the deeper offshore regions of the large and deep East African lakes, there is strong seasonality in the stability of the water column, with seasonally present stable thermal stratification followed by whole water column mixing. In offshore Lake Victoria, thermal stratification is observed between September and April, with strong vertical mixing resulting in near isothermal conditions from June until August, when there is a greater influence by the Southeast Trade Winds (Talling 1965, Beadle 1981). In the deeper offshore waters, stratification can increase mean mixed-layer irradiance and can reduce the light limitation experienced by phytoplankton during periods of deeper mixing (Mugidde *et al.* 2003); however, nutrient limitation can become more prevalent during prolonged stratification as phytoplankton draw down available nutrient stores in the mixed layer. These changes in mixing can also impact phytoplankton community composition. Talling (1986) observed a general pattern of diatom dominance after mixing and cyanobacterial dominance after restratification in the offshore of Lake Victoria, however, less seasonality in phytoplankton abundance and composition was observed in the shallow more enclosed embayments of the lake (Talling 1986), where daily whole

water column mixing with diurnal stratification are common throughout the year (Silsbe 2004, Silsbe *et al.* 2006).

The sites in this study are mostly shallow, nearshore areas of large lakes and small shallow lakes, and as would be expected, full mixing was often observed at these sites, particularly during morning sampling, when an isothermal water column was often observed. There was also evidence of diel patterns in the thermal structure of the water column, given that when temperature profiles were carried out later in the day, the top layer of the water column tended to be warmer than the lower waters and surface accumulation of buoyant cyanobacteria was often observed. At some of the deeper stations sampled (Napoleon Gulf and offshore Lake Edward in particular), I would expect that complete mixing (to site depth) combined with light extinction (attributable to algal biomass and turbidity) may lead to inadequate light for net primary production on average in the mixed layer (Mugidde *et al.* 2003, Silsbe *et al.* 2006, Loiselle *et al.* 2007).

Stratification of the Napoleon Gulf station was observed on several occasions between late September and November. Meanwhile, Lake Nkuruba's small surface area (Table 2.1), protected location within a steep-walled crater, and maximum depth (38 m) are all barriers to complete mixing, and can explain the persistence of a stable thermocline throughout the period of observation (Chapman *et al.* 1998).

Secchi depth at these study sites was strongly predicted by chlorophyll *a* concentrations (n=55, r^2_{adj} = 0.56, $P<0.001$), suggesting that changes in transparency in these lakes are strongly linked to changes in phytoplankton biomass. Generally, mean mixed layer irradiance values (based on both chlorophyll *a* and Secchi depth, Table 2.4, Figure 2.4) at all sites but Lake Nkuruba were at or near the range where potential light limitation of phytoplankton growth would be expected (Hecky and Guildford 1984, Guildford *et al.* 2000). In Lake Nkuruba, low chlorophyll and increased water transparency reduced the potential for light limitation despite a relatively deep mixed layer. Meanwhile, mean irradiance values for Napoleon Gulf and Murchison Bay suggest that light limitation may be common at these sites, with thermal stratification offering occasional relief from low-light conditions in the mixed layer of Napoleon Gulf. In Lakes George, Mburo and Saka, where water columns are generally fully mixed, high phytoplankton biomass and low light penetration can occasionally lead to light limitation of phytoplankton growth; however, despite having comparable and often much higher phytoplankton biomass than Murchison Bay or Napoleon Gulf, these lakes are shallower, and as such mean water column irradiances are generally higher than at the Lake Victoria sites.

The high variability observed for mean mixed layer irradiance in Lake Edward (both nearshore and offshore, Figure 2.4) was likely a reflection of the high seasonal variability in chlorophyll *a* concentrations (and therefore light attenuation) in this dynamic environment (Figure 2.11).

2.4.2 Nutrient and Chlorophyll Concentrations

All of these lakes are P rich relative to most temperate lake systems (Guildford and Hecky 2000), and very high TP concentrations were observed in Lake George, Lake Mburo, nearshore Lake Edward, Murchison Bay and Lake Saka, the shallowest study sites. In Murchison Bay, municipal and industrial sources contribute to the high P concentrations (LVEMP 2002; 85% of TP in Murchison Bay from these sources, 76 % of TN from these sources). Meanwhile, Napoleon Gulf has similar TP concentrations to open Lake Victoria (Hecky *et al.* 2010). The anthropogenic input of nutrients and consequent eutrophication of Lake Victoria has been well documented (Hecky 1993, Verschuren *et al.* 2002, Hecky *et al.* 2010). Although Napoleon Gulf, which is flushed by the Nile River outflow, has suffered this nutrient enrichment, it is not artificially affected by local anthropogenic wastes to the same extent as the semi-confined and poorly flushed Murchison Bay.

Shallow Lake Saka's catchment has been highly impacted by deforestation and agriculture (Crisman *et al.* 2001, Campbell *et al.* 2006). On the other hand, Lake Mburo, located within a national park, is naturally eutrophic, and the faeces of the sizeable hippopotamus population acts to increase soluble reactive phosphorus in the lake (Mbabazi *et al.* 2004). There is limited human activity in the vicinity of Lake George; and with no large anthropogenic inputs of nutrients present, it is likely that it is also a naturally eutrophic lake. Furthermore, data from over forty years ago also indicate a condition of high total phosphorus and chlorophyll *a* concentrations in Lake George (Ganf 1972, Ganf 1974). For both of the study stations in Lake Edward, much of the nutrient input is likely from the inflow of water from Lake George via the Kazinga Channel, with the offshore station being affected by mixing with the much deeper offshore waters of this large lake.

In Lake Victoria, TN concentrations are largely controlled by two processes: denitrification and atmospheric nitrogen fixation (Mugidde *et al.* 2003). Nitrogen fixation accounts for up to 80% of external nitrogen inputs to Lake Victoria (Mugidde *et al*. 2003). The rate of nitrogen fixation in this lake is largely influenced by the light conditions in the water column due to the high light requirements of this process; nitrogen fixation is therefore greater in the shallow regions of the lake where light is available throughout the mixed layer (Mugidde *et al.* 2003; Hecky *et al.* 2010). However, high phytoplankton biomass can lead to self-shading throughout the whole lake, thus limiting N-fixation (Mugidde *et al.* 2003). Nitrogen

fixation has also been shown to contribute more than half of the total nitrogen input in Lake George (Horne and Viner 1971), suggesting that the importance of nitrogen fixation to nitrogen dynamics is not unique to Lake Victoria, it also is likely that light plays an important role in limiting nitrogen fixation at all of the study sites. Phosphorus concentrations were very high in all of these lakes (with the exception of Nkuruba) and nitrogen likely limits algal biomass. Consequently in most of these lakes the upper bound on biomass should be set by nitrogen fixation by cyanobacteria which will in turn be determined by light availability.

The lower total phosphorus concentrations observed in Lake Nkuruba can be attributable to three factors: limited human impact on the lake (it is surrounded by intact rainforest; Chapman *et al.* 1998); a small catchment area: lake area ratio which limits nutrient export to the lake from the catchment; and permanent thermal stratification of the lake (Chapman et al 1998), which strongly reduces recycling of nutrients from the hypolimnion into the mixed layer. Although dissolved nutrients concentrations were very high in the hypolimnion (Poste *unpublished data*), there were no apparent substantial incursions of this nutrient rich water into the epilimnion during the study period.

Analysis of sediment cores from Lake Victoria have shown that in the offshore, initially, increased nutrient loading to Lake Victoria resulted in an increase in diatom biomass in the early 1950's (Verschuren *et al.* 1998). However, between 1960 and 1990, Si concentrations appear to have decreased drastically in the offshore due to increased Si demands and high rates of Si burial (Verschuren *et al.* 1998). These paleolimnolgical results are consistent with the drastic declines observed in water column Si in both the offshore and nearshore of the lake (Hecky *et al.* 2010), and with the low Si concentrations observed in Napoleon Gulf and Murchison Bay in the current study. Si limitation is thought to have played a role in the shift from dominance of the phytoplankton community by large diatoms (Talling 1965) to cyanobacteria (Verschuren *et al*., 1998, Kling *et al*. 2001, Hecky *et al.* 2010); however, it is important to note that cyanobacteria have always been an important component of the nearshore phytoplankton (Talling 1965). In the remainder of the study lakes, Si concentrations were several-fold higher than at the Lake Victoria sites, however, these differences did not appreciably increase the importance of diatoms in the phytoplankton communities of these lakes, with the exception of offshore Lake Edward, which will be discussed later.

The high degree of seasonal variability in nutrient concentrations and the lack of consistent seasonal patterns for these concentrations across all lakes can be explained by the importance of localized conditions at the study sites. Rainfall and mixing events can lead to large influxes of nutrients, and given

that most of the study sites are quite shallow, rapid episodic recycling of nutrients from the sediments is to be expected. Additionally, the large standing crop of phytoplankton observed in most of these lakes is capable of rapidly drawing down dissolved nutrient concentrations and redistributing nutrients between dissolved and particulate phases. The particularly high variability in nutrient concentrations at the two Lake Edward sites is likely a reflection of the dynamic nature of this region of the lake, with strong changes in the relative influence of water from Lake George (via the Kazinga Channel) and the much deeper offshore waters of the lake.

Chlorophyll *a* concentrations exhibited high variability over the study period, and several sites experienced large fluctuations in chlorophyll *a* concentrations that generally coincided with large changes in cyanobacterial biomass, likely in response to mixing events and nutrient inputs, especially by nitrogen fixation (Figures 2.11 and 2.17).

Using the trophic status classification system outlined in Vollenweider and Kerekes (1982), based on Secchi depth, total phosphorus and chlorophyll *a,* Lake Nkuruba can be classified as mesotrophic, Lake Edward offshore and Napoleon Gulf can be classified as eutrophic, and the remainder of the sites can be classified as hypereutrophic. It is important to note that the shallowest sites are hypereutrophic, and the deepest sites are eutrophic and mesotrophic. This trend largely reflects the fact that the shallowest sites experience regular whole water column mixing with attendant rapid nutrient recycling from the sediments. Also, these shallow sites allow for a higher standing crop of phytoplankton to develop before limited by self-shading (Silsbe *et al.* 2006). Based on particulate and total nutrient ratios, there was no evidence for consistent strong nitrogen or phosphorus limitation at most sites. The lack of conclusive evidence for strong nutrient limitation, combined with the detectable (and often high) dissolved nutrient concentrations in these lakes suggests that light may be the primary limiting factor for phytoplankton growth in most of these systems. However, it is important to note that moderate phosphorus deficiency was often observed at all study sites, while moderate nitrogen deficiency was uncommon at all sites except Lakes Edward and George. This likely reflects the fact that nitrogen fixation is meeting any deficiency in inorganic N at most sites, and suggest that phosphorus is more likely than nitrogen to eventually limit phytoplankton biomass, even in these phosphorus-rich systems. The role played by phosphorus in limiting primary production is also emphasized in Lake Nkuruba, where phytoplankton biomass and phosphorus concentrations were significantly lower than at other study sites, but nitrogen concentrations did not differ significantly from those observed at other sites. Furthermore, estimated

mean mixed layer light intensities in Lake Nkuruba did not indicate a high likelihood of strong light limitation of phytoplankton, suggesting that phosphorus was likely limiting phytoplankton growth.

2.4.3 Phytoplankton Community Composition

The cyanobacterial dominance observed in these study lakes is consistent with previous reports from these lakes (Ganf 1974, Verschuren *et al.* 1998, Kling *et al.* 2001, Okello *et al.* 2009, Hecky *et al.* 2010). Even in offshore Lake Edward, where diatoms were often dominant (> 50 % of total biomass), cyanobacteria made up a large portion of the phytoplankton biomass. The proportion of the total biomass that consisted of cyanobacteria tended to be highest at the hypereutrophic stations and lower at the meso/eutrophic stations (offshore Lake Edward and Napoleon Gulf), with the exception of Lake Nkuruba where cyanobacteria made up nearly all of the total biomass. Past studies found that the phytoplankton of Lake Nkuruba was dominated by small cyanobacteria and chlorophytes (Chapman *et al.* 1998); however, in the current study, chlorophytes were not found to be important contributors to the total phytoplankton biomass (Figure 2.17). The relationship between cyanobacterial dominance and lake trophic status is widely documented, as is the potential for year-round success of cyanobacteria in tropical systems (Ganf 1974).

Despite relatively high SRSi concentrations in the study lakes (with the exception of the Lake Victoria sites), only in Lake Edward did diatoms consistently make up a substantial proportion of the total phytoplankton biomass. In Lake Sakas, George, Mburo and nearshore Lake Edward, Si concentrations and mixing are likely amenable to diatom growth, however, high biomasses of buoyancy regulating cyanobacteria that are able to monopolize light are likely to generally outcompete diatoms at these sites. Meanwhile, in Lake Nkuruba, the high stability of the water column would not be favourable for diatoms, which require turbulent resuspension to stay within the euphotic zone. In Lake Edward, the prevalence of diatoms can be explained by the combination of regular mixing, relatively high transparency (especially at the offshore site) and high soluble reactive Si concentrations.

Cyanobacteria have many features that allow them to successfully dominate phytoplankton communities. Some taxa are capable of fixing atmospheric nitrogen, allowing them to thrive even when inorganic nitrogen concentrations in the water column may be low; meanwhile, other taxa are capable of buoyancy control, whereby they can regulate their position in the water column (Walsby *et al.* 1997, Mur *et al.* 1999). Buoyancy control is of particular importance for cyanobacteria in relatively shallow lakes where they can rapidly ascend to upper waters where light is adequate for growth (Mur *et al.* 1999).

Cyanobacteria are also known to be effective competitors for both nitrogen and phosphorus (Mur *et al.* 1999).

The filamentous cyanobacteria *Planktolyngbya* spp. dominated the cyanobacterial biomass in most of the study lakes. *Planktolyngbya* spp. is known to be tolerant of low light levels similar to those observed in most of the study lakes (Reynolds 2006). Despite high light requirements, *Microcystis* spp. often co-occurs with *Planktolyngbya* spp. due to the ability of this taxon to regulate buoyancy and rise to the upper levels of the water column where light is not limiting (Reynolds 2006). Several cyanobacterial taxa known to be capable of microcystin production were important contributors to the phytoplankton in these lakes, including *Microcystis* spp., *Planktothrix* spp., *Anabaena* spp. and *Cylindrospermopsis* spp.. However, the relative importance of these taxa differed between lakes.

2.4.4 Microcystin Concentrations

Microcystin concentrations consistently exceeded the World Health Organization guideline for microcystin in drinking water of 1.0 µg/L at all study sites except offshore Lake Edward, where microcystin concentrations only occasionally exceeded 1.0 µg/L, and Lake Nkuruba where concentrations were always much lower than the WHO guideline. The highest microcystin concentrations were observed at the hypereutrophic sites, with intermediate concentrations observed at the eutrophic sites, and the lowest concentrations observed in mesotrophic Lake Nkuruba.

The seasonal, spatial and inter-annual variability inherent in microcystin concentrations in water in these study lakes is demonstrated by the differences between the concentrations observed in this study and concentrations observed in previous studies. For example, Sekadende *et al.* (2005) collected water samples between May–August 2002 in Mwanza Gulf (Lake Victoria, Tanzania) and observed a range of 0–1 µg/L of microcystin, much lower than my observations in Napoleon Gulf and Murchison Bay. From 2007–2008, Okello *et al.* (2010) sampled several of the Ugandan lakes that are included in the current study. When the September to February time period is considered (to allow for direct comparison with my results), the microcystin concentrations reported by Okello *et al.* (2010) are generally lower than my observed concentrations. In particular, microcystin concentrations in Lake George were much lower in the Okello *et al.* (2010) study than in the current study. It is important to note that the sampling locations for the Lake George and Lake Edward study sites differed between the two studies, with Okello *et al.* (2010) collecting samples in a mostly-enclosed turbid embayment in Lake George (Hamukunga Bay; Okello *personal communication*), while I collected samples from the open lake. *Microcystis* biomass and toxin production may have been limited by light availability in the turbid embayment sampled by Okello

et al. (2010), leading to lower microcystin concentrations. In Lake Edward, I collected samples from near the mouth of the Kazinga channel, and then further offshore; while Okello *et al.* (2010) collected samples in a shallow turbid area in the north of the lake (Okello *personal communication*). Both the influence of the high productivity Kazinga channel and possibly lower turbidity at my chosen study site may explain the elevated microcystin concentrations observed in the current study. At the remainder of the study sites, both *Microcystis* cell numbers and biovolume were also lower in the Okello *et al.* study, pointing to the possibility that this is a result of inter-annual variability, and requiring further exploration of differences in physical and chemical variables between years.

My observed microcystin concentrations in inner Murchison Bay also exceeded those reported by Haande *et al.* (2008) where mean microcystin concentrations (in 2003–2004) were found to be 1.1 µg/L with a maximum observed concentration of 3.0 µg/L (much lower than my mean value for the study period of 7.26 µg/L). Some variability between study results may also be attributable to methodological differences in microcystin determination. In the current study, I measured microcystin using anti-Adda ELISA, while Okello *et al.* (2009) used high performance liquid chromatography-diode array detection, and Haande *et al.* (2008) reported using Abraxis anti-Adda ELISA test kits, as in the current study. Results from anti-Adda ELISA, which recognizes nearly all microcystin congeners with comparable sensitivity (Fischer *et al.* 2001, Ernst *et al.* 2009), may exceed those observed using HPLC if standards are not available for congeners that may be present (and detectable using anti-Adda ELISA). Although higher precision is achievable for specific congeners through HPLC; the low-cost, high throughput, sensitivity and reproducibility of anti-ADDA ELISA makes this method particularly attractive for extensive studies (particularly for long-term studies in locations where specialized equipment may not be available).

2.4.5 Drivers for microcystin concentration and production

There is general acceptance that the global increase in cultural eutrophication has also increased the occurrence of microcystin producing blooms, especially blooms of *Microcystis aeruginosa* (de Figueiredo *et al.* 2004). *Microcystis* biomass, chlorophyll *a*, total phosphorus and Secchi depth (all of which are strongly related to *Microcystis* biomass) were the strongest predictors of microcystin concentrations in these lakes (Figure 2.22). Field studies have shown associations between microcystin and TP, SRP, TN, N:P, chlorophyll *a*, light, and dissolved oxygen (Kotak *et al.* 2000, Kardinaal and Visser 2005, Billam *et al.* 2006); however, these relationships are highly variable, and the directionality of these relationships can differ between studies. For example, although several studies have found positive relationships

between TP and microcystin (Kotak *et al.* 2000, Giani *et al.* 2005; as well as the current study), these variables have also found to be negatively related (Oh *et al.* 2000) and even not related (Sivonen 1990).

Based on relationships between microcystin concentrations and biomass of cyanobacterial taxa known to be capable of toxin production, *Microcystis* spp., *Anabaena* spp. and *Planktothrix* spp. emerged as the most likely microcystin producers in these Ugandan lakes. However, given that *Anabaena* spp. was significantly positively related to *Microcystis* spp. ($r^2_{adj} = 0.35$, n=53, $P<0.001$), and the fact that a relationship between *Anabaena* spp. and microcystin was not observed within any sites, it is likely that the relationship between microcystin and *Anabaena* biomass was likely an artefact of the relationship between these two cyanobacterial taxa. The co-occurrence of *Microcystis* and *Anabaena* has been widely documented, and is likely attributable to nitrogen availability whereby the establishment of nitrogen-fixing cyanobacteria allows the non N-fixing *Microcystis* to use fixed nitrogen made available by nitrogen fixers, such as *Anabaena* (Paerl and Fulton 2006). The positive relationship between microcystin and *Planktothrix* spp. was likely because *Planktothrix* spp. was an important component of the cyanobacterial biomass only in Lake Saka, where microcystin concentrations were significantly higher than in other lakes, furthermore, there was no significant relationship between microcystin and *Planktothrix* biomass within Lake Saka. As such, *Microcystsis* spp. appears to have been the most important producer of microcystin in these lakes. This is consistent with previous observations of regular occurrence of the *mcy*B genotype responsible for microcystin production in *Microcystis* populations from several of the study lakes (Okello *et al.* 2010). In Lake Nkuruba, microcystin was detectable (albeit at very low levels) despite the absence of *Microcystis* spp. in the lake, suggesting that some other cyanobacterial genera may have been responsible for microcystin production. The strongest predictors for *Microcystis* spp. biomass overlapped a great deal with the predictors for microcystin, suggesting that rather than controlling microcystin concentrations directly, these variables may be indirectly controlling microcystin concentrations through influencing *Microcystis* spp. biomass.

The cell-specific microcystin concentrations (referred to as cell quotas; fg microcystin/cell *Microcystis*) observed at all study sites fell within the range of values reported in the global literature (e.g. Vasconcelos and Pereira 2001, Millie *et al*. 2009), and were similar to those reported for similar study sites by Okello *et al.* (2010), with the highest cell quotas observed in Napoleon Gulf and Lake Saka, and the lowest cell quotas observed in Lake George. However, in the current study, cell quotas tended to be higher than those observed by Okello *et al.* (2010), particularly in Lakes Edward, Lake George and Napoleon Gulf. In the case of Lakes Edward and George, this may be due to differences in sampling locations (as previously

described); meanwhile, given the high degree of temporal variability observed in microcystin cell quota in Napoleon Gulf in both studies, the difference in cell quota between these two studies may be a reflection of this variability. Okello *et al.* (2010) also found that cell quota of microcystin was strongly related to the proportion of the *mcy*B genotype in the *Microcystis* population, with higher prevalence of the *mcy*B genotype leading to higher cell specific microcystin concentrations; they also found that the proportion of *mcy*B genotype present in the *Microcystis* population differed significantly between sites. Given the strength of this relationship it is likely that much of the difference in cell quota between sites may be attributable to differences in the genotypic composition of *Microcystis* (and the resulting capacity for microcystin production). This is consistent with previous observations in the literature showing that cell quota of microcystin can vary by several orders of magnitude due to shifts in the *Microcystis* genotypic community toward more toxic strains (Zurawell *et al.* 2005). However, at the within site level there are likely to be other factors that act to moderate cell quota of microcystin through influencing the degree of microcystin production, including factors that are likely to regulate *Microcystis* growth such as light and nutrient concentrations, given the evidence that higher cell quotas tend to be observed at higher *Microcystis* growth rates (Orr and Jones 1998, Sivonen and Jones 1999, Deblois and Juneau 2010; but see Millie *et al.* 2009).

The high variability in microcystin cell quotas observed within both Napoleon Gulf and Lake Edward nearshore may reflect rapidly changing chemical and physical (especially mixing and light) conditions in these dynamic environments where exchange with open lake water can occur in addition to inputs of nutrient and chlorophyll-rich water from sheltered embayments in the case of Napoleon Gulf, and the Kazinga channel in the case of Lake Edward. Also, at both of these sites, there is some evidence that light may be an important factor in determining cell quota of microcystin. In Napoleon Gulf, mean water column irradiance has a significant positive relationship with cell quota; while in Lake Edward, Secchi depth has a significant positive relationship with cell quota. Additionally, in Napoleon Gulf, microcystin cell quota and mean water column light intensity share a similar seasonal trend of relatively high values in Sep–Oct (when stratification and relatively low phytoplankton biomass allow for high light conditions in the mixed layer) and declining values throughout the reminder of the study period.

The negative relationships observed (both among and occasionally within sites) between cell quota and *Microcystis* biomass (as well several other correlated variables including total biomass, Cyanophyta biomass, chlorophyll *a*, and Secchi depth) suggest that when *Microcystis* biomass is lower, microcystin production tends to be higher. These results are consistent with previous reports in the literature of

negative relationships between cell quota and *Microcystis* spp. biomass (reviewed in Kardinaal and Visser 2005). These results may also reflect the importance of light in determining microcystin cell quota since in many of these lakes the phytoplankton biomass is dominated by *Microcystis* spp., and where biomass is low, reduced light attenuation by phytoplankton and higher light availability would be expected, allowing for more rapid growth of *Microcystis*. Since microcystin production is thought to be highest where conditions are most favourable for growth (Orr and Jones 1999, Briand 2005), adequate light conditions would be expected to increase microcystin production. Although several studies have reported generally negative relationships between photon irradiance and microcystin cell quota (Utkilen and Gjolme 1992, Wiedner *et al.* 2003, Deblois and Juneau 2010), this relationship is largely observed at high photon irradiance, where cellular production of microcystin may be inhibited, and both Wiedner *et al.* (2003) and Deblois and Juneau (2010) observe an increase in cell quota of microcystin with increasing light up to the point where maximum growth rate is achieved, with a negative relationship between light and cell quota after this point. In the current study, there was evidence that light limitation of phytoplankton growth was common, suggesting the potential for increased cell quota of microcystin with increasing irradiance in these low transparency systems. Also, the lack of significant relationships between nutrient concentrations and microcystin cell quotas may further support the importance of light availability in determining cellular production of microcystin.

Based on the results of this study, I propose a general explanatory framework for microcystin production in these lakes whereby: 1) at the shallow study sites, regular recycling of nutrients from the sediments during mixing (as well as anthropogenic inputs of nutrients in the cases of Lake Saka and Murchison Bay) lead to high nutrient concentrations; 2) high nutrient concentrations lead to high phytoplankton (and particularly cyanobacterial) biomass; 3) phytoplankton growth (and biomass) is limited due to self-shading, with shallower sites able to support higher phytoplankton biomass because of higher mean mixed layer irradiance than deeper sites with comparable phytoplankton biomass; 4) low light conditions favour *Microcystis* spp. which is able to regulate its position in the water column through buoyancy control; 5) microcystin concentrations are determined by *Microcystis* biomass in addition to cell quota of microcystin (which is in turn determined by the genotypic composition of *Microcystis* as well as *Microcystis* growth rate, which is likely to be determined by light availability in these systems). These processes give us insight into why microcystin concentrations (and *Microcystis* biomass) are particularly high at the shallowest study sites (Lake Edward nearshore, Lake George, Lake Mburo, Murchison Bay, Lake Saka). In Lake Saka in particular, the combination of conditions favourable for high *Microcystis* biomass as well as the prevalence of toxigenic genotypes of *Microcystis* in this lake (Okello

et al. 2010) can explain the extremely high microcystin concentrations observed in this lake, where concentrations were occasionally more than 100-fold higher than the WHO recommended guideline for microcystin in drinking water.

2.4.6 Health and Management Implications

The microcystin concentrations observed in this study consistently (and often substantially) exceeded the WHO guideline for microcystin in drinking water in all lakes but Lake Nkuruba (Figure 2.19). These concentrations suggest the potential for detrimental health effects for the human and animal populations that rely on these water bodies. Although Okello *et al.* (2010) suggest that because much of the microcystin present in these study lakes belongs to the MC-RR congener, known to be less toxic than MC-LR (which is a common microcystin congener in temperate lakes), water from these lakes pose less of a risk to human consumers or livestock than in European lakes. However, the concentrations regularly encountered at several of the study sites greatly exceed the WHO recommended guideline for drinking water, confirming that even if the microcystin congeners present in these lakes are not the most toxic congeners, at very high concentrations (such as those observed in the current study) they can still pose a substantial risk to consumers. Additionally, Okello *et al.* (2010) found several previously uncharacterized microcystin congeners in their samples, for which toxicity remains unknown.

In Uganda, many households in riparian communities collect raw lake water and boil it prior to consumption. Given that microcystin is a heat stable compound (Harada 1996), boiling water does not reduce the risk of exposure to microcystin. However, throughout the whole study period, most of the microcystin measured was cell-bound, and as such, filtration through a cloth to remove the colonial cyanobacteria (notably *Microcystis* spp.) would likely be a simple and effective way of reducing the risk of exposure for consumers, given that cloth filtration has been shown to be effective at removing pathogenic bacteria (notably cholera) and phytoplankton from water (Colwell *et al.* 2003).

Given the prevalence of *Anabaena* spp., *Planktothrix* spp., and *Cylindrospermopsis* spp. in these lakes, it is highly plausible that other cyanotoxins, including anatoxin and cylindrospermopsin are being produced in these systems, and could, in addition to acting independently, have interactive effects with microcystin (Codd *et al.* 2005). These results highlight the importance of monitoring cyanotoxin concentrations in lake water, of assessing the efficacy of microcystin removal by municipal water treatment systems, and of educating the public about the risks of cyanotoxins in drinking water while offering practical solutions for reducing the risk of exposure.

Table 2.1 General morphological characteristics of the study lakes and sampling sites. Much of this information is reproduced from Table 1.1 (Chapter 1) of this thesis.

Lake	Code	Max. Lake Depth (m)	Mean Lake Depth (m)	Site Depth (m)	Area (km^2)	Volume (km^3)
Lake Edward	~	120	33	~	2325	76.7
Nearshore	EdN	~	~	3.5	~	~
Offshore	EdO	~	~	7.3	~	~
Lake George	G	7	2.4	2.8	250	0.5
Lake Mburo	Mb	4	2	3.2	13	0.325
Lake Victoria	~	75	39	~	66368	2598
Inner Murchison Bay	Mu	7	3.2	5.2	18	0.113
Napoleon Gulf	Na	20.5	7.9	17.5	26.5	0.22
Lake Nkuruba	Nk	38	16	33.4	0.03	0.000481
Lake Saka	S	8.5	3.6	3.2	0.15	0.000054

Table 2.2 Summary of physicochemical observations for the study lakes. Results are reported in the format of mean ± standard deviation, and units are indicated. Abbreviations used include SD (Secchi Depth), Chl *a* (chlorophyll *a*), TP (total phosphorus), TN (total nitrogen), NH_4-N (ammonium nitrogen), SRP (soluble reactive phosphorus), SRSi (soluble reactive Si), PartSi (particulate Si), PartP (particulate phosphorus), and PC, PN and PP (particulate carbon, nitrogen and phosphorus, these abbreviations are used for particulate nutrient ratios).

	SD (m)	Chl *a* (µg/L)	TP (µg/L)	TN (µg/L)	NH_4-N (µg/L)	SRP (µg/L)	SRSi (µg/L)	PartSi (µg/L)	PartP (µg/L)	PC:PN (molar)	PC:PP (molar)	PN:PP (molar)	TN:TP (molar)
Edward Nearshore	0.50 ± 0.25	66.3 ± 46.2	129.1 ± 54.7	1707 ± 643	5.4 ± 3.8	10.3 ± 6.1	7437 ± 1262	12.5 ± 6.5	201.6 ± 116.0	9.6 ± 1.0	152.5 ± 34.9	16.2 ± 4.5	33.2 ± 15.8
Edward Offshore	1.05 ± 0.27	21.3 ± 22.8	58.9 ± 9.2	1013 ± 276	4.8 ± 4.1	10.6 ± 5.2	6040 ± 1232	12.2 ± 3.8	128.1 ± 149.0	11.0 ± 1.1	172.4 ± 15.5	15.9 ± 2.4	37.7 ± 6.6
George	0.37 ± 0.08	138.0 ± 39.1	186.5 ± 26.2	1462 ± 1008	5.9 ± 4.0	9.9 ± 4.9	7969 ± 1616	15.0 ± 8.3	287.3 ± 90.8	9.3 ± 1.2	232.2 ± 54.3	24.8 ± 4.5	16.1 ± 10.3
Mburo	0.48 ± 0.10	48.6 ± 10.1	106.8 ± 11.1	1934 ± 964	6.9 ± 4.0	10.5 ± 3.0	6861 ± 426	9.5 ± 1.5	149.9 ± 34.0	8.2 ± 0.7	172.5 ± 42.5	21.1 ± 5.0	42.0 ± 25.1
Murchison Bay	0.72 ± 0.14	96.5 ± 38.1	100.3 ± 22.5	2108 ± 742	29.3 ± 30.4	7.2 ± 5.2	805 ± 313	12.3 ± 5.1	104.0 ± 33.0	6.5 ± 0.2	175.2 ± 51.4	27.3 ± 7.9	50.2 ± 22.7
Napoleon Gulf	1.40 ± 0.23	24.7 ± 18.4	60.0 ± 16.2	1644 ± 1022	23.8 ± 41.6	11.5 ± 11.0	757 ± 296	10.4 ± 8.3	50.7 ± 8.2	6.5 ± 0.6	136.2 ± 24.3	21.2 ± 5.1	62.2 ± 41.3
Nkuruba	1.80 ± 0.39	6.2 ± 2.2	35.6 ± 8.6	1323 ± 750	6.4 ± 6.5	8.2 ± 3.7	5510 ± 1087	1.1 ± 0.5	24.0 ± 3.4	7.7 ± 0.8	179.5 ± 55.7	23.1 ± 5.9	81.6 ± 39.7
Saka	0.44 ± 0.11	90.0 ± 36.3	175.0 ± 32.2	2440 ± 1675	8.5 ± 5.5	24.7 ± 14.7	9046 ± 2091	11.2 ± 5.1	161.0 ± 45.3	7.5 ± 0.8	186.0 ± 50.1	25.3 ± 9.4	32.3 ± 22.4

Table 2.3 Statistical comparisons between sampling sites for physicochemical variables. Rows show the lakes for which values for the given variable are significantly lower (ANOVA, P<0.05) than for the lake indicated at the top of the column. Codes found in Table 2.1.

	EdO	EdN	G	Mb	Mu	Na	Nk	S
Temperature	Nk, S	Nk, S	Nk, S	S	Nk, S	Nk, S	~	~
Secchi Depth	EdN, G, Mb, S	~	~	~	EdN, G, S	EdN, G, Mb, Mu, S	EdN, EdO, G, Mb, Mu, S	~
Mean Water Column Irradiance	Na	Na, Mu	~	Mu, Na	~	~	Mu, Na	~
TP	~	EdO, Na, Nk	EdN, EdO, Mb, Mu, Na, Nk	EdO, Nk	EdO, Nk	Nk	~	EdO, Mb, Mu, Na, Nk
TN	~	~	~	~	~	~	~	~
SRSi	Mu, Na	Mu, Na	Mu, Na	Mu, Na	~	~	Mu, Na	Mu, Na
NH_4-N	~	~	~	~	~	~	~	~
SRP	~	~	~	~	~	~	~	~
Chlorophyll *a*	~	~	EdN, EdO, Mb, Na, Nk	EdO, Nk	EdO, Na, Nk	~	~	Na, Nk
PC:PN	Mb, Mu, Na, Nk, S	Mu, Na, S	Mu, Na	Mu	~	~	~	~
PC:PP	~	~	~	~	~	~	~	~
PN:PP	~	~	~	~	~	~	~	~
TN:TP	~	~	~	~	~	~	~	~
Total Biomass	Nk	Nk	EdN, Na, Nk	Nk	Na, Nk	~	~	Na, Nk
Cyanophyta Biomass	~	~	EdN, EdO, Na, Nk	Nk	Nk	~	~	EdN, EdO, Na, Nk
Microcystis Biomass	Nk	Nk	Nk	Nk	Nk	Nk	Nk	Nk
MC	~	Nk	EdO, Nk	Nk	EdO, Nk	~	~	EdO, Mb, Na, Nk
MC Cell Quota	~	~	~	~	~	Mb	~	~

Table 2.4 Mean mixed layer irradiance for all study lakes. Light attenuation (k_{PAR}) was estimated based on chlorophyll *a* concentrations (Silsbe *et al.* 2006), and mean mixed layer irradiance was calculated with an assumed incident surface irradiance of 50 000 mmol photons/m^2/day (Guildford *et al.* 2000). Abbreviations used: z_{mix} = mixing depth, SD = Secchi depth, mean % I_s = mean proportion of irradiance incident on the lake's surface that is present in the mixed layer (as % of surface irradiance), and $\bar{I}_{mixed}$ = mean mixed layer irradiance (in mmol photons/m^2/min). Values reported are in the form of mean ± standard deviation.

Lake	Mean z_{mix} (m)	SD (m)	Chl *a* (µg/L)	k_{PAR} (m^{-1})	Mean % I_x	$\bar{I}_{mixed}$
Edward Nearshore	3.5	0.50 ± 0.25	66.3 ± 46.2	1.7 ± 0.7	22.8 ± 11.1	7.9 ± 3.9
Edward Offshore	7.3	1.05 ± 0.27	21.3 ± 22.8	0.9 ± 0.5	19.8 ± 11.6	6.9 ± 4.0
George	2.8	0.37 ± 0.08	138.0 ± 39.1	2.6 ± 0.4	15.8 ± 8.1	5.5 ± 2.8
Mburo	3.2	0.48 ± 0.10	48.6 ± 10.1	1.5 ± 0.2	20.8 ± 2.2	7.2 ± 0.8
Murchison Bay	5.2	0.72 ± 0.14	96.5 ± 38.1	2.0 ± 0.6	10.0 ± 3.5	3.5 ± 1.2
Napoleon Gulf	13.5	1.40 ± 0.23	24.7 ± 18.4	1.0 ± 0.4	9.8 ± 6.3	3.4 ± 2.2
Nkuruba	8.2	1.80 ± 0.39	6.2 ± 2.2	0.5 ± 0.1	24.8 ± 7.2	8.6 ± 2.5
Saka	3.2	0.44 ± 0.11	90.0 ± 36.3	2.0 ± 0.5	16.0 ± 2.8	5.6 ± 1.0

Table 2.5 Summary of correlations between physical, chemical and biological variables across all sites (using all data, n=55). Values reported are correlation coefficients, and those in bold are statistically significant at the P<0.05 level, while those with asterisks are significant at the P<0.01 level. Most of the abbreviations for the variables found in this table are defined in the text, with the exception of: 1/SD (inverse Secchi Depth), light (mean mixed layer irradiance, based on chlorophyll *a* concentrations), T. B. (total phytoplankton biomass), C. B. (Cyanophyta biomass), and M. B. (*Microcystis* spp. biomass).

	Temp	1/SD	Light	TP	TN	SRP	NH_4	SRSi	PartSi	PartP	Chl *a*	PC:PN	PC:PP	PN:PP	TN:TP	T. B.	C. B.	M. B.	MC
1/SD	-0.03	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
Light	-0.16	0.12	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
TP	-0.02	**0.89***	-0.12	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
TN	0.01	**0.32**	-0.03	**0.27**	~	~	~	~	~	~	~	~	~	~	~	~	~	~	~
SRP	-0.18	0.13	0.07	0.21	**0.34**	~	~	~	~	~	~	~	~	~	~	~	~	~	~
NH4-N	-0.08	-0.13	-0.27	0.09	-0.01	**-0.43***	~	~	~	~	~	~	~	~	~	~	~	~	~
SRSi	-0.26	**0.47***	**0.64***	**0.29**	0.11	0.22	**-0.29**	~	~	~	~	~	~	~	~	~	~	~	~
PartSi	**0.40***	**0.52***	-0.24	**0.61***	0.15	0.15	0.04	-0.15	~	~	~	~	~	~	~	~	~	~	~
PartP	0.16	**0.88***	-0.11	**0.84***	**0.28**	0.10	0.03	**0.36**	**0.65***	~	~	~	~	~	~	~	~	~	~
Chl *a*	0.07	**0.75***	**-0.43***	**0.82***	**0.35***	0.04	0.23	0.05	**0.56***	**0.82***	~	~	~	~	~	~	~	~	~
PC:PN	**0.28**	**0.28**	**0.62***	0.08	-0.03	0.13	**-0.52***	**0.62***	0.19	0.25	-0.19	~	~	~	~	~	~	~	~
PC:PP	-0.01	**0.36***	0.04	0.24	0.14	0.14	-0.26	**0.33**	0.07	0.18	**0.32**	0.21	~	~	~	~	~	~	~
PN:PP	-0.20	0.16	**-0.37***	0.17	0.16	0.04	0.10	-0.07	-0.09	-0.00	**0.42***	**-0.47***	**0.76***	~	~	~	~	~	~
TN:TP	0.03	**-0.37***	0.06	**-0.50***	**0.70***	0.14	-0.03	-0.12	**-0.32**	**-0.37***	**-0.29**	-0.09	-0.05	0.02	~	~	~	~	~
T. B.	0.01	**0.67***	-0.21	**0.73***	0.17	0.17	-0.03	0.09	**0.65***	**0.67***	**0.74***	-0.02	**0.33**	**0.32**	**-0.39***	~	~	~	~
C. B.	-0.09	**0.60***	**-0.29**	**0.67***	0.16	0.13	0.03	0.06	**0.44***	**0.60***	**0.74***	-0.21	**0.35**	**0.46***	**-0.36**	**0.93***	~	~	~
M. B.	-0.21	**0.40***	-0.23	0.53*	0.12	0.05	0.20	0.01	**0**.04	**0.44***	**0.72***	-0.27	0.27	**0.45***	-0.23	**0.61***	**0.62***	~	~
MC	-0.17	**0.60***	**-0.37***	**0.74***	**0.32**	0.25	0.22	0.03	**0.50***	**0.64***	**0.80***	-0.26	0.27	**0.42***	-0.26	**0.67***	**0.67***	**0.70***	~
Cell quota	-0.17	-0.37	-0.03	-0.28	-0.11	0.14	-0.07	-0.14	-0.06	**-0.35**	**-0.41***	0.02	-0.18	-0.22	0.08	**-0.37**	**-0.39***	**-0.49***	0.02

Table 2.6 Summary of mean (± s.d.) *Microcystis* biomass, *Microcystis* cell numbers, microcystin concentrations (in whole water) and microcystin cell quota for all study sites. No *Microcystis* was observed in Lake Nkuruba.

Lake	*Microcystis* biomass (mg/L)	*Microcystis* (x 10^9 cells/L)	Microcystin (µg/L)	Cell Quota (fg MC/cell *Microcystis*)
Edward Nearshore	8.24 ± 8.54	0.30 ± 0.39	5.81 ± 5.86	24.9 ± 20.0
Edward Offshore	3.63 ± 8.01	0.11 ± 0.24	0.97 ± 1.10	165.1 ± 149.7
George	221.3 ± 483.7	10.15 ± 17.08	8.54 ± 6.36	7.8 ± 9.9
Mburo	7.3 ± 6.1	8.61 ± 6.91	2.48 ± 0.96	19.0 ± 34.3
Murchison Bay	99.4 ± 168.8	4.82 ± 7.78	7.26 ± 5.73	9.8 ± 11.9
Napoleon Gulf	2.1 ± 2.5	0.06 ± 0.08	1.75 ± 1.26	119.5 ± 164.3
Saka	73.9 ± 137.1	1.32 ± 2.04	61.2 ± 73.4	112.6 ± 171.1

Table 2.7 Summary of significant relationships (P<0.05) between physical and environmental variables and *Microcystis* biomass, microcystin concentrations and microcystin cell quota at both the among lake level (including all data, n=55) and the within lake level.

	Positive Relationships	Negative Relationships
***Microcystis* spp. Biomass**		
Among All Lakes	Chl *a*, TP, PartP	Depth, Secchi depth
Lake Edward Nearshore	Chl *a*, PartP	~
Lake Edward Offshore	~	~
Lake George	Secchi, TP	Temperature
Lake Mburo	~	~
Murchison Bay	~	Secchi
Napoleon Gulf	Chl *a*, Temperature	~
Lake Nkuruba	~	~
Lake Saka	PC:PP, TN:TP, TN	~
Microcystin Concentrations		
Among All Lakes	Chl *a*, TP, PartP, PartSi, PN:PP, *Microcystis,* Cyanophyta, Total Biomass	Depth, TN, Secchi
Lake Edward Nearshore	Chl *a*	~
Lake Edward Offshore	PC:PN, *Microcystis*	~
Lake George	TP	~
Lake Mburo	~	~
Murchison Bay	TP, SRP	TN, TN:TP
Napoleon Gulf	Temperature, *Microcystis*	~
Lake Nkuruba	SRSi	~
Lake Saka	TN	~
Cell Quota of Microcystin		
Among All Lakes	Depth, Secchi	*Microcystis*, Cyanophyta, Chl *a*, Total Biomass
Lake Edward Nearshore	SRP, Total Biomass, Secchi	~
Lake Edward Offshore	~	*Microcystis*
Lake George	~	~
Lake Mburo	~	*Anabaena*, SRSi
Murchison Bay	~	~
Napoleon Gulf	Mean Mixed Layer Irradiance	*Microcystis*, Chl *a*
Lake Nkuruba	~	~
Lake Saka	~	~

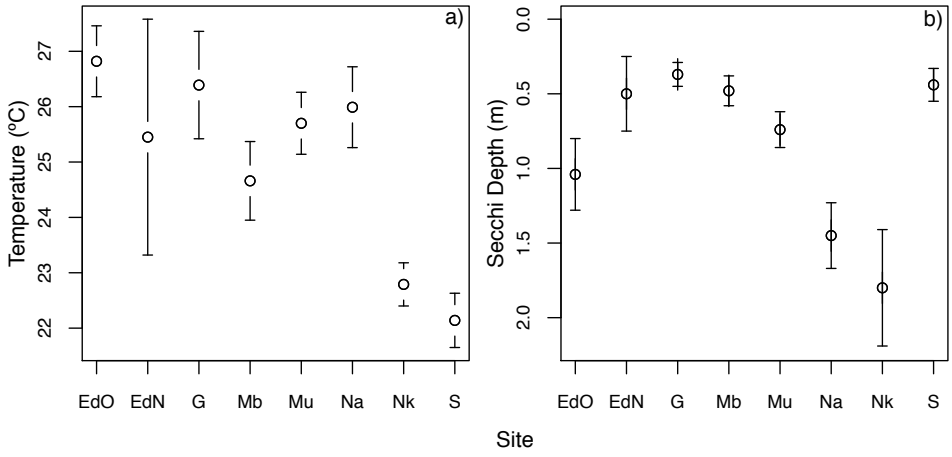

Figure 2.1 Mean values (± s.d.) for a) mean water column temperature and b) Secchi depth for all sites. Site codes are found in Table 2.1. (Open circles = means, bars = s.d.). Note the reversed y-axis for the Secchi depth figure.

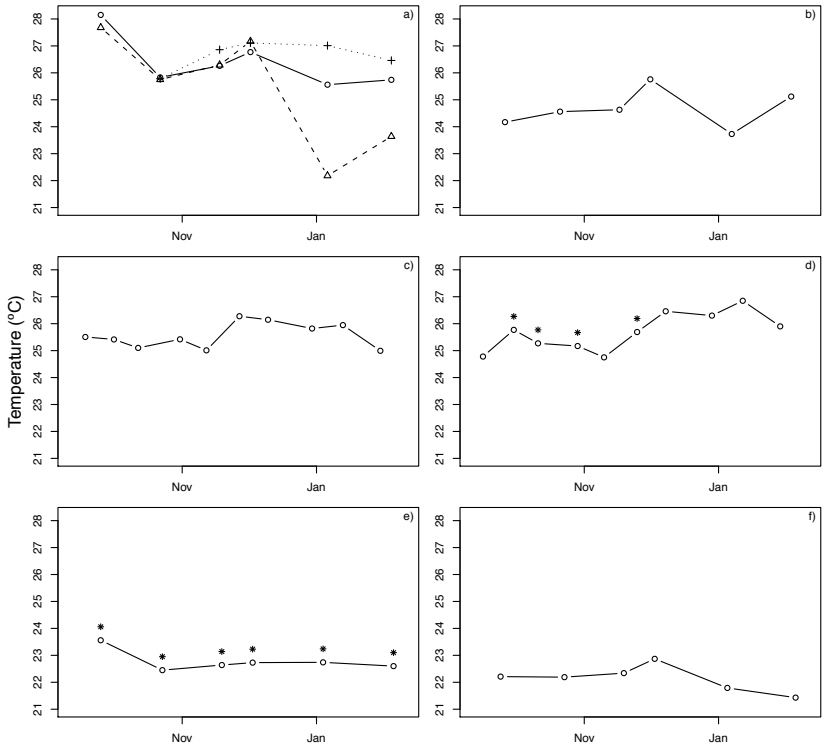

Figure 2.2 Seasonality in mean water column temperatures for a) Lake Edward offshore (+ with dotted line), Lake Edward nearshore (Δ with dashed line) and Lake George (O with solid line); b) Lake Mburo, c) Murchison Bay, d) Napoleon Gulf, e) Lake Nkuruba and f) Lake Saka. Asterisks represent stable thermal stratification.

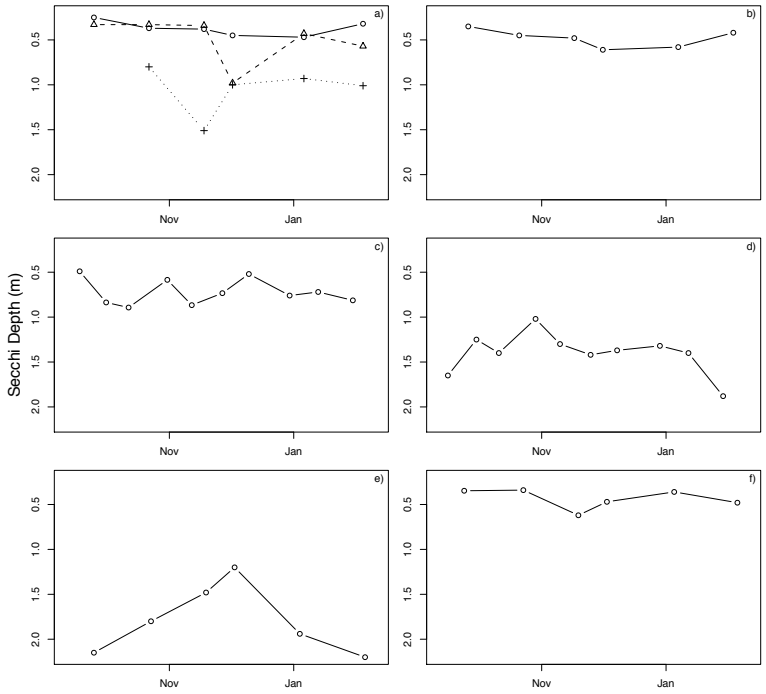

Figure 2.3 Seasonality in Secchi depth (y-axis reversed) for a) Edward offshore (+), Edward nearshore (Δ), George (O); b) Mburo; c) Murchison Bay; d) Napoleon Gulf; e) Nkuruba; f) Saka.

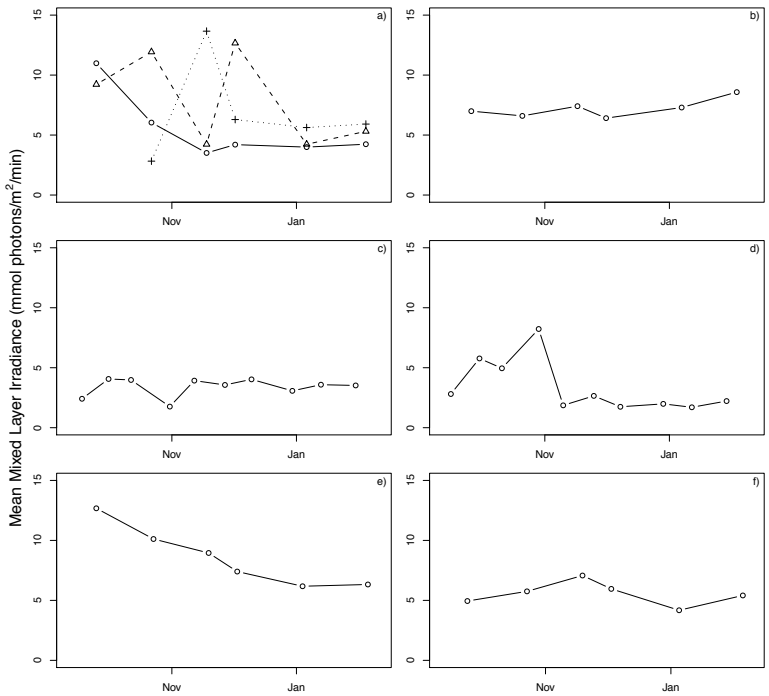

Figure 2.4 Seasonality in mean mixed layer irradiance as estimated from chlorophyll *a* for each site. Symbols and labels as in Figure 2.3.

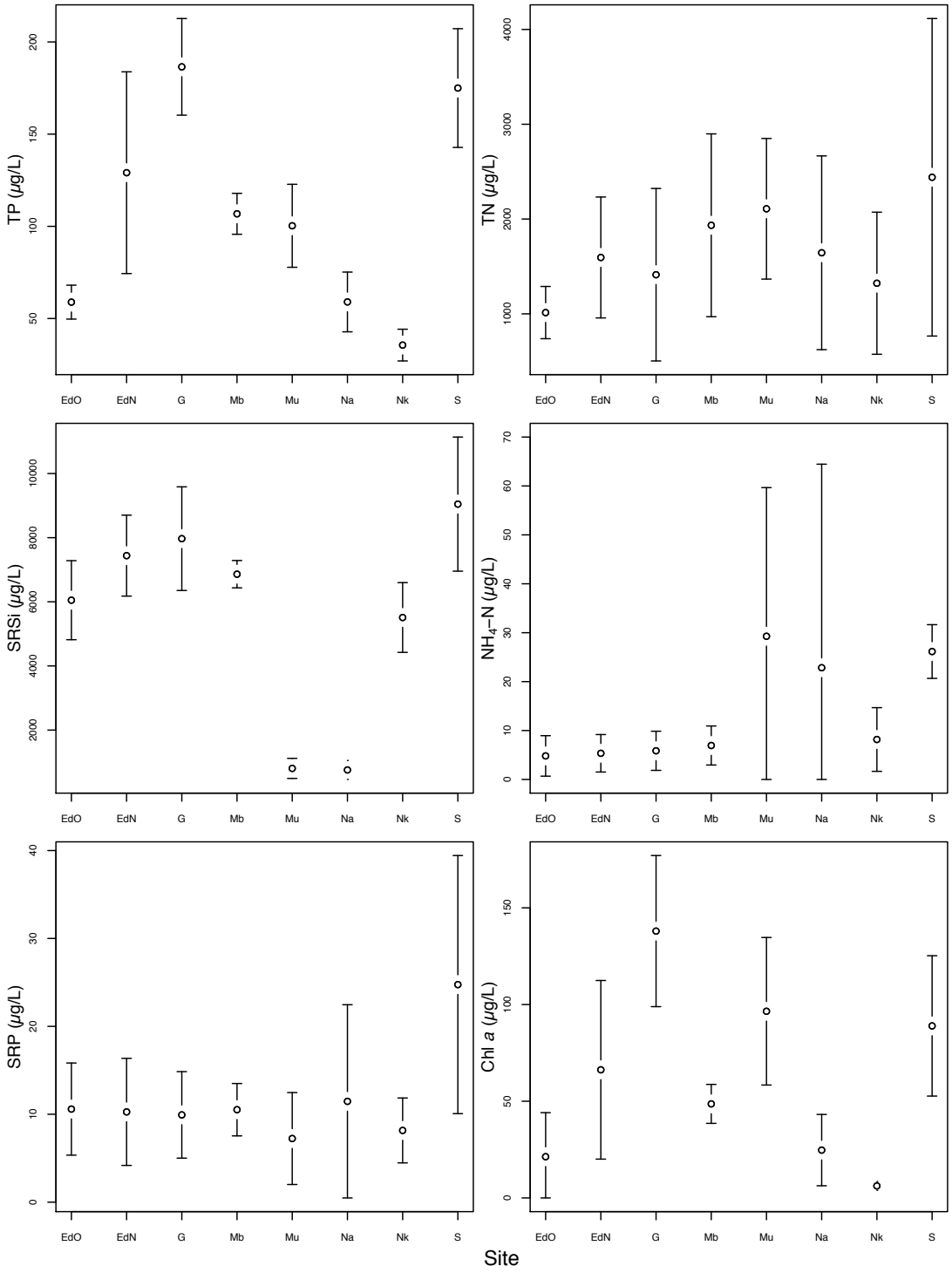

Figure 2.5 Mean values (± s.d.) for total nutrients, dissolved nutrients and Chlorophyll *a* at all sites. Site codes are found in Table 2.1. (Open circles = means, bars = s.d.).

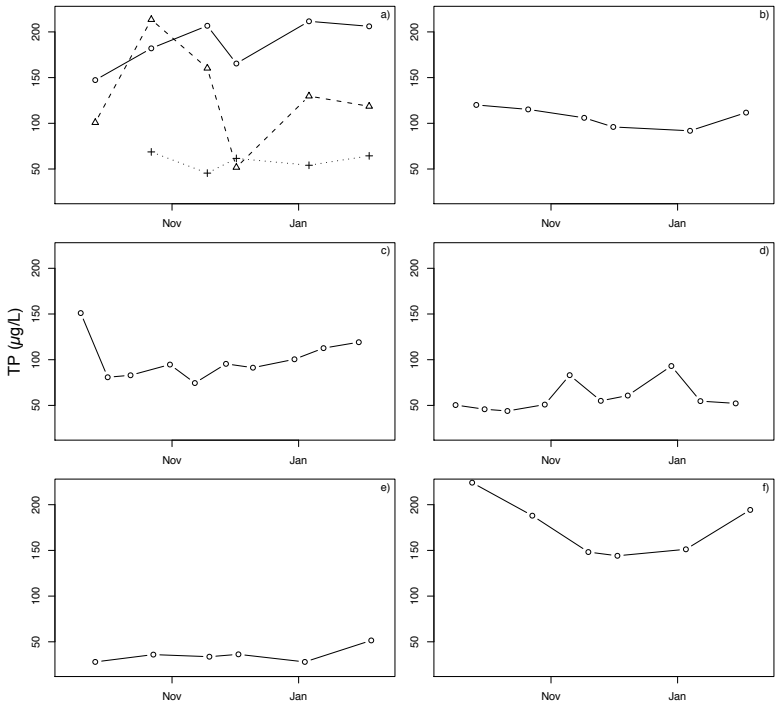

Figure 2.6 Seasonality in TP for a) Edward offshore (+), Edward nearshore (Δ), George (O); b) Mburo; c) Murchison Bay; d) Napoleon Gulf; e) Nkuruba; f) Saka.

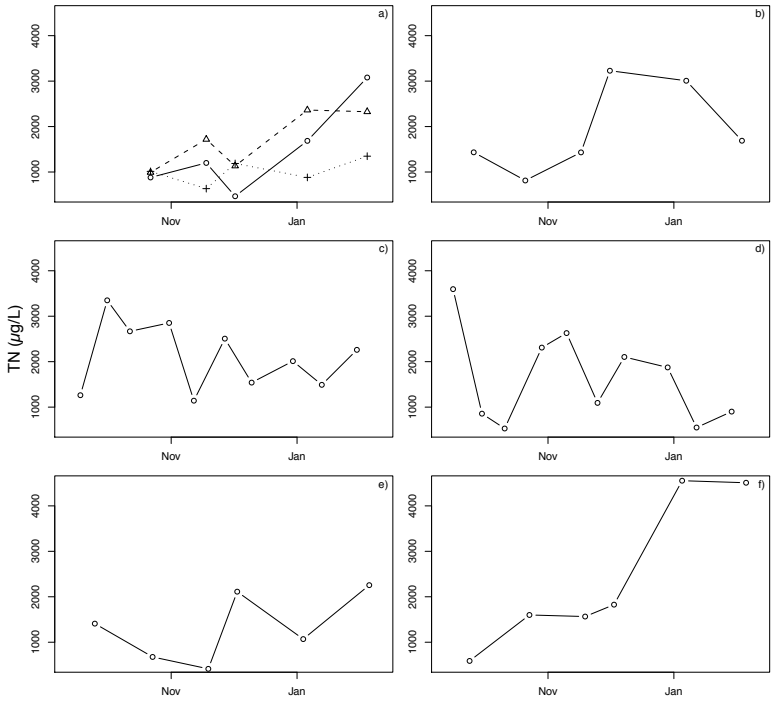

Figure 2.7 Seasonality in TN for each site. Symbols and labels as in Figure 2.6.

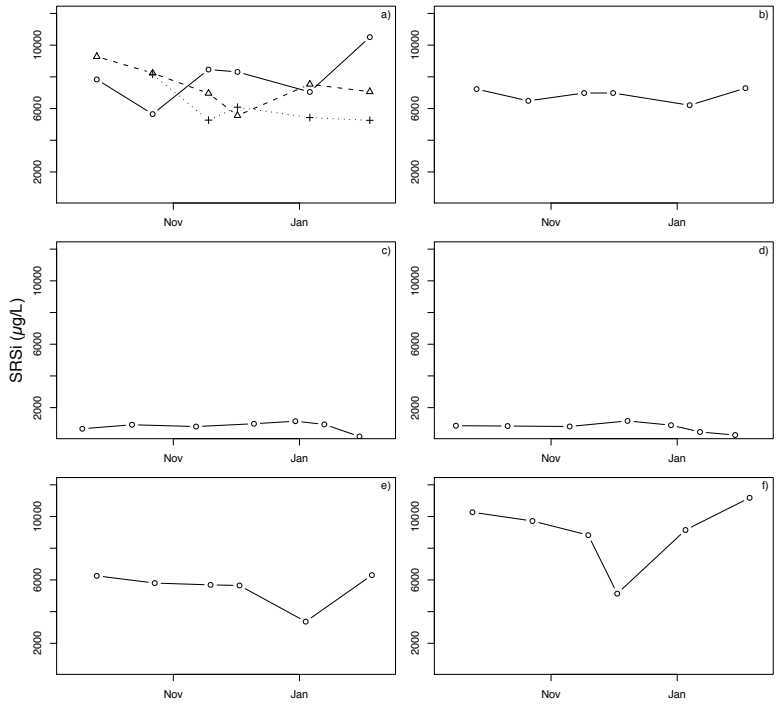

Figure 2.8 Seasonality in SRSi for a) Edward offshore (+), Edward nearshore (Δ), George (O); b) Mburo; c) Murchison Bay; d) Napoleon Gulf; e) Nkuruba; f) Saka.

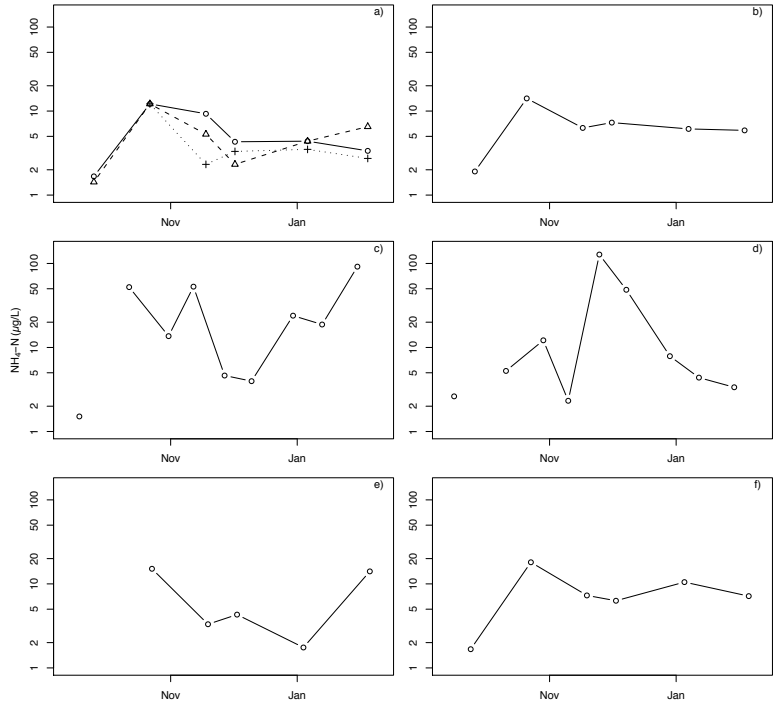

Figure 2.9 Seasonality in NH_4-N for each site. Symbols and labels are as in Figure 2.8.

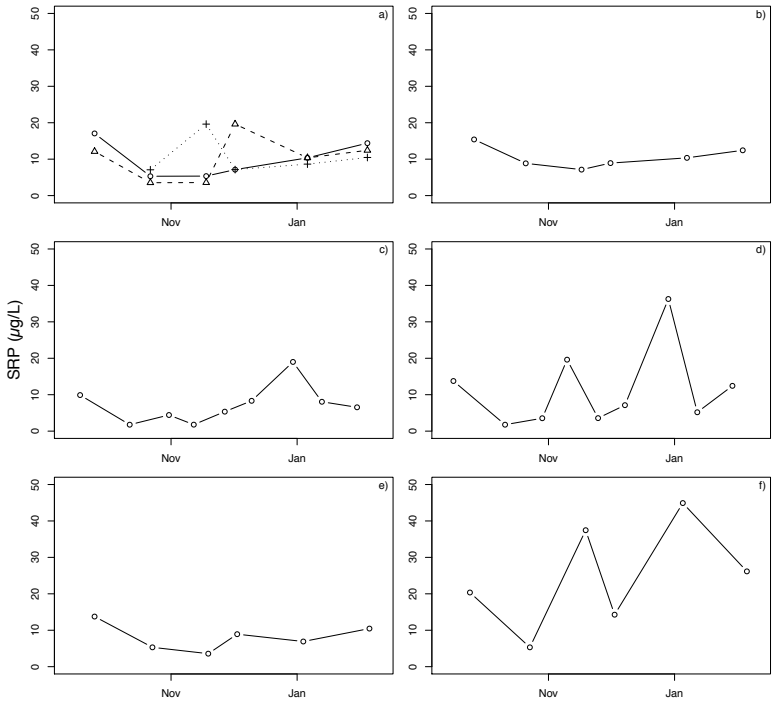

Figure 2.10 Seasonality in SRP for a) Edward offshore (+), Edward nearshore (Δ), George (O); b) Mburo; c) Murchison Bay; d) Napoleon Gulf; e) Nkuruba; f) Saka.

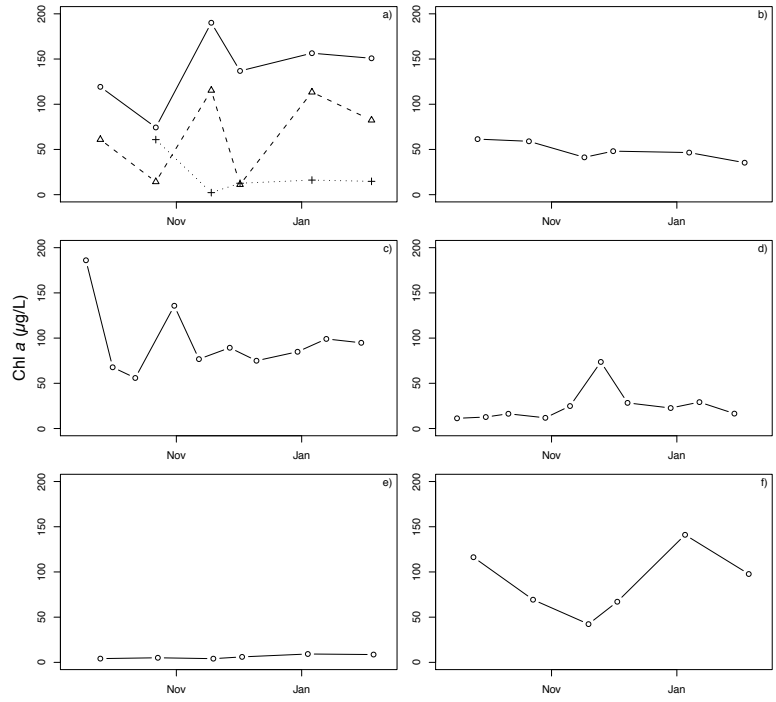

Figure 2.11 Seasonality in Chlorophyll *a* for each site. Symbols and labels are as in Figure 2.10.

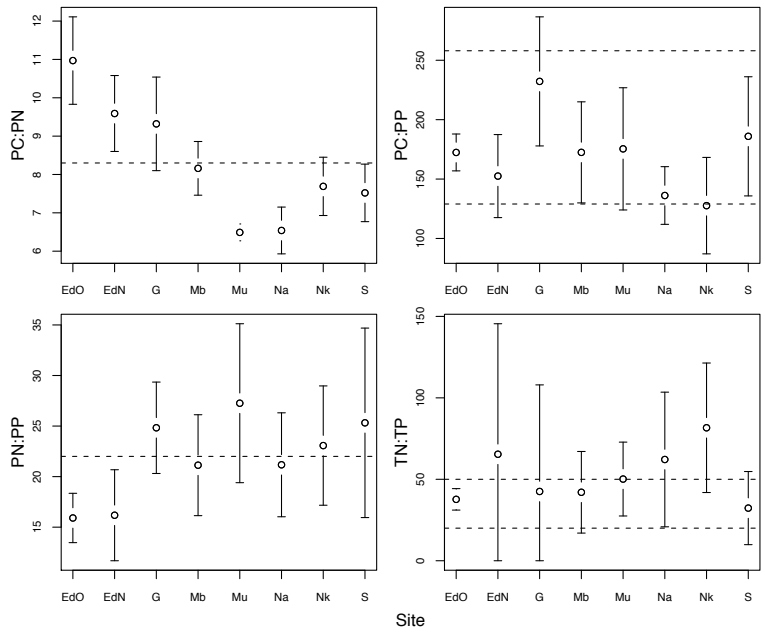

Figure 2.12 Mean values (± s.d.) for particulate and total nutrient (C, N, P) ratios for all sites. Site codes are found in Table 2.1. (Open circles = means, bars = s.d.).

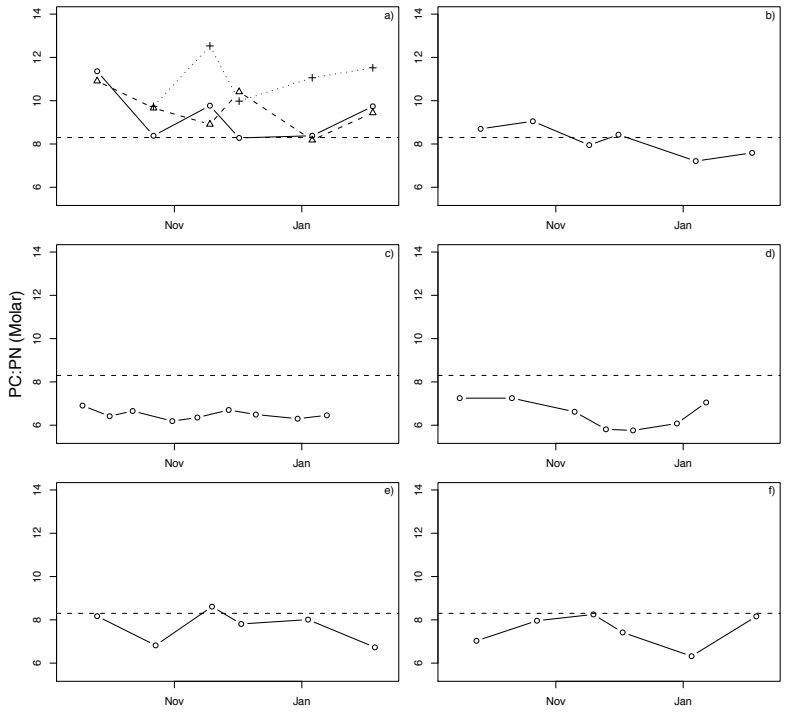

Figure 2.13 Seasonality in PC:PN for a) Edward offshore (+), Edward nearshore (Δ), George (O); b) Mburo; c) Murchison Bay; d) Napoleon Gulf; e) Nkuruba; f) Saka. The dashed line represents the boundary above which moderate N-deficiency would be expected.

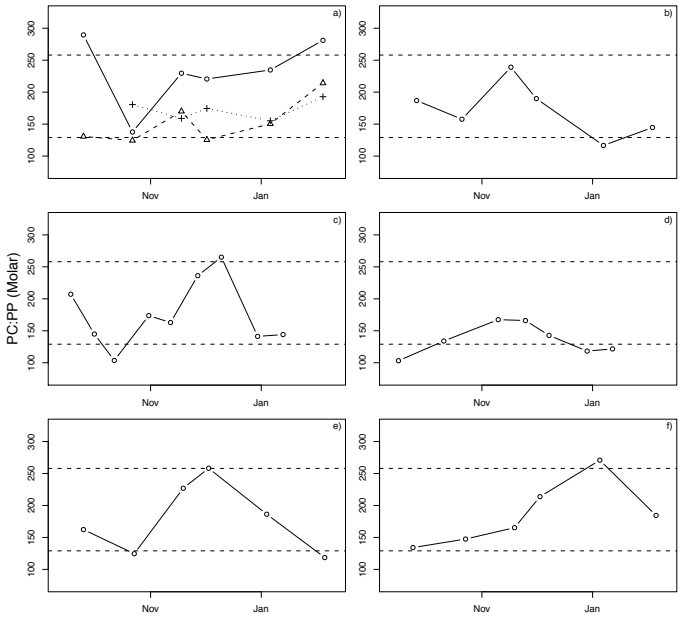

Figure 2.14 Seasonality in PC:PP for a) Edward offshore (+), Edward nearshore (Δ), George (O); b) Mburo; c) Murchison Bay; d) Napoleon Gulf; e) Nkuruba; f) Saka. In the area bounded by the two dashed lines moderate P-deficiency would be expected, with values above and below this region indicating extreme and no P-deficiency respectively.

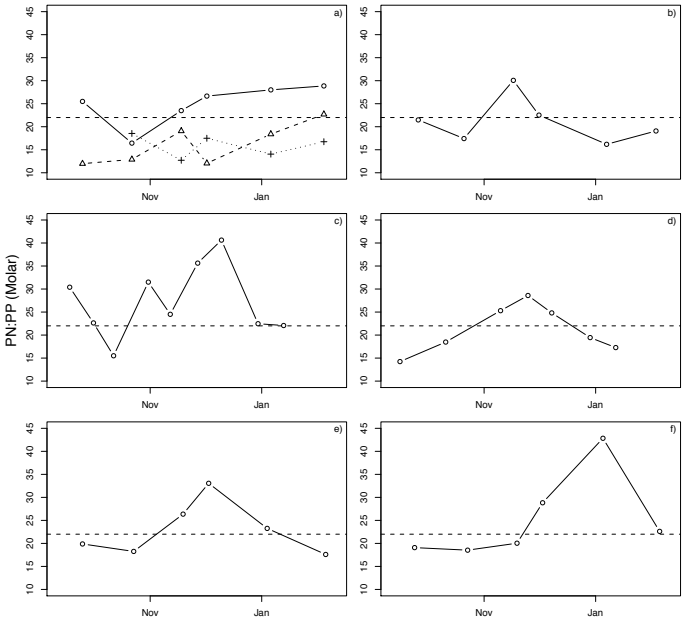

Figure 2.15 Seasonality in PN:PP for each site. Symbols and labels are as in Figure 2.14. Values above the dashed line are taken to indicate possible P-deficiency, while values below this line suggest no P-deficiency.

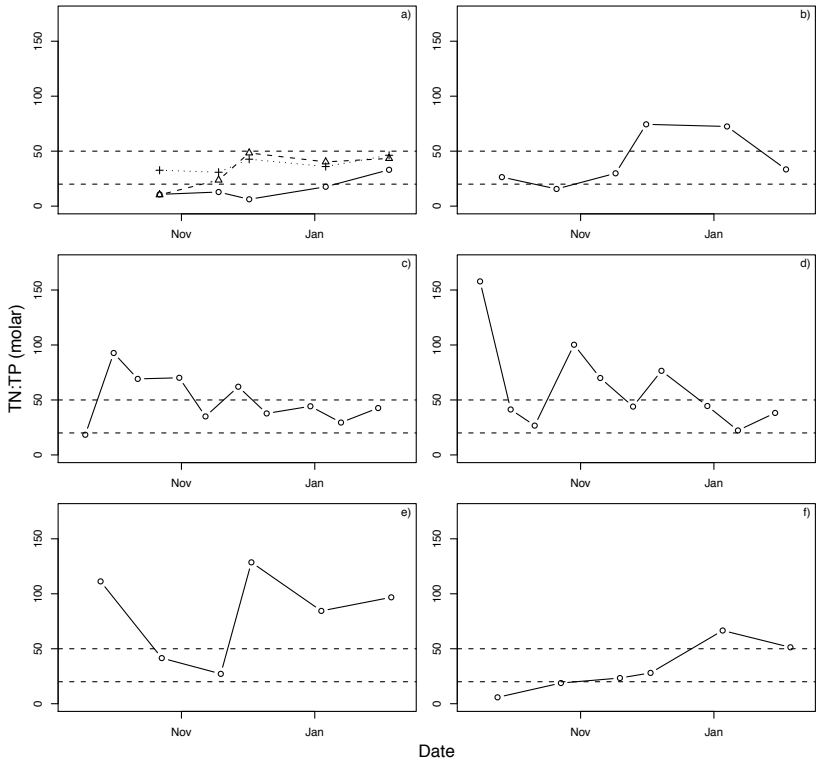

Figure 2.16 Seasonality in TN:TP for a) Edward offshore (+), Edward nearshore (Δ), George (O); b) Mburo; c) Murchison Bay; d) Napoleon Gulf; e) Nkuruba; f) Saka. In the area bounded by the two dashed lines possible N and P colimitation (or no deficiency) would be expected, with values above and below this region indicating possible P deficiency and possible N deficiency respectively.

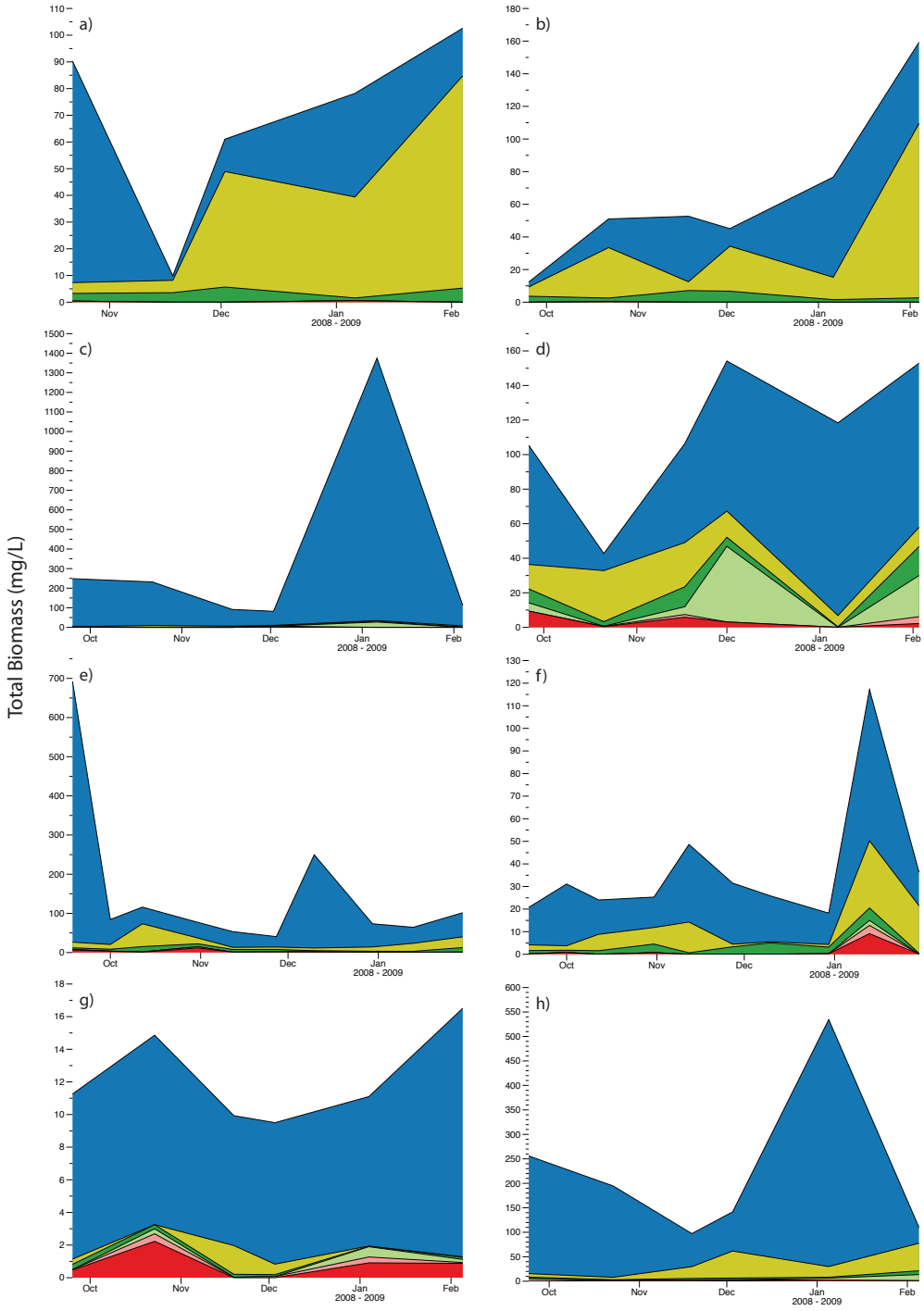

Figure 2.17 Seasonality in phytoplankton community composition for a) Edward offshore, b) Edward nearshore, c) George, d) Mburo, e) Murchison Bay, f) Napoleon Gulf, g) Nkuruba and h) Saka. Note that the y-axis scales differ. Blue = Cyanophyceae, yellow = Baccilariophyceae, green = Chlorophyceae, light green = Euglenophyceae, pink = Dinophyceae, and red = Cryptophyceae.

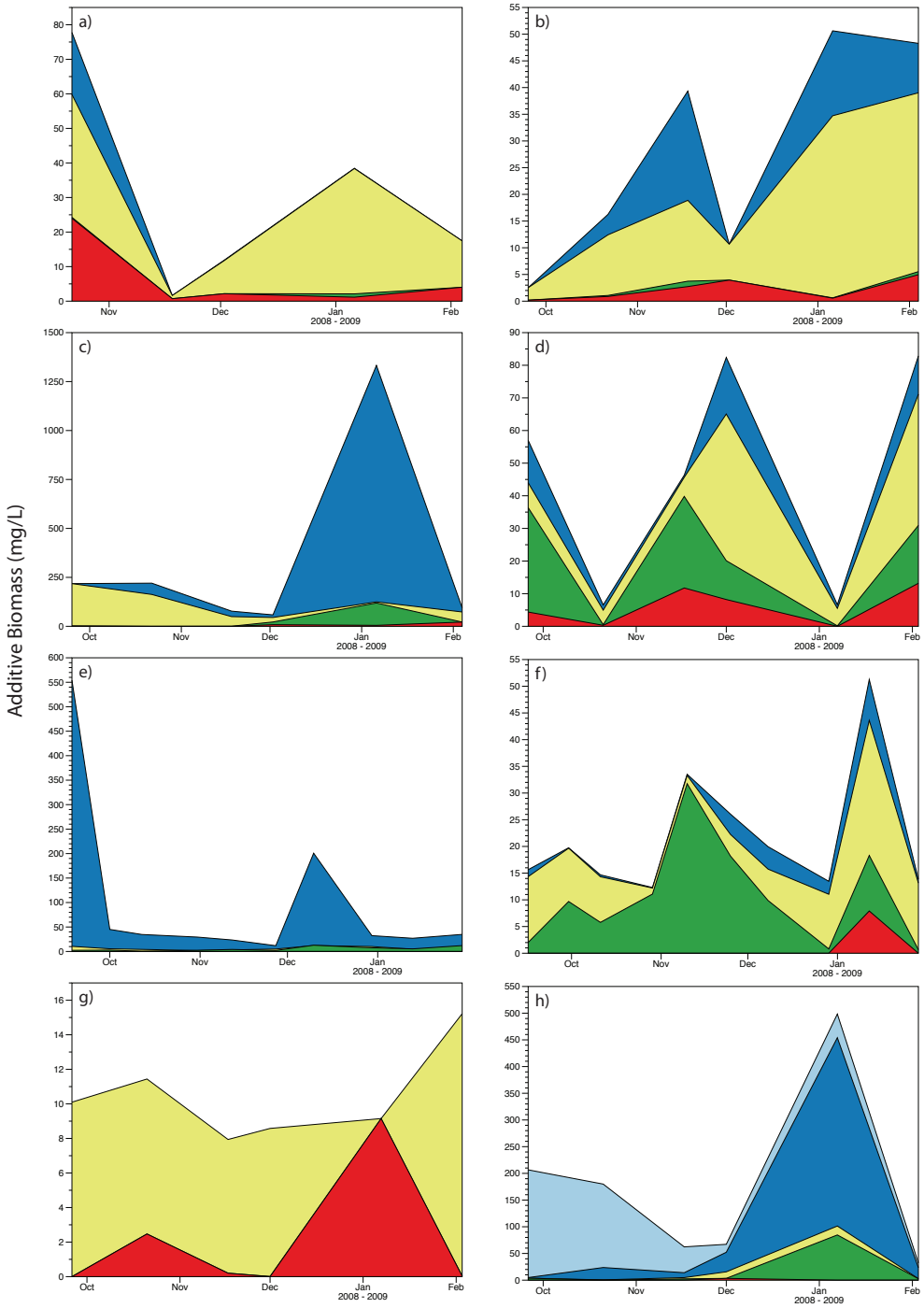

Figure 2.18 Seasonality in cyanobacterial community composition for a) Edward offshore, b) Edward nearshore, c) George, d) Mburo, e) Murchison Bay, f) Napoleon Gulf, g) Nkuruba and h) Saka. Note that the y-axis scales differ. Blue = *Microcystis*, yellow = *Planktolyngbya*, green = *Anabaena*, red = *Cylindrospermopsis*, and light blue = *Planktothrix*.

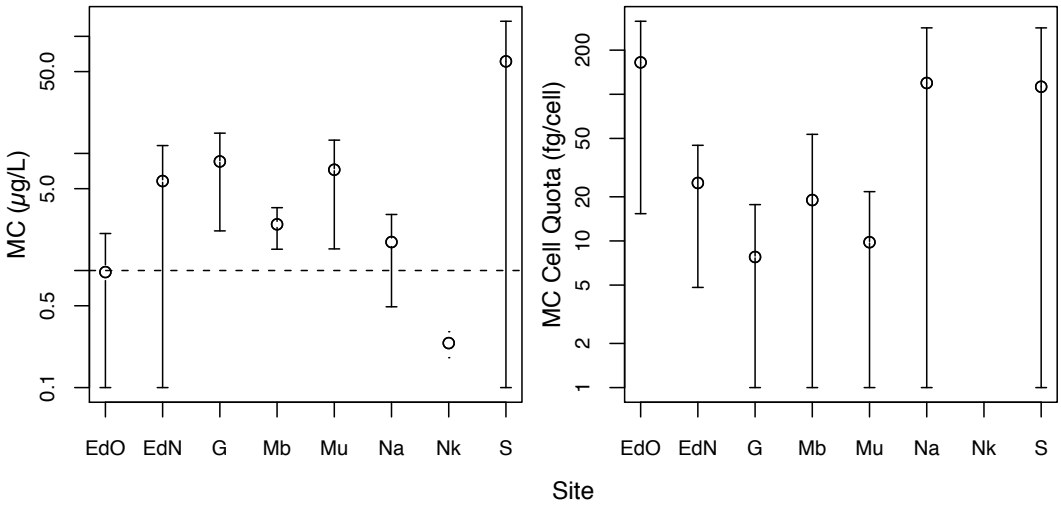

Figure 2.19 Mean values (± s.d.) for a) microcystin concentrations and b) cell quota (fg MC/cell *Microcystis*) for all sites. Site codes are found in Table 2.1. (Open circles = means, bars = s.d.). The dashed line in plot a) represents the WHO guideline for MC in drinking water.

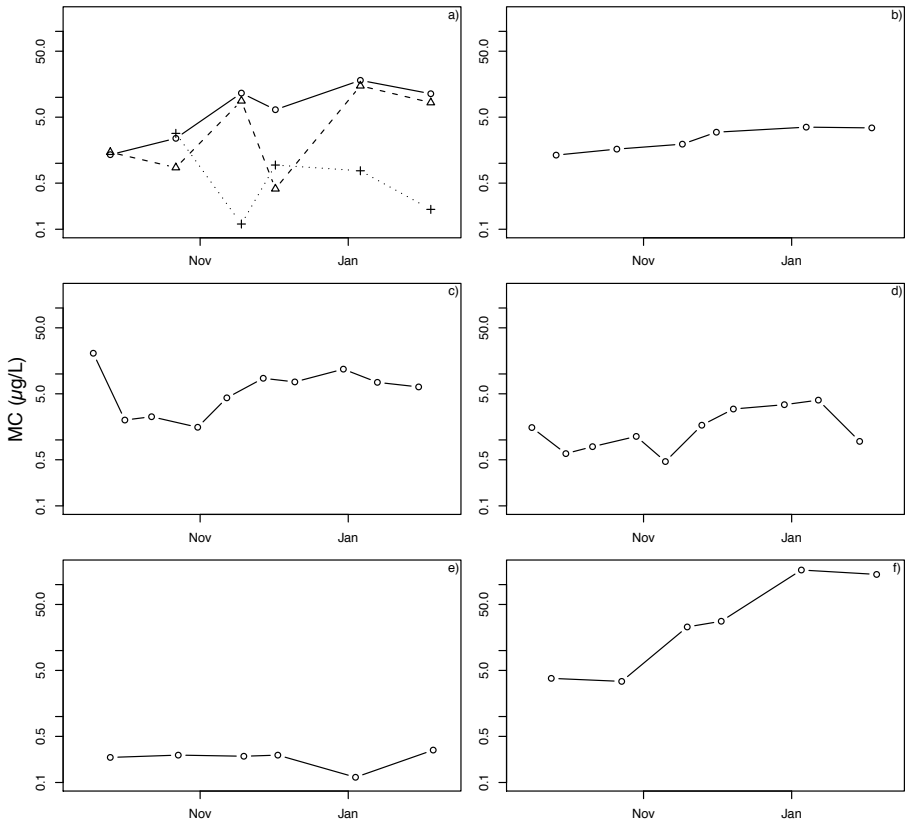

Figure 2.20 Seasonality in microcystin concentrations for a) Edward offshore (+), Edward nearshore (Δ), George (O); b) Mburo; c) Murchison Bay; d) Napoleon Gulf; e) Nkuruba; f) Saka.

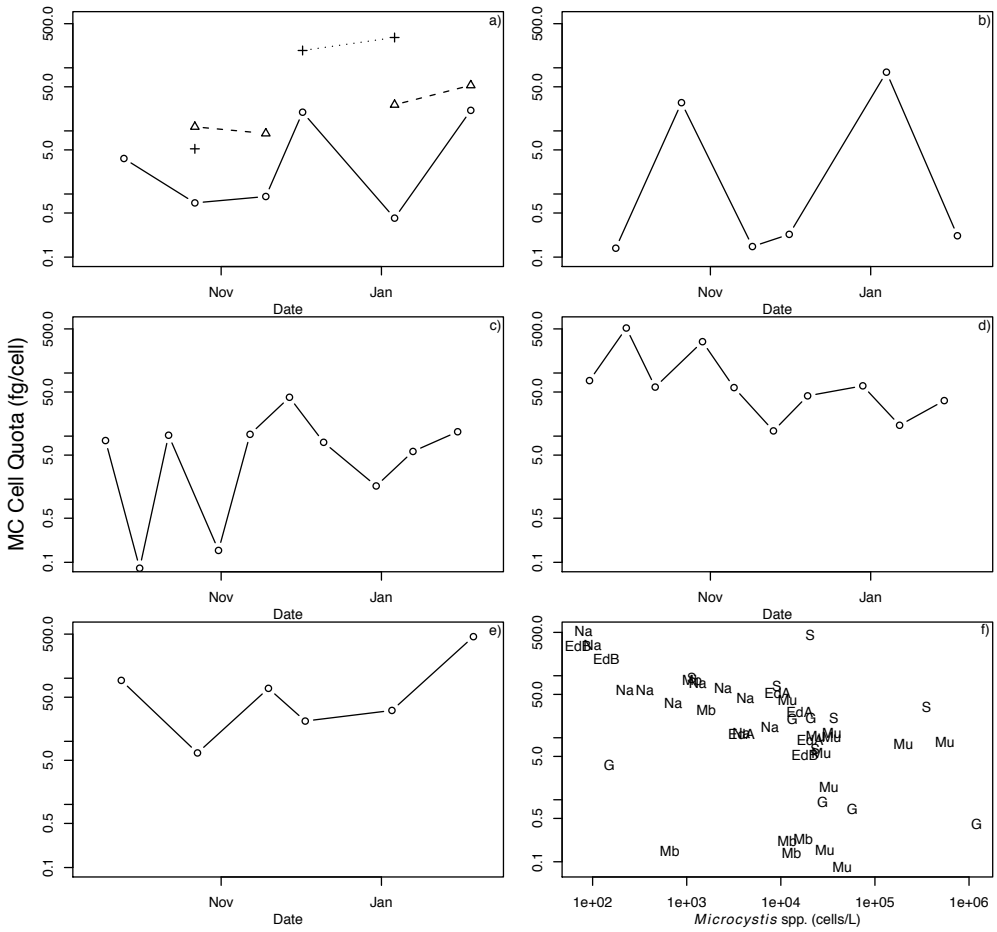

Figure 2.21 Seasonality in cell quota of microcystin (fg MC/cell *Microcystis*) for a) Lake Edward offshore (+ with dotted line), Lake Edward nearshore (Δ with dashed line) and Lake George (O with solid line); b) Lake Mburo, c) Murchison Bay, d) Napoleon Gulf, and e) Lake Saka. Note that Lake Nkuruba is not displayed since *Microcystis* was not present. In panel f) the negative relationship between cell quota of microcystin and *Microcystis* spp. biomass (both log-transformed) is shown (r^2_{adj} = 0.22, P<0.001).

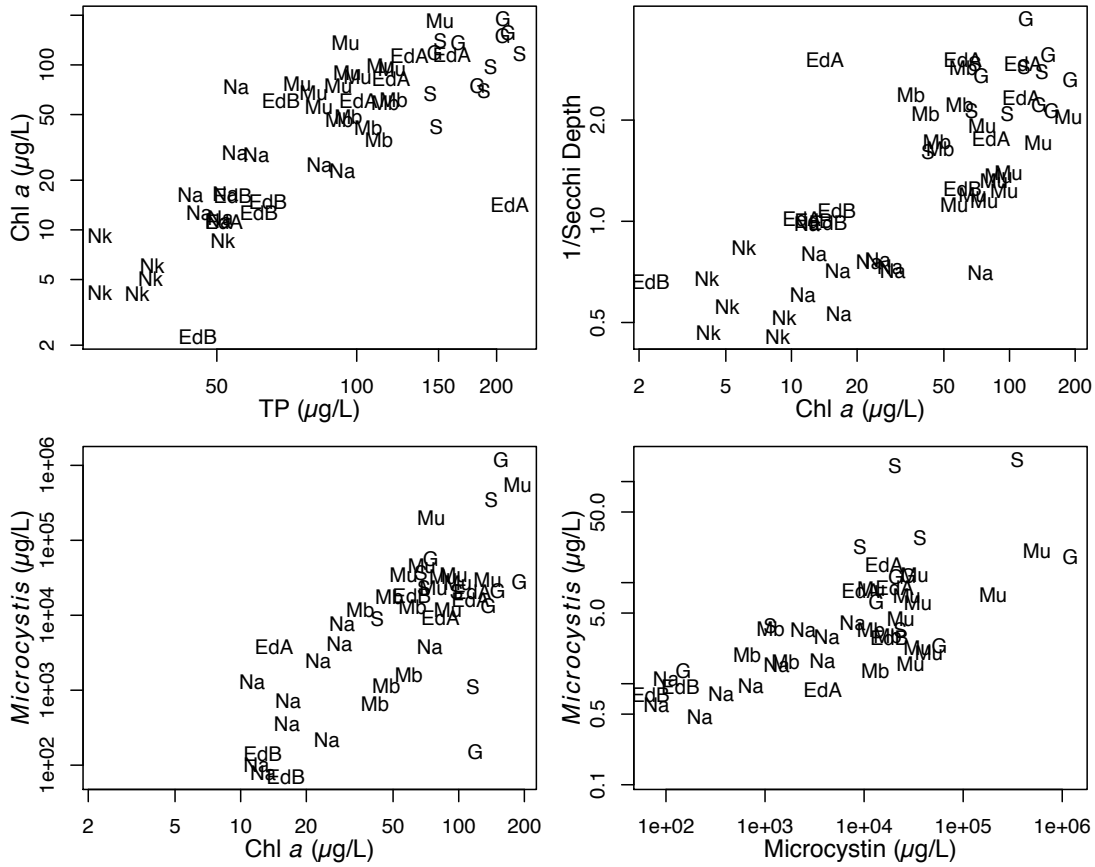

Figure 2.22 Relationships between a) chlorophyll *a* and TP (r^2_{adj} = 0.47, P<0.001), b) inverse Secchi depth and chlorophyll *a* (r^2_{adj} = 0.76, P<0.001), c) chlorophyll *a* and *Microcystis* spp. biomass (r^2_{adj} = 0.51, P<0.001), and d) *Microcystis* spp. biomass and microcystin concentrations in water (r^2_{adj} = 0.48, P<0.001).

Chapter 3
Characterization and comparison of food web structure in several Ugandan lakes using stable isotope analysis

3.1 Introduction

Understanding the food web structure of lakes is essential for several reasons. Lakes in East Africa are critical sources of fish both for export and for local consumption. By understanding the current fish community composition and trophic relationships, sound management decisions can be made. Also, by having baseline data for aquatic food web structure, changes in this structure, which may be precipitated by a warming climate, or by anthropogenic influences on lakes, can be monitored and the implications of these changes can be assessed. In addition, it is critical to be familiar with the underlying food web structure in order to understand the trophic transfer of compounds (including contaminants and toxins) in aquatic systems.

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios can be used to characterize food webs and trophic interactions (Peterson and Fry 1987, Fry 1991). Stable isotope ratios of carbon ($\delta^{13}C$) give an indication of the primary source of organic carbon to consumers; and the source signature is largely retained or only slightly enriched by trophic transfers (change of 0 to 1‰; Peterson and Fry 1987, Cabana and Rasmussen 1994, Hecky and Hesslein 1995). There are several factors that influence $^{13}C/^{12}C$ of aquatic primary producers, including isotopic fractionation during photosynthesis, photosynthetic pathway, $\delta^{13}C$ of $CO_{2(aq)}$, growth rate, boundary layer for diffusion of CO_2, and $CO_{2(aq)}$ concentrations (Hecky and Hesslein 1995); while the ratio in terrestrial plants is more dependent on the photosynthetic pathway alone. C-4 plants (including some aquatic macrophytes such as *Cyperus papyrus*) have $\delta^{13}C$ values of -12 to -14 ‰, while C-3 plants (including the water hyacinth, *Eichhornia cassipes*) have $\delta^{13}C$ values of -26 to -28 ‰ (Hecky and Hesslein 1995, Campbell *et al.* 2003a). Under idealized conditions of no carbon limitation of growth and full equilibration with the atmosphere, phytoplankton would be expected to have $\delta^{13}C$ values of -37 ‰; however, these values are rarely achieved, particularly in tropical systems, where $CO_{2(aq)}$ is lower and growth rates are higher (Hecky and Hesslein 1995). Due to CO_2 diffusion limitation by a boundary layer, benthic periphyton tend to have higher $\delta^{13}C$ values than phytoplankton, giving insight into the relative importance of benthic and pelagic energy for a consumer (Hecky and Hesslein 1995). However, colonial and filamentous phytoplankton including cyanobacteria can have significant boundary layers that can enrich their stable carbon isotopic signature. Furthermore,

when growth rates are high, instantaneous ^{12}C-limitation can lead to higher (enriched) $\delta^{13}C$ values in phytoplankton.

Stable nitrogen isotopic ratios ($\delta^{15}N$) are useful for characterizing the trophic level of an organism, because with each trophic transfer, organisms selectively excrete the lighter nitrogen isotope (^{14}N), leaving behind an enriched (more positive) $\delta^{15}N$ signal in the consumer (Minagawa and Wada 1984, Peterson and Fry 1987). On average, with each trophic transfer there is an increase of 3.4 ± 1.1 ‰ in $\delta^{15}N$ (Minagawa and Wada 1984); however, this may vary based on food quality or the nutritional state of the consumers. For example, starvation can lead to retention of the heavier isotope, yielding a higher than expected $\delta^{15}N$ value (Haubert *et al.* 2005). Baseline $\delta^{15}N$ values for primary producers and microbes in a system can be influenced by the input of nitrogen from human or animal waste ($\delta^{15}N$: 10–20 ‰), organic soil nitrogen ($\delta^{15}N$: -2–9 ‰), commercial fertilizers ($\delta^{15}N$: -2.5–2 ‰), and cyanobacterial fixation of atmospheric nitrogen ($\delta^{15}N$: 0 ‰) (Harrington 1998). As such, $\delta^{15}N$ values can provide information on the primary source of the N supporting an organism as well its trophic position. Stable isotope analysis is particularly valuable because, unlike gut content analysis, which yields only a 'snapshot' of the organism's diet, stable isotope analysis allows for an integrated long-term description of diet (Hesslein *et al.* 1993, Kidd *et al.* 1995).

Results of stable isotope analysis can also be used to calculate the realized trophic level of an organism based on its $\delta^{15}N$ value relative to a baseline established for the study site (Vander Zanden *et al.* 1997, Post 2002), and by calculating the realized trophic level of the top predator in the lake, one can gain an estimate of food chain length (Vander Zanden *et al.* 2007) if the baseline value is known or can be estimated. Calculated trophic levels for a species can be compared between lakes to gain insight into differences in dietary habits, and in the potential for the accumulation of contaminants or toxins. Food chain lengths can also be compared between lakes.

Stable isotope analysis has been successfully applied in food-web contaminant studies in several tropical, temperate and arctic systems (Kidd *et al.* 1995, Atwell *et al.* 1998, Campbell *et al.* 2003b, Kidd *et al.* 2003, Campbell *et al.* 2006, Campbell *et al.* 2008). In the current study, the food web structure as determined using stable isotope analysis will be used to explore the accumulation and trophic transfer of both mercury (Chapter 4) and the cyanotoxin microcystin (Chapter 5) in several East African lakes. To my knowledge, this is the first study to pair characterization of microcystin concentrations in fish with stable isotope analysis.

Among East African lakes of interest to the current study, food webs have been described using stable isotope analysis for Napoleon and Winam Gulfs in Lake Victoria (Campbell *et al.* 2003), Ugandan crater lakes Saka and Nkuruba (Campbell *et al.* 2006), Lake Albert (Campbell *et al.* 2005), and Lake Mburo (Mbabazi *et al.* 2004). The current study revisits all of these sites except Winam Gulf, and also includes sites where stable isotope analysis of food webs has not previously been done, including the tropical great lake Edward, Lake George and Murchison Bay, an embayment in northern Lake Victoria. By including all of these sites I will gain insight into the temporal stability of food webs that have already been described, as well as data that are directly comparable to my microcystin and mercury concentrations (Chapters 4, 5 this thesis). Also, food chain lengths and trophic levels have not previously been calculated based on $\delta^{15}N$ values for any of these sites, yielding further insight into the structure of these food webs, the differences between these sites, and the factors that may determine food chain length in tropical lakes. The inclusion of Lake Edward, George and Murchison Bay will also yield important baseline data on food web structure for these sites, which all support economically and locally important fisheries.

3.2 Methods

Fish samples were collected from Lake Albert in April and May of 2007. Some samples were collected from Butiaba (in the northeast of the lake) in April of 2007, and were purchased directly from fishermen as they landed with their catch. Meanwhile, the majority of the samples were collected from Lake Albert near Kaiso (Ngassa spit), in the central eastern portion of the lake, in May of 2007 in conjunction with Uganda's National Fisheries Resources Research Institute using gillnets.

Fish and food web samples from Lakes Edward, George, Mburo, Victoria (Murchison Bay and Napoleon Gulf), Nkuruba and Saka were collected between September 2008 and February 2009. Fish were purchased directly from fishermen (either while still on the water, or immediately after landing) and general location of the catch was confirmed (although this was not always possible for Murchison Bay). Plankton samples were collected using vertical net hauls (20 µm mesh for phytoplankton; 80 µm and 153 µm for zooplankton), and samples were subsequently filtered onto pre-combusted quartz-fibre filters (nominal pore size 0.7 µm). Debris was visually removed from phytoplankton and zooplankton samples, and samples were examined microscopically to confirm sample composition. *Chaoborus*, a dipteran zooplanktivore, was visually detected and separated from zooplankton samples. Epilithic algae from Lake Nkuruba was scraped off of rocks in the littoral zone, resuspended in deionized water and filtered onto precombusted quartz-fibre filters. Benthic invertebrates were collected using a ponar grab. Because these samples were also used for mercury analysis, trace metal clean sampling protocols were used throughout.

Subsamples of dorsolateral muscle tissue were taken from fish for use in stable isotope analysis. For intermediate-sized haplochromine cichlids, whole fillets of axial muscle tissue were collected for analysis. For very small haplochromine cichlids, whole fish, with the viscera removed, were kept. For *Rastrineobola argentea* and other very small fish (<5 cm), it was not possible to separate muscle tissue or remove the viscera, and whole fish were used for analysis. Whole invertebrates were used for analysis, except for gastropods (from Murchison Bay and Lake Nkuruba), where the shells were removed.

Fish and invertebrate samples were oven-dried at 60 ºC for at least 24 hours (until weights stabilized) and then were ground into a fine powder using a ball mill grinder. Filters with plankton and periphyton samples were oven-dried as for fish and invertebrate samples, but were not ground. Samples were not acidified prior to analysis, as these samples were all biotic, and were not expected to contain appreciable amounts of carbonate. Stable carbon and nitrogen isotopic ratios were determined using a Micromass Isochrom Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS) at the Environmental Isotope Laboratory, University of Waterloo, ON, Canada. To determine variability between runs, standards for carbon (IAEA-CH6 (sugar), EIL-72 (cellulose) and EIL-32 (graphite)) and nitrogen (IAEA-N1 and IAEA-N2, both ammonium sulphate) were analyzed. Meanwhile, one in every ten samples was run in duplicate to measure variability within runs. Mean standard deviations for standard material are ± 0.2 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$. Mean standard deviations of duplicated samples were ± 0.05 ‰ for $\delta^{13}C$ and ± 0.21 ‰ for $\delta^{15}N$.

The delta notation used ($\delta^{15}N$ and $\delta^{13}C$) represents the difference (in parts per thousand, ‰) between the measured isotopic ratio of the sample and the isotopic ratio of the reference standard (PeeDee belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$), and is calculated using the following equation (as in Campbell 2001):

Eq. 3.1 $$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample} - R_{standard})/(R_{standard})]\times 1000$$

where $R={}^{13}CO_2/{}^{12}CO_2$ for $\delta^{13}C$ or $R={}^{15}N/{}^{14}N$ of N_2 for $\delta^{15}N$.

Food web structure was graphically displayed by plotting $\delta^{15}N$ values against $\delta^{13}C$ values. Then, corrections for $\delta^{15}N$ values were made where there were strong baseline relationships between $\delta^{15}N$ and $\delta^{13}C$. Trophic level (TL) was calculated for all samples based on their $\delta^{15}N$ values relative to a baseline organism with an assumed trophic level (ideally a long-lived primary consumer; Vander Zanden *et al.* 2007). Meanwhile, food chain length (FCL) was calculated for all lakes based on the mean $\delta^{15}N$ value of the top predator in the lake relative to the mean $\delta^{15}N$ value of a baseline organism with an assumed trophic level. The equations used for TL and FCL calculations are as follows:

Eq. 3.2 $TL_{sample} = (\delta^{15}N_{sample} - \delta^{15}N_{baseline})/3.4 + \lambda$

Eq. 3.3 $FCL = (\delta^{15}N_{top\ predator} - \delta^{15}N_{baseline})/3.4 + \lambda$

where λ is the assumed trophic level of the baseline organism, and 3.4 is the assumed isotopic fractionation of nitrogen from one trophic level to the next (Minagawa and Wada 1984, Cabana and Rasmussen 1996, Vander Zanden *et al.* 2007, Gantner 2009). Linear regression was used in order to explore whether there were relationships between FCL and productivity (chlorophyll *a* and total phosphorus concentrations), ecosystem size (lake area) or productivity × ecosystem size (information used in these regressions is found in Table 3.1) for these sites (Post *et al.* 2000, Vander Zanden *et al.* 2007).

To determine whether there were differences in baseline nitrogen isotopic ratios, $\delta^{15}N$ values were regressed against calculated trophic level for all lakes. Given that isotopic fractionation was assumed to be 3.4 ‰ per trophic level for all lakes, these regressions have identical slopes; however, the differences between the intercepts for these regression lines represent differences in baseline $\delta^{15}N$ values between lakes.

To explore possible differences in baseline $\delta^{13}C$ values between lakes, an ANOVA was carried out comparing the $\delta^{13}C$ values for net phytoplankton (>20 μm) between the study sites. Similarly, an ANOVA was carried out comparing $\delta^{13}C$ values for tilapiine cichlids (including *Oreochromis esculentus*, *O. leucostictus*, *O. niloticus*, *O. variabilis* and *T. zilli*) between the study sites. Generally, plant material forms an important part of the diet of tilapiine cichlids (Greenwood 1958). Despite the variability in diet expected among tilapiine cichlids both within and among sites, given that fish integrate isotopic values over longer time periods, I can gain a clearer understanding of average $\delta^{13}C$ values in phytoplankton by examining these primary consumers. I also explored the relationship between observed stable isotopic ratios for both phytoplankton and tilapiine cichlids through linear regression (i.e. $\delta^{13}C$ of tilapiines vs. $\delta^{13}C$ of phytoplankton, and likewise for $\delta^{15}N$).

To determine whether fish species were occupying similar trophic positions in different lakes, an analysis of variance (ANOVA) was carried out for each fish species comparing calculated trophic levels between sites. Post-hoc tests were carried out to determine pair-wise significant differences between lakes. To confirm that differences in the fish length distributions within species between sites were not introducing a bias into my comparison of trophic level, ANOVAs were also carried out for each fish species comparing total length between sites.

Regressions between stable carbon and nitrogen isotopic ratios and total length were done for each species in each lake. These results indicate whether these fish experience ontogenetic changes in dietary C and N sources as they grow. I also carried out analyses of covariance for each species ($\delta^{15}N$ or $\delta^{13}C$~site + total length + site × total length) in order to determine whether shifts in diet with growth are similar or different between lakes.

All statistical analyses were carried out using R, version 2.11.1 (R Development Core Team 2010).

3.3 Results

There was a strong negative relationship between $\delta^{15}N$ and $\delta^{13}C$ of phytoplankton in Lake Edward (Figure 3.2c, $r^2_{adj} = 0.92$, $P<0.001$). These differences in isotopic ratios were attributable to spatial trends, with phytoplankton with significantly lower $\delta^{15}N$ ($P<0.05$) and higher $\delta^{13}C$ ($P<0.001$) at the near shore site (where the Kazinga Channel enters Lake Edward) than at the offshore site. Carbon and nitrogen isotopic ratios for near shore Lake Edward phytoplankton were not significantly different than those observed in Lake George. Due to this trend in baseline $\delta^{15}N$ values and in order to accurately calculate trophic level and food chain length, the $\delta^{15}N$ values from Lake Edward were corrected such that the baseline nitrogen isotopic ratio was equal for all samples (using the mean $\delta^{15}N$ value for Lake Edward offshore phytoplankton as the new baseline). The $\delta^{15}N$ correction equation applied was as follows:

Eq. 3.4 $$\delta^{15}N_{corrected} = \delta^{15}N_{sample} + (\delta^{13}C_{po} - \delta^{13}C_{sample}) \times b$$

$\delta^{15}N_{corrected}$: corrected $\delta^{15}N$ value for the sample

$\delta^{15}N_{sample}$: original $\delta^{15}N$ value for the sample

$\delta^{13}C_{po}$: $\delta^{13}C$ value for offshore Lake Edward (-18.97 ‰)

$\delta^{13}C_{sample}$: $\delta^{13}C$ value for the sample

b: $\delta^{15}N$ vs $\delta^{13}C$ regression slope for L. Edward phytoplankton (-0.37986)

Lake Edward's food web based on the corrected $\delta^{15}N$ values (Figure 3.2d) can be compared with the uncorrected food web (Figure 3.2b). The corrected $\delta^{15}N$ values for Lake Edward were used in all subsequent analyses, calculations and plots. No other corrections were necessary given that for no other lake was there a strong relationship observed between $\delta^{15}N$ and $\delta^{13}C$ values for phytoplankton. No phytoplankton samples were available for Lake Albert (another large lake with multiple water sources such as the Victoria Nile, which enters the lake near Butiaba), and so this relationship could not be tested there.

Trophic level (TL, not to be confused with total length: L) and food chain length (FCL) calculations were carried out using equations 3.2 and 3.3 and the information in Table 3.3. Because this study compared TL and FCL between lakes, it was important to be consistent in my choice of a baseline organism for $\delta^{15}N$ values. I was not able to obtain molluscs or definitively herbivorous zooplankton samples from all lakes. As such, I chose to use net phytoplankton as a baseline organism for trophic level calculation, and I collected phytoplankton samples on at least a monthly basis (often twice-monthly) over a six-month period in order to capture the temporal variability in phytoplankton $\delta^{13}C$ and $\delta^{15}N$ values. Due to high variability in the highly eutrophic Murchison Bay (Silsbe *et al.* 2005), $\delta^{15}N$ values (range 4.0–9.2 ‰), zooplankton collected with a 153 μm net was used as a baseline (7.7 ‰), incidentally the mean $\delta^{15}N$ value for *Bellamya* sp., a gastropod, was identical to that observed for the zooplankton, suggesting that this is a reliable baseline $\delta^{15}N$ value representative of primary consumers. Standard deviations for phytoplankton $\delta^{15}N$ values ranged from 0.2–1.0 ‰ at the sites where it was used as a baseline (Table 3.2). In Lake Albert, plankton samples were not available; therefore, *Brycinus nurse* was used as a baseline organism because as an obligate zooplanktivore, a trophic level of 3 can be assumed. However, because Lake Albert's food web was not sampled exhaustively, several known piscivores are absent or underrepresented in my sample set, so I did not calculate FCL for Lake Albert. Food chain lengths (FCL, expressed in number of trophic levels between primary producers and top piscivores) ranged from 2.8–4.3. FCL values were lowest in Murchison Bay (2.8) and highest in Lakes Edward (4.3), George (3.9) and Saka (3.6). Intermediate FCL values were observed for Napoleon Gulf (3.4), Lake Mburo (3.3), and Lake Nkuruba (3.2). There were no statistically significant relationships found between FCL and productivity (chlorophyll *a* and total phosphorus concentrations; Table 3.1), ecosystem size (lake area; Table 3.1), or productivity × ecosystem size.

When $\delta^{15}N$ is plotted against trophic level, the differences among sites in y-intercepts are indicative of differences in baseline $\delta^{15}N$ values between lakes. For my study lakes, baseline $\delta^{15}N$ values spanned a range of nearly 6 ‰ (Figure 3.3). Baseline $\delta^{15}N$ values were lowest in Lakes Saka, George and Mburo, intermediate in Lakes Nkuruba, Edward, Albert and Napoleon Gulf, and highest in Murchison Bay.

Baseline $\delta^{13}C$ was compared between sites in two ways. First, I carried out an ANOVA on $\delta^{13}C$ values from phytoplankton from all sites (Figure 3.4a). Then I carried out an ANOVA on $\delta^{13}C$ values from tilapiine cichlids (including *Oreochromis esculentus*, *O. leucostictus*, *O. niloticus*, *O. variabilis* and *Tilapia zilli*) from all sites (Figure 3.4b). Among phytoplankton samples, $\delta^{13}C$ values were significantly lower in Lake Nkuruba (-26.0 ± 0.9 ‰) than in all other lakes (ANOVA, $P<0.001$). Napoleon Gulf (-16.9

± 2.5 ‰), offshore Lake Edward (-19.0 ± 2.8 ‰) and Lake Saka (-20.7 ± 1.5 ‰) were not significantly different from one another, but were significantly higher than Lake Nkuruba, and significantly lower than the remainder of the sites. The highest $\delta^{13}C$ values were observed in Lake George (-9.6 ± 1.6 ‰) and nearshore Lake Edward (-11.3 ± 2.4 ‰) where values were significantly higher than nearly all other sites. Murchison Bay had intermediate $\delta^{13}C$ values (-15.6 ± 1.5 ‰) that were not significantly different than those observed in Lake Mburo or Napoleon Gulf.

Among tilapiine cichlids there was generally less variability in $\delta^{13}C$ values than for phytoplankton (Figure 3.4). The relative position of mean $\delta^{13}C$ values for tilapiine cichlids was similar to what was observed for phytoplankton samples. Lake George had significantly higher $\delta^{13}C$ values than at any other site. $\delta^{13}C$ values in tilapiines from Lake Mburo were the second highest of any site, and were significantly different than all other sites. Similarly, Napoleon Gulf's tilapiine cichlids had the third highest $\delta^{13}C$ values of all sites, and were significantly different than all other sites. The only sites that were not significantly different than all other sites were Lake Edward, Lake Albert and Murchison Bay, which grouped together as significantly lower than Lakes George, Mburo and Napoelon Gulf and significantly higher than all other lakes. The lowest $\delta^{13}C$ values in tilapiine cichlids were observed in Lake Nkuruba followed by Lake Saka. There was a strong positive relationship ($r^2_{adj} = 0.93$, $P<0.001$, slope=1.05) between site means for $\delta^{13}C$ of tilapiine cichlids and $\delta^{13}C$ of phytoplankton (Figure 3.5a). Meanwhile, there was a significant positive relationship ($r^2_{adj} = 0.68$, $P<0.05$, slope=0.67) between site means of $\delta^{15}N$ of tilapiine cichlids and $\delta^{15}N$ of phytoplankton (Figure 3.5b).

In comparing the calculated trophic levels for fish species in different lakes (Figure 3.6), I found that some fish species were located at similar calculated trophic levels (not significantly different in an ANOVA at $P<0.05$) in all lakes where they were present (including *Bagrus docmac*, *Clarias gariepinus*, *Oreochromis esculentus*, and *Synodontis afrofischeri*). Meanwhile, other species of fish did exhibit significant (ANOVA, $P<0.05$) differences in calculated trophic levels between lakes. *Protopterus aethiopicus* had a higher TL in Lake George than in Murchison Bay; however, no other significant differences were seen in TL for *P. aethiopicus* between sites. Unlike *Synodontis afrofischeri,* which were at similar trophic levels in both of the embayments in northern Lake Victoria, *Synodontis victoriae* were at a significantly higher TL in Napoleon Gulf than in Murchison Bay. *Lates niloticus* from Murchison Bay appeared to be feeding at lower trophic levels than in Lakes Albert or Saka. Similarly, in Napoleon Gulf *L. niloticus* had significantly lower calculated TL values than in Lake Saka.

Among the tilapiine cichlids, *Oreochromis leucostictus* from Napoleon Gulf had a lower mean TL value than in Lakes Albert or Nkuruba; also, *O. leucostictus* collected from Lake Albert were significantly smaller than those collected from any other lake except for Lake Nkuruba (ANOVA, $P<0.05$). Similarly, *T. zilli* from Lake Nkuruba had a higher mean TL than in Lake Albert, Murchison Bay or Napoleon Gulf, and *T. zilli* from Lake Nkuruba were significantly smaller than those collected from Murchison Bay (ANOVA, $P<0.05$). For both *O. leucostictus* and *T. zilli*, I observed significant negative relationships between trophic level and total length ($r^2_{adj} = 0.16$ and $P<0.05$ for both) across all sites. For *Oreochromis niloticus*, significantly higher TL values were observed in Lake Edward, Mburo and Saka than in Napoleon Gulf or Murchison Bay, while TL values for *O. niloticus* from Lake Mburo exceeded those observed in Lake George.

The haplochromine cichlids sampled also exhibited differences in trophic level between sites. *Haplochromis squamipinnis* had significantly higher mean calculated trophic level in Lake Edward than in Lake George, however, *H. squamipinnis* individuals collected from Lake Edward also had significantly higher mean total length than those from Lake George (ANOVA, $P<0.05$). I also sampled unidentified haplochromines from several other lakes (referred to as *Haplochromis* (?) spp. in this study, and taken to exclude *H. squamipinnis*). In Lakes Edward and George these fish were significantly larger (ANOVA, $P<0.05$) and at a significantly higher trophic level (ANOVA, $P<0.05$) than those from any other site. Although there were no other significant differences in trophic level for *Haplochromis* spp. between sites, in Lake Saka, the *Haplochromis* spp. sampled were significantly smaller than at any site but Napoleon Gulf.

Relationships between stable carbon and nitrogen isotopic values and total length are summarized in Table 3.4 and shown in Figure 3.7. *Lates macrophthalamus* in Lake Albert and *L. niloticus* in Napoleon Gulf both demonstrated significant increases in $\delta^{15}N$ values with growth (r^2_{adj} values of 0.96 and 0.40 respectively). Meanwhile, a positive trend (although not statistically significant) was observed between $\delta^{15}N$ and total length for *L. niloticus* from Lake Albert and Murchison Bay (Figure 3.7a). No such relationship was observed for Lake Saka. In Lake Edward, $\delta^{15}N$ values for both *Haplochromis* sp. and *Protopterus aethiopicus* increased significantly with length (r^2_{adj} values of 0.67 and 0.64 respectively)), while $\delta^{13}C$ values decreased significantly with total length (r^2_{adj} values of 0.72 and 0.50 respectively). Significant positive relationships were observed between $\delta^{13}C$ and total length for *L. macrophthalamus* and *Schilbe intermedius* in Lake Albert, and *O. niloticus* in Lake Saka; while a significant negative relationship was observed between $\delta^{13}C$ and total length for *O. leucostictus* in Lake Nkuruba.

Oreochromis niloticus experienced an increase in $\delta^{15}N$ values with increasing length in Lakes George (r^2_{adj}=0.24), Mburo (r^2_{adj}=0.27) and Murchison Bay (r^2_{adj}=0.22).

The slopes and directions of the $\delta^{15}N$–total length regressions for *O. niloticus* differed greatly between sites, and were significantly higher in Lakes George and Mburo than in Murchison Bay (ANCOVA, P<0.01), and were not significantly different between Lakes George and Mburo (Figure 3.7b). ANCOVAs comparing the relationship between both $\delta^{15}N$ and total length and $\delta^{13}C$ and total length between sites revealed many significant differences in intercepts between these regressions.

For *Lates* spp. (including *L. macrophthalamus* and *L. niloticus*) the regression slope for $\delta^{15}N$ vs. total length was lower in Lake Saka than in Napoleon Gulf (ANCOVA, P<0.05), however there were no other significant differences in slope for *Lates* spp. between any other sites (including Albert, Murchison Bay, Napoleon Gulf and Lake Saka). This indicates that despite the lack of statistically significant relationships between $\delta^{15}N$ and total length observed for *Lates* spp. at most sites, an increase in $\delta^{15}N$ with growth appears to be occurring at similar rates at all sites except for Lake Saka.

3.4 Discussion

3.4.1 Differences in baseline $\delta^{13}C$ and $\delta^{15}N$ values

The differences in observed baseline $\delta^{13}C$ and $\delta^{15}N$ values between study sites are attributable to several processes. Different nitrogen sources (including human and animal waste, organic soil nitrogen, commercial fertilizers and atmospheric nitrogen) have distinctive $\delta^{15}N$ signals, with nitrogen from human and animal waste having high $\delta^{15}N$ values compared to organic soil nitrate, and atmospheric nitrogen (either incorporated in commercial fertilizers or fixed by N-fixing cyanobacteria) having $\delta^{15}N$ values that approximate 0 ‰ (Harrington 1998). As such, anthropogenic input of nutrients to lakes as well as rates of cyanobacterial N-fixation can influence the $\delta^{15}N$ values observed in phytoplankton as well as the rest of the food web. The lowest $\delta^{15}N$ values were observed in Lakes Saka, George, and Mburo, which are all hypereutrophic lakes where N-fixing cyanobacteria are present (Chapter 2, this thesis). While Lake Saka's catchment has been heavily impacted by deforestation and agriculture (Crisman and Chapman 2001), Lakes George and Mburo are not likely to be receiving large amounts of human waste, given that both lakes are at least partially contained within protected areas, although animal waste may be of importance to these lakes (Lehman *et al.* 1998, Mbabazi *et al.* 2004). Conversely, Murchison Bay had the highest baseline $\delta^{15}N$ values, which reflects the large amount of human waste that the inner bay receives

from the nearby city of Kampala (projected 2010 population of 1.8 million; Nyakaana *et al.* 2007), however, the observed baseline $\delta^{15}N$ value for phytoplankton in the inner bay is still well below published $\delta^{15}N$ values for dissolved inorganic nitrogen from human waste (10–20 ‰), suggesting that this influence may in part be counteracted by high levels of atmospheric nitrogen fixation. $\delta^{15}N$ values for phytoplankton in Murchison Bay were much more variable than at any other study site, reflecting temporal and spatial variability in pulses of anthropogenic nutrients as well as blooms of N-fixing cyanobacteria. Based on human population densities and their likely impact, of all sites, Murchison Bay, Napoleon Gulf and Lake Saka would be expected to receive the most nitrogen from human waste. Although, Napoleon Gulf is expected to be well-flushed owing to its location at the outflow of Lake Victoria to the Victoria Nile river, and may experience less local anthropogenic influence than either Murchison Bay or Lake Saka. Lake Saka may also be receiving commercial fertilizer (which typically has $\delta^{15}N$ values ranging from -2.5 to 2.0 ‰; Harrington 1998) given that there is a prison farm within the catchment (Crisman and Chapman 2001). At all sites, differences in input of anthropogenic and natural nitrogen sources, as well as rates of atmospheric nitrogen fixation were responsible for the range of baseline $\delta^{15}N$ values observed among (and likely within) lakes.

Differences in baseline $\delta^{13}C$ values are similarly indicative of several different processes and carbon sources, with C-4 plants and C-limited primary producers (particularly benthic periphyton, rapidly growing phytoplankton, and colonial cyanobacteria which may have increased boundary layers limiting diffusion of CO_2) having higher $\delta^{13}C$ values, and phytoplankton that is not affected by substantial carbon limitation (i.e. in more oligotrophic lakes with lower phytoplankton biomass) having much lower $\delta^{13}C$ values (Hecky and Hesslein 1995). In the current study, phytoplankton tended to have lower $\delta^{13}C$ values in less productive lakes (e.g. Lake Nkuruba and Lake Edward offshore); however, hypereutrophic Lake Saka also had relatively low $\delta^{13}C$ values. It is possible that $CO_{2(aq)}$ concentrations in Lake Saka are high enough to reduce the possibility of instantaneous carbon limitation, leading to lower $\delta^{13}C$ values in Lake Saka than those observed in the other hypereutrophic lakes included in this study. These increased $CO_{2(aq)}$ concentrations may be driven by high rates of decomposition of biogenic carbon in this hypereutrophic lake, or by the cooler temperatures (where CO_2 is more soluble; Lourey *et al.* 2004) observed in Lake Saka relative to the other highly productive lakes sampled (see chapter 2 of this thesis). Lakes Mburo and George were also possibly receiving a subsidy of C-4 plant detritus (*C. papyrus* is prevalent in both lakes) from the extensive adjacent wetlands as well as the faeces of the large hippopotamus populations resident in these lakes (Mbabazi *et al.* 2004); and this source of enriched organic carbon would only reinforce the

food web dependence on isotopically heavy carbon. Furthermore, hippopotamus faeces may provide nutrients, which can sustain high phytoplankton growth, leading to C-limited photosynthesis and more positive $\delta^{13}C$ values in the food web.

The processes leading to differences in baseline $\delta^{15}N$ and $\delta^{13}C$ values are particularly evident in Lakes George and Edward, where $\delta^{15}N$ values were low in Lake George, intermediate in nearshore Lake Edward near the mouth of the Kazinga Channel, and higher in offshore Lake Edward. This decreasing trend is likely attributable to lower rates of atmospheric nitrogen fixation in offshore Lake Edward than in Lake George. Also, in near shore Lake Edward, higher light availability in the shallower water may allow for higher rates of N-fixation (which has high light requirements; Mugidde *et al.* 2003) than in the deeper mixed layer depth in offshore Lake Edward. The opposite trend was observed for $\delta^{13}C$ values, where the highest values were in Lake George, and the lowest values were in offshore Lake Edward, possibly due to the decreasing influence of both ^{12}C-limited primary productivity supported by high nutrient concentrations and CO_2 derived from the degradation of isotopically heavy C-4 plant detritus.

3.4.2 Lake Albert Food Web Structure

Lake Albert's food web has been previously described using stable isotope analysis paired with gut content analysis (Campbell *et al.* 2005). The food web that emerged from the Campbell *et al.* (2005) study was triangular, with piscivorous fish (particularly *Hydrocynus forskahlii*) at the apex, integrating both benthic and pelagic dietary sources, and was very similar to the food web structure observed in the current study. Although there were some differences in $\delta^{15}N$ and $\delta^{13}C$ values between fish collected by Campbell *et al.* (2005) and those included in the current study, the relative trophic positions of the fish were similar in both studies, and were consistent with expected trophic positions based on published diets. As in Campbell *et al*. (2005), there was a great deal of overlap in $\delta^{15}N$ values between species, suggesting a high rate of omnivory and few obligate feeding relationships. Hecky *et al.* (2010) observed that in Lake Victoria, nearshore phytoplankton tended to have higher $\delta^{13}C$ and lower $\delta^{15}N$ values (due to differences in mixing depths, with deeper mixing depths leading to light limitation of both phytoplankton biomass and atmospheric nitrogen fixation). This is a general trend that is likely extends to other large Ugandan lakes (including Lakes Albert and Edward). While the current study confirms this trend in Lake Edward, given that I do not have data on the isotopic ratios of phytoplankton in Lake Albert, it is difficult to assess spatial patterns in phytoplankton $\delta^{13}C$ and $\delta^{15}N$ for this large lake.

The tilapiine cichlids had the lowest $\delta^{15}N$ values of all fish from Lake Albert, which is consistent with their known dietary preference for phytoplankton, benthic algae, detritus and occasionally invertebrates (Greenwood 1958). These fish displayed a wide range in $\delta^{13}C$ values in Lake Albert suggesting the inclusion of both benthic and pelagic food sources (or possibly nearshore and offshore sources). In particular, *T. zilli* and some *O. leucostictus* had enriched $\delta^{13}C$ values relative to other fish sampled, indicating a possible dietary preference for macrophytic debris, benthic primary consumers, or the possibility that these fish are feeding closer to shore. The range in $\delta^{15}N$ values observed for tilapiine cichlids (*O. leucostictus* in particular) suggests that primary consumers may be an important source of prey for some of these fish, however this range could indicate spatial differences in feeding locations as well.

Brycinus nurse, *Neobola bredoi* and *Alestes baremose* are all known to rely heavily on zooplankton (Greenwood 1958). As expected, the $\delta^{15}N$ values for these fish are similar to one another, and to the values observed for other secondary consumers (including *Schilbe intermedius* and *Barbus bynni*), while these fish all had higher $\delta^{15}N$ values than the detritivorous tilapiine cichlids.

The piscivorous fish sampled included *Bagrus bayad*, *Lates niloticus*, *Lates macrophthalamus*, and *Hydrocynus forskahlii*. The $\delta^{13}C$ values for *B. bayad* suggest the inclusion of benthic sources of primary carbon, which is consistent with the published diet for this fish, which includes both small fish and insect larvae from inshore areas (Greenwood 1958). Both *L. niloticus* and *L. macrophthalamus* had $\delta^{15}N$ values consistent with feeding on primary consumers, however one 70 cm long individual of *L. macrophthalamus* had a much higher $\delta^{15}N$ value (11.1 ‰) than any other fish sampled, suggesting that this fish may be feeding on zooplanktivorous fish. *Hydrocynus forskahlii* also appeared to be consistently piscivorous. Based on calculated trophic levels, all of the piscivorous fish sampled appear to be feeding mostly on primary consumers, with some fish (particularly the largest *L. macrophthalamus*) feeding on secondary consumers. Due to the lack of data on the range of $\delta^{13}C$ values present in phytoplankton and benthic "algae" from Lake Albert, it is particularly difficult to determine the source of differences in $\delta^{13}C$ values in fish at this site.

3.4.3 Lakes Saka and Nkuruba Food Web Structure

The food web structures for Lakes Saka and Nkuruba were described, based on stable isotope analysis, by Campbell *et al.* (2006). As for Lake Albert, my results were similar to those previously reported. In both Lake Saka and Lake Nkuruba, the $\delta^{13}C$ values observed in fish closely reflect the range of values

observed in phytoplankton in each of these lakes, suggesting strong reliance of these food webs on pelagic primary production.

Zooplankton collected with an 80 µm net had carbon and nitrogen isotopic ratios (calculated trophic level of 2.0) consistent with a diet of phytoplankton, while zooplankton collected with an 153 µm net had a calculated trophic level of 2.3, suggesting that these samples included both herbivorous and carnivorous zooplankton. *Chaoborus* from this lake appears to be feeding on both phytoplankton and zooplankton in their different instar stages.

For the three species of fish in the lake, $\delta^{15}N$ and $\delta^{13}C$ values were very similar to those observed by Campbell *et al.* (2006). Based on $\delta^{13}C$ values, all fish in Lake Nkuruba appear to be very strongly dependent on planktonic primary carbon. With respect to $\delta^{15}N$ values, *O. leucostictus* tended to have lower values than *T. zilli*, indicating differences in the relative importance of primary producers and primary consumers in the diet of these fish. Meanwhile, *P. reticulata*, a zoobenthivore (Campbell *et al.* 2006), had $\delta^{15}N$ values consistent with its role as a secondary consumer, although given the low $\delta^{13}C$ values observed for this fish, the primary consumers upon which it feeds are likely primarily reliant on pelagic primary production.

Campbell *et al.* (2006) found that in Lake Saka, *L. niloticus* occupied the top trophic position, but did not appear to be relying on haplochromine cichlids as major prey, as they have done in the past in Lake Victoria (Ogutu-Ohwayo 1990). The carbon and nitrogen isotopic ratios observed for fish from Lake Saka in the current study are once again very similar to those observed by Campbell *et al.* (2006). In the current study, *O. niloticus* appears to be feeding on a range of dietary items including phytoplankton, detritus and benthic invertebrates. Surprisingly, one Nile tilapia (*O. niloticus*) had a higher $\delta^{15}N$ value than the highest value observed for *L. niloticus*, which may reflect feeding on fish eggs, or on higher trophic level benthic invertebrates (which is supported by the high $\delta^{13}C$ value observed for this individual).

The haplochromine cichlids sampled in this study (*Astatoreochromis alluaudi* as well as other unidentified haplochromine cichlids) grouped together isotopically, although these fish occupied a broad range of $\delta^{15}N$ and $\delta^{13}C$ values. The isotopic ratios observed within this group suggest that they are feeding on a range of detritus, phytoplankton and primary consumers. While in some lakes *Astatoreochromis alluaudi* is known to feed predominantly on molluscs, in Lake Saka, this species is known to feed on insects, plant matter and possibly yolk-sac brood (Binning *et al.* 2009). Meanwhile the other haplochromines included are known to be mostly primary consumers (Campbell *et al.* 2006). *Lates*

niloticus had somewhat higher $\delta^{15}N$ values than the other fish sampled, although there was a great deal of overlap in $\delta^{15}N$ between *L. niloticus* and haplochromine cichlids, indicating that *L. niloticus* in Lake Saka do not feed exclusively on secondary consumers (e.g. haplochromine cichlids), but are incorporating primary consumers into their diet as well. Also, both the *A. alluaudi* and *Haplochromis* (?) spp. yolk-sac brood had much lower $\delta^{13}C$ values than their parents or than bulk phytoplankton samples, likely as a result of their high lipid content (lipids are known to have lower $\delta^{13}C$ values than muscle tissue, Post *et al.* 2007).

3.4.4 Napoleon Gulf Food Web Structure

The food web structure of Napoleon Gulf based on stable isotope analysis was described by Campbell *et al.* (2003). The current study included a wider range of species than previously sampled. With the exception of *Yssichromis laparograma*, I sampled all species included in Campbell *et al.* (2003), as well as *Astatoreochromis alluaudi*, *Bagrus docmac*, *Brycinus sadleri*, *Mormyrus kannume*, *Oreochromis leucostictus*, *Oreochromis variabilis*, *Synodontis afrofischeri*, and *Synodontis Victoriae*.

In eutrophic systems, benthic production is often greatly reduced (Vadeboncoeur 2003). In Napoleon Gulf, the $\delta^{13}C$ values observed in fish span a range that is comparable to that observed for phytoplankton at this study site; which gives support to the expectation that the food web in this gulf is primarily supported by planktonic primary carbon sources. The differences in $\delta^{13}C$ values in fish are likely a reflection of the inherent seasonal and spatial variability in $\delta^{13}C$ values in phytoplankton from Napoleon Gulf, as well as differences in $\delta^{13}C$ between different phytoplankton taxa (which were not analyzed separately). Also, spatial trends in both $\delta^{13}C$ and $\delta^{15}N$ of particulate organic matter have been observed in Lake Victoria, with higher $\delta^{13}C$ and lower $\delta^{15}N$ values nearshore as compared to offshore; due to differences in phytoplankton biomass, nitrogen fixation rates and mixing depths (Hecky *et al.* 2010). It is very likely that this pattern is also true for the other large lakes sampled (Albert and Edward). As such, differences in stable isotopic ratios between species may reflect differences in preferred feeding locations. Species with particularly enriched $\delta^{13}C$ values included *B. docmac*, *M. kannume*, the three *Oreochromis* spp., *R. argentea* and *T. zilli*. Meanwhile *B. sadleri*, *Haplochromis* sp., *L. niloticus*, *P. aethiopicus*, and the two *Synodontis* spp. had lower $\delta^{13}C$ values.

The three *Oreochromis* species sampled (*O. leucostictus*, *O. niloticus* and the rare *O. variabilis*) had $\delta^{15}N$ values consistent with a diet based on phytoplankton and detritus. *Tilapia zilli* had a calculated trophic level of 2.5, suggesting a diet including phytoplankton/detritus as well as some primary

consumers. Haplochromine cichlids from Napoleon Gulf appeared to have a dietary range consistent with a diet mostly based on primary consumers.

Rastrineobola argentea had $\delta^{15}N$ values that were below what would be expected based on obligate zooplanktivory, which may suggest the inclusion of some lower $\delta^{15}N$ food sources, including phytoplankton/detritus, or chironomids (which had $\delta^{15}N$ values comparable to phytoplankton in Napoleon Gulf). Meanwhile *Brycinus sadleri*, a fish known to primarily consume chironomid pupae (Greenwood 1958), had a $\delta^{15}N$ value consistent with feeding on low $\delta^{15}N$ chironomids. Isotopic ratios for *Mormyrus kannume* grouped tightly together, with $\delta^{15}N$ values suggesting a diet of primary consumers with a narrow range in $\delta^{15}N$ and $\delta^{13}C$ values. *Synodontis afrofischeri*, *S. Victoriae*, and *Astatoreochromis alluaudi* are all known molluscivores, with the *Synodontis* species known to include some insect larvae in their diets (Greenwood 1958, Mbabazi 2004b). This published dietary characterization is consistent with calculated trophic levels based on $\delta^{15}N$ values, which were close to 3 (secondary consumers) for all of these species.

Lates niloticus in Napoleon Gulf had $\delta^{15}N$ values consistent with feeding on primary consumers, with little dietary incorporation of secondary consumers. This is similar to what was observed by Campbell *et al.* (2003). *Protopterus aethiopicus* had a large range in $\delta^{15}N$ values, consistent with a range in prey from invertebrates up to fish. One *P. aethiopicus* specimen had a calculated trophic level of 4.3, suggesting that this fish was feeding on secondary consumers, likely haplochromine cichlids or juveniles of other species (given that *P. aethiopicus* tend to eat smaller fish; Greenwood 1958).

3.4.5 Murchison Bay Food Web Structure

I am not aware of any study to date that has used stable isotope analysis to elucidate food web structure in Murchison Bay in northern Lake Victoria. Both Napoleon Gulf and Murchison Bay have similar species assemblages and many of the same species were sampled in both embayments. In Murchison Bay there was a great deal of overlap in $\delta^{15}N$ values between the species sampled. As such, no obligate feeding relationships emerged, and omnivory is likely very common in the bay. Many of the fish species in Murchison Bay exhibited larger ranges in $\delta^{15}N$ values than in Napoleon Gulf, which is possibly a reflection of the broad range of $\delta^{15}N$ values (4.0–9.2 ‰) observed in phytoplankton from Murchison Bay. Also, it is possible that some fish had been feeding primarily outside of inner Murchison Bay, where there may be different baseline $\delta^{15}N$ values due to reduced influence of anthropogenic nitrogen, and different rates of atmospheric nitrogen fixation.

Despite some species being known to rely more heavily on benthic sources of carbon, in Murchison Bay all fish samples were depleted in ^{13}C relative to phytoplankton (between 0.3 and 3.1 ‰ lower). This may be a further indication of the movement of fish in and out of the inner bay. $\delta^{13}C$ values in the outer bay would be expected to be lower (due to lower phytoplankton biomass and lower associated ^{12}C-limited photosynthesis than in the inner bay), and fish incorporating carbon from outside of the inner bay would reflect this lower $\delta^{13}C$ source. All benthic invertebrates sampled in Murchison Bay had lower $\delta^{13}C$ values than phytoplankton, although given that each of these organisms were sampled on only one occasion, this difference may be due to the high variability in $\delta^{13}C$ of phytoplankton from Murchison Bay.

The three species of tilapiine cichlids sampled (*O. leucostictus*, *O. niloticus* and *T. zilli*) had large ranges in $\delta^{15}N$ values, which could be attributable to differences in their degree of omnivory, as well as differences in the relative amount of time spent feeding outside of the inner bay. The range of $\delta^{15}N$ values observed suggests that some fish sampled were feeding exclusively on phytoplankton, while others were feeding more heavily on primary consumers. There were some fish with $\delta^{15}N$ values that were similar to those observed for phytoplankton in the bay. This may be due to higher levels of feeding by these individual fish on ^{15}N-depleted nitrogen-fixing cyanobacteria, which are known to be an important component of the phytoplankton community in Murchison Bay (see Chapter 2, this thesis). Based on calculated trophic levels, *O. leucostictus* and *T. zilli* appeared to incorporate more primary consumers in their diet than did *O. niloticus*. Haplochromine cichlids in Murchison Bay had a calculated trophic level consistent with a diet based on primary consumers, with the possibility of inclusion of some detritus/phytoplankton in their diets.

As in Napoleon Gulf, *Rastrineobola argentea* had $\delta^{15}N$ values that were lower than expected given that it is known to be a zooplanktivore. However, I purchased these fish in the market and could not confirm their catch location. If they were from outside of inner Murchison Bay, they may have lower $\delta^{15}N$ values due to reduced incorporation of ^{15}N enriched anthropogenic nitrogen sources. Alternatively, they may be ingesting phytoplankton in addition to zooplankton, given that the size range of cyanobacterial colonies can overlap with that of zooplankton.

Calculated trophic levels for *Synodontis afrofischeri* were higher and more variable than for *S. victoriae* in Murchison Bay. Both species exhibited a range in $\delta^{15}N$ values that suggested that while some of these fish were feeding mostly on primary consumers, others appeared to be feeding at a much lower trophic

level. Given the extremely low $\delta^{15}N$ values observed for chironomids for Murchison Bay, it is possible that these form an important source of food for some *Synodontis*.

Protopterus aethiopicus had a mean calculated trophic level of 2.8 in Murchison Bay, consistent with a diet based on primary consumers, likely including both invertebrates and small fish. *Lates niloticus* specimens from Murchison Bay had the largest range in $\delta^{15}N$ values observed for any species sampled in the bay and had calculated trophic level values ranging from 1.6–3.2. There are several factors that are likely influencing this variability including ontogenetic dietary shifts, variability in baseline $\delta^{15}N$ within inner Murchison Bay, and the importance of food sources from outside of the inner bay, where baseline $\delta^{15}N$ is expected to differ.

3.4.6 Lake Mburo Food Web Structure

Mbabazi *et al.* (2004) provided a description of the food web in Lake Mburo based on stable isotope analysis, with results that were very similar to those found in the current study. Calculated trophic levels for fish were consistent with the isotopic and gut content analysis results from Mbabazi *et al.* 2004, with *Clarias gariepinus*, *Bagrus docmac*, *Haplochromis* (?) sp., and *Protopterus aethiopicus* approximately occupying the role of a secondary consumer (TL of 3). Given the wide ranges in carbon and nitrogen isotopic values observed for many of these species (particularly for *C. gariepinus* and *P. aethiopicus*), and the overlapping values between species, omnivory appears to be common in Lake Mburo. The three *Oreochromis* species sampled had $\delta^{15}N$ values that suggested a diet mostly of primary producers and detritus, although the range in $\delta^{15}N$ values within these species suggests variable rates of dietary inclusion of primary consumers by these fish (especially for *O. niloticus*). Also, based on $\delta^{13}C$ values, *O. leucostictus* appeared to be relying more on phytoplankton as compared to the other *Oreochromis* species, whose enriched $\delta^{13}C$ signatures suggest inclusion of more C-4 detritus or benthic algae in their diet, however, these fish may just be reflecting variability in phytoplankton $\delta^{13}C$ values. In addition to *O. niloticus* and *O. esculentus*, *B. docmac* and *Haplochromis* (?) spp., had mean $\delta^{13}C$ values that were enriched relative to phytoplankton, while chironomids had $\delta^{13}C$ values that were depleted relative to phytoplankton.

Three individual fish had isotopic signatures that were unexpected and very different from the other fish observed. One *O. niloticus* had a very high $\delta^{15}N$ value, which may be due to an individual dietary preference for either high $\delta^{15}N$ invertebrates or possibly fish eggs. Meanwhile, one *C. gariepinus* and one *P. aethiopicus* also had outlying stable isotopic ratios. Both of these fish had very depleted $\delta^{13}C$ values

relative to other fish or even the lowest values observed for phytoplankton, while the *C. gariepinus* had a much higher $\delta^{15}N$ value than any other fish sampled. Given the flexible feeding habits of these fish, they may have been consuming eggs from secondary consumers (although even feeding at this trophic level would not account for the high $\delta^{15}N$ value observed for the *C. gariepinus* individual, which had a calculated trophic level of nearly 5). In Lake Saka, I observed that haplochromine cichlid brood had much lower $\delta^{13}C$ values than their parents or other fish, which is likely to be the case in Lake Mburo as well, given that brood are lipid rich, and lipids tend to be isotopically depleted relative to muscle tissue (Post *et al.* 2007).

3.4.7 Lakes George and Edward Food Web Structure

Lake Edward is one of the least studied large lakes of the world. To the northeast, Lake George, a shallow hypereutrophic lake is connected to Lake Edward via the Kazinga channel. To my knowledge, stable isotope analysis has not previously been used to explore food web structure in either lake. Comparing the food web structure of Lakes Edward and George is of considerable interest given that although they have very similar species assemblages (and are connected), the two lakes have very different limnological characteristics (particularly with respect to morphology, chemistry and phytoplankton biomass).

In hypereutrophic Lake George, low light conditions will limit the spatial extent of benthic primary production (Vadeboncoeur *et al.* 2003), and as such, Lake George's food web should be largely based on planktonic primary production. Based on $\delta^{13}C$ values, many fish from Lake George appear to be incorporating carbon that is more isotopically enriched than the phytoplankton samples analyzed. However, given the high variability of $\delta^{13}C$ in phytoplankton and the hypereutrophic conditions in Lake George, which can lead to C-limited photosynthesis, a primarily planktonic diet cannot be discounted for the apparently isotopically-enriched fish in this lake. It is also possible that some fish incorporate C-4 plant detritus from Lake George's extensive papyrus wetlands. Mbabazi *et al.* (2004) found that fish in Lake Mburo appeared to rely on a diet of both phytoplankton and some papyrus detritus, with papyrus detritus tending to have similar $\delta^{13}C$ values and much higher $\delta^{15}N$ values than phytoplankton. However, given that I do not have live or detrital papyrus samples, the possible dietary inclusion of this C-4 plant is difficult to confirm.

In Lake George, tilapiine cichlids all had isotopic signatures consistent with feeding on a mix of phytoplankton, detritus and primary consumers. $\delta^{15}N$ values for *O. niloticus* and *O. leucostictus* indicated that some fish were likely feeding almost exclusively on primary producers, while others were reliant on

primary consumers. Meanwhile, based on $\delta^{15}N$ values, *T. zilli* and *O. esculentus* (a native tilapia) both appeared to be feeding primarily on phytoplankton.

C. gariepinus and *P. aethiopicus* had very similar ranges in $\delta^{13}C$ and $\delta^{15}N$. *C. gariepinus* is known to feed on small fish, mollusks, insects and detritus, while *P. aethiopicus* is known to feed on small fish, insects, and gastropods (Greenwood 1958). The range in $\delta^{15}N$ values observed for these fish was consistent with their published diet, with dietary sources including fish, benthic invertebrates and detritus. There was one *P. aethiopicus* and one *C. gariepinus* that had very depleted $\delta^{13}C$ values when compared to the rest of the food web, these fish also had higher $\delta^{15}N$ values. It is possible that these fish may have migrated from L. Edward, where baseline $\delta^{15}N$ values tend to be higher and $\delta^{13}C$ values tend to be lower. There are also some fish (including one each of *C. gariepinus*, *O. niloticus*, *O. esculentus* and *T. zilli*) that have intermediate values of $\delta^{13}C$, in between what is normally seen for Lake George and what was seen for the two outlying fish described above. These fish may have spent time in both Lake Edward and Lake George, or may be feeding preferentially on phytoplankton that is experiencing higher isotopic discrimination against ^{13}C (or on primary consumers feeding on such phytoplankton).

Bagrus docmac from Lake George exhibited a narrow range of $\delta^{15}N$ and $\delta^{13}C$ values, and appeared to be feeding primarily on small fish with the inclusion of some primary consumers. The haplochromine cichlids (including *Haplochromis squamipinnis* as well as unidentified *Haplochromis* (?) spp.) in Lake George along with *B. docmac* were at the highest trophic level in the lake. Based on $\delta^{15}N$ values, piscivory appears to be common among Lake George's haplochromine cichlids. *H. squamipinnis* is known to eat small fish and insects (Mbabzi *et al.* 2004), a putative diet that is consistent with its $\delta^{13}C$ and $\delta^{15}N$ isotopic values. In this lake, the range in $\delta^{15}N$ for these fish is likely a reflection of the variability in the proportion of the diet that is made up by fish. Based on $\delta^{15}N$ values and calculated trophic level, the unidentified *Haplochromis* (?) sp. appears to be a tertiary consumer that is likely piscivorous based on the food web sampled. One specimen of *Haplochromis* (?) sp. in Lake George that had a much higher $\delta^{15}N$ value than any other fish sampled, suggesting the dietary inclusion of other haplochromine cichlids, or possibly of fish eggs.

In Lake Edward there was a very wide range in $\delta^{13}C$ values. This appears to be due to the influence of inflowing water from Lake George via the Kazinga Channel, with baseline carbon and nitrogen isotopic values in nearshore Lake Edward intermediate between the baseline values observed in Lake George and those observed in offshore Lake Edward. As observed for Lake Victoria by Hecky *et al.* (2010),

phytoplankton from nearshore Lake Edward tended to have higher $\delta^{13}C$ and lower $\delta^{15}N$ than in offshore Lake Edward; likely due to differences in nutrient concentrations, phytoplankton biomass, and mixing depth. The wide range in observed carbon and nitrogen isotopic ratios in Lake Edward's food web suggests that fish are relying on carbon from both nearshore and offshore sources, or are spending time feeding in the Kazinga Channel or Lake George which have higher levels of phytoplankton productivity. It is also possible that toward the enriched end of the $\delta^{13}C$ spectrum, fish may be consuming benthic algal sources of carbon. However, given that in eutrophic lakes, benthic primary production is likely reduced, and since the range in $\delta^{13}C$ observed in fish is within the range of $\delta^{13}C$ observed for phytoplankton from Lake Edward, phytoplankton likely dominates the base of Lake Edward's food web, with fish that have elevated $\delta^{13}C$ values likely feeding primarily in the nearshore of the lake or the Kazinga Channel.

Based on $\delta^{13}C$ values of fish in Lake Edward, isotopically enriched sources of carbon appear to be very important for several species. In particular, *B. bynni*, *B. docmac* and *P. aethiopicus* are feeding primarily on high $\delta^{13}C$ carbon sources (either nearshore or possibly benthic), while *O. niloticus*, *O. leucostictus*, haplochromine cichlids and *C. gariepinus* are more reliant on low $\delta^{13}C$ sources. For zooplankton, the carbon source appeared to be entirely offshore and pelagic, and their $\delta^{15}N$ values were consistent with their role as a primary consumer.

Based on $\delta^{15}N$ values, among tilapiine cichlids, both *O. niloticus* and *O. leucostictus* appeared to have dietary ranges extending from exclusive consumption of phytoplankton/detritus, to exclusive consumption of primary consumers. *Barbus bynii* appeared to rely more heavily on ^{13}C enriched carbon sources than many of the other fish sampled. These fish are known to feed on aquatic plants, mollusks and insects (Greenwood 1958), however, based on their $\delta^{15}N$ signature, they appear to be relying primarily on a diet of primary consumers, rather than a diet heavily based on aquatic plants.

P. aethiopicus, *B. docmac*, and *C. gariepinus* all have wide ranges in carbon isotopic ratios, suggesting dietary variation between individual fish, which is consistent with their known omnivory (Greenwood 1958). Based on their high $\delta^{15}N$ values, these fish appear to be predominantly feeding on fish, or a mix of invertebrates and fish. As in Lake George, the haplochromine cichlids had the highest $\delta^{15}N$ values observed for the whole food web, suggesting a high degree of piscivory for both *H. squamipinnis* and *Haplochromis* (?) sp. Given that mean trophic level values for these cichlids are higher than TL = 4, it is possible that their diets may include other haplochromines, or potentially fish eggs.

Within the species sampled, individual fish exhibited a wide range of $\delta^{13}C$ and $\delta^{15}N$ values, suggesting intra-specific differences in feeding preferences with regard to location (particularly nearshore vs. offshore) and prey.

3.4.8 Food Chain Length

The difference in $\delta^{15}N$ values ($\Delta \delta^{15}N$) between phytoplankton and the fish with the highest mean values can provide an indicator of food chain length. $\Delta \delta^{15}N$ ranged from a low of 4.8 ‰ in Murchison Bay to a high of 11.1 ‰ in Lake Edward (Table 3.3). While differences in $\Delta \delta^{15}N$ values between lakes may reflect real differences in food chain length, they may also reflect spatial differences in baseline $\delta^{15}N$ values and differences in the trophic fractionation of stable nitrogen isotopes. Additionally, herbivory has been shown to be associated with lower nitrogen isotopic fractionation than carnivory (Vander Zanden *et al.* 2001), suggesting that where herbivory is common (e.g. in hypereutrophic lakes with many primary consumers), lower isotopic fractionation may be expected. Aside from the confounding effects of variability of baselines at the within lake level as well as differences in fractionation of stable nitrogen isotopes, $\Delta \delta^{15}N$ may reflect the prevalence of piscivory in top predators and the degree of omnivory in these food webs. In Lakes Edward and George, where $\Delta \delta^{15}N$ values were particularly high, the top predators appeared to be feeding very heavily on tertiary consumers (likely fish). While, in Murchison Bay, the extremely low $\Delta \delta^{15}N$ value may reflect either a high degree of omnivory in the top predators of this lake, or preferential feeding on herbivorous fish by piscivores. However, due to the high variability of phytoplankton $\delta^{15}N$ in Murchison Bay, and the fact that fish may be feeding outside of the sewage enriched (high $\delta^{15}N$) inner bay where phytoplankton samples were collected, it is difficult to be confident that the $\delta^{15}N$ values that I am using as the "base" of the food web are accurate.

Food chain length can also be calculated as the number of trophic levels between primary producers and top predators by assuming consistent isotopic fractionation of nitrogen from one trophic level to the next. While this is somewhat artificial given that fractionation is known to vary widely both within and between systems (Vander Zanden *et al.* 2001 and see above), 3.4 ‰ is a widely used value in the literature that allows for between system comparisons.

There are several hypotheses concerning the factors that determine food chain length in aquatic systems (see Post *et al.* 2000, Post *et al.* 2007, Vander Zanden *et al.* 2007). Many studies have suggested that available energy (productivity) limits the number of trophic levels (these studies are reviewed in Vander Zanden *et al.* 2007). Ecosystem size has also been suggested as a predictor of FCL, where larger

ecosystems support longer food chains (Vander Zanden *et al.* 1999, Post *et al.* 2000). The productive space hypothesis suggests that food chains will be longer in lakes that have higher productivity × ecosystem size (Schoener 1989, Vander Zanden *et al.* 2007). However, when the calculated food chain lengths in the current study were regressed against productivity (both chlorophyll *a* and total phosphorus concentrations; Table 3.1), ecosystem size (lake area; Table 3.1), and productivity × ecosystem size, no statistically significant relationships emerged, suggesting that these factors are not important drivers of food chain length in these lakes.

Vander Zanden *et al.* (2007) explored global patterns in food chain lengths for lakes, streams and marine systems, and found a mean FCL in lakes of 3.95 ± 0.5. In the current study, mean FCL was 3.5 ± 0.5 trophic levels (range: 2.8–4.3), similar to that observed by Vander Zanden *et al.* (2007), although slightly lower, which is consistent with the global study's (not statistically significant) observation of lower food chain lengths in the tropics than in temperate and arctic lakes (Vander Zanden *et al.* 2007). This pattern is likely due to the prevalence of both herbivory and omnivory in these productive tropical lakes relative to higher latitude lakes (Jeppesen *et al.* 2010), however the Ugandan ichthyofauna is known to include several strongly piscivorous fish (Greenwood 1958).

3.4.9 Comparing trophic levels within species among lakes

As with calculated food chain lengths, calculated trophic levels may be strongly influenced by inaccurate estimates of baseline organism $\delta^{15}N$ values and by isotopic fractionation rates that differ from the assumed rate of 3.4 ‰ per trophic level for $\delta^{15}N$. However, the ability to compare calculated trophic level for a given species within sites is particularly appealing, as long as possible sources of error are not ignored.

Differences in calculated trophic level between sites were observed for several species. For *P. aethiopicus*, which had a higher trophic level in Lake George than in Murchison Bay, this may be due to increased piscivory in Lake George as compared to Murchison Bay. However, in Murchison Bay, the baseline $\delta^{15}N$ value was based on organisms collected in inner Murchison Bay, which receives ^{15}N enriched sewage, while large mobile organisms may be feeding outside of the inner bay, where the effect of sewage on $\delta^{15}N$ of phytoplankton is expected to be less pronounced. However, this is further complicated by the opposite effect that mixing depth has on baseline isotopic ratios, with phytoplankton from deeper-mixing offshore sites tending to have higher baseline $\delta^{15}N$ values than nearshore due to decreased rates of atmospheric nitrogen fixation (Hecky *et al.* 2010). This makes it difficult to ascertain

whether differences in trophic level for a species between sites reflect dietary differences or differences in $\delta^{15}N$ baseline values for individual fish, which are overlooked by assuming a standard baseline value at a study site. This may have been the case for *S. victoriae*, which had a higher calculated trophic level in Napoleon Gulf than in Murchison Bay, even though they may have had identical diets in both embayments. Similarly, the low calculated trophic levels for *L. niloticus* in Murchison Bay relative to other sites could be indicative of time spent feeding outside of inner Murchison Bay rather than an actual difference in trophic level. The high calculated trophic levels for *L. niloticus* from Lake Saka were likely due to the dietary inclusion of haplochromine cichlids, which are small secondary consumers in Lake Saka. This contrasts with *L. niloticus* in Lakes Albert, Murchison Bay and Napoleon Gulf, where these fish seem to be feeding mostly on primary consumers. These observations are consistent with the results of dietary studies in northern Lake Victoria, which have described a shift in a diet based primarily on haplochromine cichlids to higher levels of omnivory and reliance on the prawn *Caridina nilotica* (Ogutu-Ohwayo 1990).

The significant negative relationships between trophic level and total length for both *O. leucostictus* and *T. zilli*, explain why for *O. leucostictus* calculated trophic level was highest in Lakes Albert and Nkuruba, where fish were the smallest of any site. Similarly, *T. zilli* were smallest in Lake Nkuruba, and had the highest mean TL of any site. These fish may be feeding more heavily on benthic invertebrates while at small sizes, and shifting to a diet based more heavily on phytoplankton and detritus as they grow. However, another possible explanation for the negative relationship observed between calculated trophic level and total length for these species is that the smaller fish were sampled in higher transparency lakes (see Chapter 2, this thesis), where benthic primary productivity would be expected to be higher (Vadeboncoeur *et al.* 2003). *O. niloticus* appeared to be feeding at lower trophic levels in Napoleon Gulf and Murchison Bay than in Lakes Edward, Mburo or Saka. This may either be as a result of increased dietary reliance on low $\delta^{15}N$ cyanobacteria, or in the case of Murchison Bay, may be due to feeding outside of these embayments where there would be less influence of high $\delta^{15}N$ nitrogen from human waste.

The differences in calculated trophic level (and total length) for the unidentified haplochromine cichlids are likely due to the fact that I was sampling different species in the different lakes, with piscivorous haplochromines in Lakes George and Edward (TL = 4), and fish with calculated trophic levels of approximately 3 in the other lakes, but with likely differences in dietary preferences, size, and morphology. The sizes and types of haplochromine cichlids caught in these lakes were also reflective of

sampling style, with gill-nets used in Lakes Mburo, Edward and George, fish traps used in Lake Saka, and primarily as by-catch from the *Rastrineobola argentea* fishery in northern Lake Victoria.

The strong (nearly 1:1) relationship between $\delta^{13}C$ of phytoplankton and $\delta^{13}C$ of tilapiine cichlids across all study sites suggests that tilapiine cichlids are strongly reliant on pelagic phytoplankton as a food source. This is further supported by the strong relationship between $\delta^{15}N$ values of both phytoplankton and tilapiine cichlids across lakes.

3.4.10 Ontogenetic dietary shifts

Some fish (such as *Lates*) are known to experience ontogenetic dietary shifts as they grow and are able to consume larger prey, while others are not expected to experience such shifts (Campbell *et al.* 2003). For all *Lates* spp. from all sites with the exception of Lake Saka, positive relationships between $\delta^{15}N$ and total length suggested that these fish were shifting from lower trophic level prey to higher trophic level prey as they grew. These relationships were not always statistically significant; however, regression slopes for these relationships did not generally differ between sites. In Lake Saka, there was no relationship observed between total length and $\delta^{15}N$ for *L. niloticus*, which suggests that in this lake these fish are not experiencing significant dietary shifts with growth, however, only 4 individual fish were collected from this lake, making it difficult to draw any conclusions about dietary shifts with growth. Also, as previously outlined, in Lake Saka, *L. niloticus* appeared to have different dietary habits than at other study sites.

In Lake Edward, both *P. aethiopicus* and *Haplochromis* sp. experienced an increase in $\delta^{15}N$ values with total length, likely due to increasing reliance on piscivory. A concurrent decrease in $\delta^{13}C$ values with growth may also reflect that larger fish tend to rely more on food sources from offshore Lake Edward than from the nearshore. These relationships were not observed in other lakes suggesting that this relationship is not universal. In Lake Saka, the significant positive relationship between $\delta^{13}C$ and total length for *O. niloticus* suggests that as these fish grow, they incorporate more ^{12}C limited sources of carbon in their diet (possibly benthic, or C-limited pelagic sources). In Lake Albert, where significant positive relationships were observed between $\delta^{13}C$ and total length (for *L. macrophthalamus* and *S. intermedius*) there were few replicates, and the range in $\delta^{13}C$ values was not very broad (Table 3.2), making it difficult to ascertain the importance of these observed relationships. However, these trends may reflect an increase in reliance on nearshore carbon, which is likely to be enriched in ^{13}C relative to offshore carbon as was observed in the current study for Lake Edward, and has been previously reported for Lake Victoria (Hecky *et al.* 2010).

The significant positive relationships observed between $\delta^{15}N$ and total length for *O. niloticus* from Lakes George, Mburo and Murchison Bay are possibly due to increasing dietary incorporation of primary consumers, or more ^{15}N enriched phytoplankton as these fish grow. Based on differences in slopes of the $\delta^{15}N$–total length relationships, in Murchison Bay, *O. niloticus* did not experience as rapid of a shift in $\delta^{15}N$ values with growth as in Lakes George and Mburo, which may reflect that as fish grow, they feed more outside of the inner bay on lower $\delta^{15}N$ nitrogen sources, alternatively, they may be preferentially ingesting N-fixing cyanobacteria in Murchison Bay. Given the differences between sites in the slopes and directions of regressions between $\delta^{15}N$ and total length, there do not appear to be universal trends in dietary shifts with growth among *O. niloticus*.

3.4.11 General Conclusions

The food webs observed for the Ugandan lakes included in this study had several general characteristics: with the exception of Lakes George and Edward, predominantly piscivorous fish appeared to be rare, meanwhile omnivory was very common, with few obligate feeding relationships observed. There were also strong differences in baseline $\delta^{15}N$ and $\delta^{13}C$ between sites. Differences in $\delta^{15}N$ were dependent on several factors, including the influence of high $\delta^{15}N$ human waste, rates of atmospheric N-fixation by cyanobacteria, and the prevalence of other nitrogen sources. Baseline $\delta^{13}C$ values were largely dependent on the degree of carbon isotopic fractionation during photosynthesis, with high phytoplankton biomass and growth rates tending to lead to reduced isotopic discrimination during photosynthesis due to instantaneous carbon limitation. This study would have benefitted from better characterization of stable isotopic ratios in long-lived primary consumers, or more frequent measurements of phytoplankton in order to determine reliable baseline values that account for strong spatial and temporal variation.

The sites included in this study were all eutrophic or hypereutrophic, with the exception of mesotrophic Lake Nkuruba. Stable isotope ratios in fish generally reflected a dietary reliance on planktonic primary production (with $\delta^{13}C$ values of phytoplankton spanning the range of the consumer food web at all study sites), with no strong evidence that benthic carbon plays an important role in supporting these food webs. This is consistent with the general expectation that in eutrophic lakes, benthic primary production tends to be reduced due to high planktonic chlorophyll *a*, reduced transparency, and a concurrent decrease in substrate that is adequately lit for benthic photosynthesis (Vadeboncoeur *et al.* 2003). Decreased transparency can also increase the prevalence of omnivory through reducing the selectivity of foraging.

Given the strong similarities in food web structure and dietary linkages observed in the current study with previously reported results from Napoleon Gulf, crater lakes Saka and Nkuruba, Lake Mburo and Lake Albert, there appears to be temporal stability in the food webs of these systems. While in Murchison Bay, Lake Edward and Lake George this study provides important information about the aquatic food webs at these sites which can help to inform fisheries management decisions and can act as a baseline to which potential future changes in species assemblages or trophic relationships can be compared. Results of stable isotope analysis and knowledge of food web structure in these lakes can also provide context for the assessment of accumulation and trophic transfer of compounds including mercury (Chapter 4, this thesis) and microcystin (Chapter 5, this thesis). The fact that in all study lakes (including mesotrophic Lake Nkuruba), food webs appeared to be supported primarily by phytoplankton is of direct relevance to the potential for food web exposure to microcystin. Given that in these study lakes *Microcystis* spp. (a planktonic cyanobacterial taxa) appears to be the dominant microcystin producer (Chapter 2, this thesis), the reduced importance of benthic trophic pathways in these lakes may reduce the ability of the consumer food web to lower their exposure to microcystin through alternative feeding strategies.

Table 3.1 Selected site characteristics of study lakes (all data in this table are taken from Chapter 2 of this thesis, with the exception of Lake Albert, where data are taken from Mugidde *et al.* 2007). Total phosphorus and chlorophyll *a* concentrations for Lake Edward are for the offshore station. These values were used to explore potential relationships between calculated food chain length (FCL) and lake productivity and size.

Lake	Area (km^2)	Total Phosphorus (μg/L)	Chlorophyll *a* (μg/L)
Lake Albert	5600	32.3 ± 2.4	19.2 ± 3.4
Lake Edward	2325	58.9 ± 9.2	21.3 ± 22.8
Lake George	250	186.5 ± 26.2	138.0 ± 39.1
Lake Mburo	13	106.8 ± 11.1	48.6 ± 10.1
Inner Murchison Bay (Victoria)	18 (66368)	100.3 ± 22.5	96.5 ± 38.1
Napoleon Gulf (Victoria)	26.5 (66368)	60.0 ± 16.2	24.7 ± 18.4
Lake Nkuruba	0.03	35.6 ± 8.6	6.2 ± 2.2
Lake Saka	0.15	175.0 ± 32.2	89.0 ± 36.3

Table 3.2 Results of stable isotope analysis and trophic level calculations for fish and food web samples. Published diet is based on Greenwood (1958), and dietary items are labeled as follows: Ph: photosynthesis, P: phytoplankton, Z: zooplankton, HNF: hetertrophic nanoflagellates, I: insects, BI: benthic invertebrates, M: macrophytes, Mo: mollusks, F: fish, PB: benthic "algae", D: detritus, G: gastropods. Asterisks indicate that each replicate represents 5 fish pooled. Each benthic invertebrate sample also represents several pooled individuals.

Name	n	Code	Published Diet	$\delta^{15}N$ (‰) $\mu \pm$ s.d	$\delta^{13}C$ (‰) $\mu \pm$ s.d.	Trophic Level $\mu \pm$ s.d.
Lake Albert						
Alestes baremose	1	Ab	Z, I, F	8.9	-20.0	2.9
Barbus bynii	2	Bb	M, Mo, I	7.9–7.9	-19.9 to -19.2	2.6–2.6
Bagrus bayad	2	Bba	F	8.6–9.4	-18.3 to -17.8	2.8–3.1
Brycinus nurse	13	Bnu	Z	9.1 ± 0.3	-19.0 ± 0.6	3.0 ± 0.1
Hydrocynus forskahlii	2	Hf	F	9.3–10.2	-18.6 to -18.6	3.1–3.3
Labeo horie	1	Lh	D	8.1	-19.3	2.7
Lates macrophthalamus	4	Lm	F, BI	9.4 ± 0.9	-19.4 ± 0.6	3.1 ± 0.3
Lates niloticus	6	Ln	F, BI, Z, P	9.5 ± 0.9	-19.1 ± 0.6	3.1 ± 0.3
Oreochromis leucostictus	2	Ol	P, D	7.3–8.5	-19.0 to -16.1	2.5–2.8
Oreochromis niloticus	1	On	P, D, BI	6.4	-18.5	2.2
Neobola bredoi	6	Rb	Z	8.7 ± 0.4	-19.7 ± 0.5	2.9 ± 0.1
Schilbe intermedius	3	Si	F, I	8.6 ± 0.2	-19.5 ± 0.1	2.8 ± 0.1
Tilapia zilli	6	Tz	P, PB, D, M	6.6 ± 0.7	-15.8 ± 2.3	2.3 ± 0.2
Lake Edward						
Barbus bynni	9	Bb	M, Mo, I	8.6 ± 0.7	-12.1 ± 1.2	3.1 ± 0.2
Bagrus docmac	9	Bd	BI, F	11.4 ± 1.5	-14.2 ± 2.4	3.9 ± 0.5
Clarias gariepinus	9	Cg	F, Mo, I, D	9.7 ± 1.3	-15.2 ± 2.0	3.4 ± 0.4
Haplochromis (?) spp.	6	H	I	12.0 ± 1.6	-15.4 ± 2.0	4.1 ± 0.5
Haplochromis squamipinnis	10	Hs	F, I	12.6 ± 1.1	-16.2 ± 2.0	4.3 ± 0.3
Oreochromis leucostictus	4	Ol	P, D	6.5 ± 0.4	-16.6 ± 0.9	2.5 ± 0.1
Oreochromis niloticus	15	On	P, D, BI	6.9 ± 1.0	-17.1 ± 1.2	2.6 ± 0.3
Protopterus aethiopicus	10	Pa	I, G, F	9.2 ± 0.8	-13.3 ± 2.4	3.3 ± 0.2
Nearshore phytoplankton	4	p_n	Ph	-1.4 ± 0.6	-11.3 ± 2.4	1
Offshore phytoplankton	5	p_o	Ph	1.5 ± 0.7	-19.0 ± 2.8	1
Zooplankton (>80 µm)	3	z_{80}	P, Z, HNF	4.0 ± 0.9	-20.0 ± 2.8	1.7 ± 0.3
Zooplankton (>153 µm)	3	z_{153}	P, Z, HNF	4.5 ± 0.7	-21.0 ± 1.7	1.9 ± 0.2
Lake George						
Bagrus docmac	10	Bd	BI, F	8.3 ± 0.7	-8.4 ± 1.6	3.8 ± 0.2
Clarias gariepinus	9	Cg	F, Mo, I, D	6.2 ± 1.2	-9.7 ± 6.1	3.2 ± 0.3
Haplochromis (?) spp.	7	H	I	8.6 ± 1.1	-7.1 ± 2.5	3.9 ± 0.3
Haplochromis squamipinnis	6	Hs	F, I	7.4 ± 1.1	-8.6 ± 2.1	3.6 ± 0.3
Oreochromis esculentus	2	Oe	P, D	2.8–2.9	-16.7 to -9.5	2.2–2.2
Oreochromis leucostictus	6	Ol	P, D	3.6 ± 0.7	-10.1 ± 2.1	2.5 ± 0.2
Oreochromis niloticus	18	On	P, D, BI	2.7 ± 0.7	-5.9 ± 2.6	2.2 ± 0.2
Protopterus aethiopicus	9	Pa	I, G, F	6.8 ± 1.0	-10.5 ± 6.4	3.4 ± 0.3
Tilapia zilli	1	Tz	P, PB, D, M	3.0	-16.0	2.3
Phytoplankton	5	p	Ph	-1.3 ± 0.4	-9.6 ± 1.6	1

Name	n	Code	Published Diet	$\delta^{15}N$ (‰) μ ± s.d	$\delta^{13}C$ (‰) μ ± s.d.	Trophic Level μ ± s.d.
Chaoborus	1	c	Z	3.8	-9.4	2.5
Lake Mburo						
Bagrus docmac	1	Bd	BI, F	6.0	-10.5	2.9
Clarias gariepinus	7	Cg	F, Mo, I, D	7.2 ± 2.4	-12.8 ± 2.9	3.3 ± 0.7
Haplochromis (?) spp.	10	H	I, Z, P	6.0 ± 0.7	-10.8 ± 0.8	2.9 ± 0.2
Oreochromis esculentus	10	Oe	P, D	4.1 ± 0.5	-10.6 ± 0.9	2.3 ± 0.1
Oreochromis leucostictus	10	Ol	P, D	4.1 ± 0.6	-13.5 ± 1.1	2.4 ± 0.2
Oreochromis niloticus	15	On	P, D, BI	4.8 ± 1.5	-11.0 ± 1.2	2.6 ± 0.4
Protopterus aethiopicus	10	Pa	I, G, F	6.3 ± 1.7	-12.9 ± 3.1	3.0 ± 0.5
Phytoplankton	6	p	Ph	-0.5 ± 0.2	-12.6 ± 1.3	1
Chironomidae	1	ch	P, D, BI	2.4	-14.3	1.8
Lake Victoria (Murchison Bay)						
Clarias gariepinus	1	Cg	F, Mo, I, D	8.8	-18.7	2.3
Haplochromis (?) spp.	9	H	P, I	9.8 ± 1.1	-16.7 ± 0.8	2.6 ± 0.3
Lates niloticus	18	Ln	F, I, BI, Z, P	9.4 ± 1.6	-16.2 ± 1.2	2.5 ± 0.5
Oreochromis leucostictus	5	Ol	P, D	8.5 ± 1.1	-17.1 ± 0.8	2.2 ± 0.3
Oreochromis niloticus	24	On	P, D, BI	7.7 ± 1.5	-16.3 ± 1.5	2.0 ± 0.4
Protopterus aethiopicus	9	Pa	I, G, F	10.5 ± 1.1	-16.1 ± 1.3	2.8 ± 0.3
Rastrineobola argentea	1*	Ra	Z	8.6	-15.9	2.3
Synodontis afrofischeri	8	Sa	Mo, BI	10.2 ± 1.5	-17.0 ± 0.6	2.7 ± 0.5
Synodontis victoriae	7	Sv	Mo, BI	9.0 ± 0.8	-16.1 ± 2.3	2.4 ± 0.2
Tilapia zilli	7	Tz	P, PB, D, M	8.7 ± 1.3	-17.0 ± 1.5	2.3 ± 0.4
Phytoplankton	21	p	Ph	5.6 ± 1.0	-15.6 ± 1.5	1.4
Zooplankton (>80 μm)	1	z_{80}	P, Z, HNF	7.6	-15.3	2.0
Zooplankton (>153 μm)	1	z_{153}	P, Z, HNF	7.7	-15.3	2.0
Mayflies (*Povilla*)	1	g	P, D	6.1	-19.1	1.5
Leeches	1	h	BI	9.2	-17.3	2.4
Snails (*Bellamya*)	1	b	P	7.7	-17.8	2.0
Chironomidae	1	ch	P, D, BI	3.7	-17.6	0.8
Lake Victoria (Napoleon Gulf)						
Astatoreochromis alluaudi	1	Aa	Mo	7.4	-16.0	2.8
Bagrus docmac	1	Bd	BI, F	9.3	-14.2	3.4
Brycinus sadleri	1	Bs	I	6.5	-16.7	2.5
Haplochromis (?) spp.	8	H	I, Z, P	7.6 ± 0.7	-15.4 ± 1.5	2.8 ± 0.2
Lates niloticus	23	Ln	F, I, BI, Z, P	7.9 ± 1.0	-15.3 ± 1.2	2.9 ± 0.3
Mormyrus kannume	5	Mk	BI	8.1 ± 0.3	-14.6 ± 0.5	3.0 ± 0.1
Oreochromis leucostictus	2	Ol	P, D	4.3–4.6	-15.4 to -12.9	1.9–2.0
Oreochromis niloticus	24	On	P, D, BI	4.6 ± 0.7	-13.8 ± 1.0	2.0 ± 0.2
Oreochromis variabilis	9	Ov	P, D	4.0 ± 0.5	-14.5 ± 0.6	1.8 ± 0.2
Protopterus aethiopicus	10	Pa	I, G, F	8.2 ± 1.7	-16.4 ± 0.9	3.0 ± 0.5
Rastrineobola argentea	5*	Ra	Z	6.9 ± 0.4	-14.0 ± 0.8	2.6 ± 0.1
Synodontis afrofischeri	3	Sa	Mo, BI	8.3 ± 0.1	-15.4 ± 0.04	3.1 ± 0.02
Synodontis victoriae	4	Sv	Mo, BI	7.5 ± 0.4	-16.9 ± 0.3	2.8 ± 0.1
Tilapia zilli	10	Tz	P, PB, D, M	6.4 ± 1.3	-13.6 ± 1.5	2.5 ± 0.4
Phytoplankton	10	p	Ph	1.3 ± 1.0	-16.9 ± 2.5	1.0
Zooplankton (>80 μm)	3	z_{80}	P, Z, HNF	5.3 ± 0.4	-15.8 ± 1.8	2.2 ± 0.1

Name	n	Code	Published Diet	$\delta^{15}N$ (‰) μ ± s.d	$\delta^{13}C$ (‰) μ ± s.d.	Trophic Level μ ± s.d.
Zooplankton (>153 μm)	3	z_{153}	P, Z, HNF	5.2 ± 0.3	-15.3 ± 2.0	2.2 ± 0.1
Chironomidae	1	ch	P, D, BI	2.8	-17.2	1.4
Lake Nkuruba						
Oreochromis leucostictus	5	Ol	P, D	6.1 ± 0.6	-28.1 ± 0.9	2.6 ± 0.2
Poecelia reticulata	1*	Pr	BI	7.9	-28.2	3.2
Tilapia zilli	9	Tz	P, PB, M	7.5 ± 1.3	-26.0 ± 0.9	3.0 ± 0.4
Phytoplankton	6	p	Ph	0.5 ± 0.5	-27.8 ± 0.7	1.0
Epilithic Phytoplankton	1	p_b	Ph	2.0	-11.3	1.4
Zooplankton (>80 μm)	6	z_{80}	P, Z, HNF	3.9 ± 0.8	-28.7 ± 1.0	2.0 ± 0.2
Zooplankton (>153 μm)	6	z_{153}	P, Z, HNF	5.1 ± 0.5	-29.1 ± 0.7	2.3 ± 0.2
Chaoborus	1	c	Z	5.8	-26.4	2.5
Snails	1	g	P	3.0	-26.7	1.7
Lake Saka						
Astatoreochromis alluaudi	10	Aa	Mo	5.7 ± 0.7	-20.1 ± 1.0	3.1 ± 0.2
Barbus neumayerii	1	Bn	BI, M, D	5.7	-23.8	3.1
Haplochromis (?) spp.	10	H	I, Z, P	5.3 ± 0.7	-20.5 ± 0.8	3.0 ± 0.2
Lates niloticus	4	Ln	F	7.3 ± 0.4	-19.7 ± 0.3	3.6 ± 0.1
Oreochromis niloticus	16	On	P, D, I	3.1 ± 1.3	-18.9 ± 0.8	2.4 ± 0.4
Tilapia zilli	2	Tz	P, PB, D, M	2.9–4.1	-21.2 to -18.8	2.3–2.7
Phytoplankton	5	p	Ph	-1.6 ± 0.2	-20.7 ± 1.5	1.0
A. alluaudi yolk-sac brood	1	aa_b	Yolk	4.7	-21.9	2.9
Haplochromis (?) spp. yolk-sac brood	1	h_b	Yolk	3.6	-25.0	3.1

Table 3.3 Summary of baseline and top predator organisms used in trophic level (TL) and food chain length (FCL) calculations (see text for equations). "δ^{15}N Range" refers to the difference between the δ^{15}N values of top piscivores and of phytoplankton at a study site.

Lake	Baseline Organism	Baseline Organism Trophic Level	Top Predator	δ^{15}N Range (‰)	Food Chain Length
Lake Albert	*Brycinus nurse*	3	Not Determined	~	~
Lake Edward	Phytoplankton	1	*Haplochromis squamipinnis*	11.1	4.3
Lake George	Phytoplankton	1	*Haplochromis* spp.	9.9	3.9
Lake Mburo	Phytoplankton	1	*Clarias gariepinus*	7.7	3.3
Murchison Bay	Zooplankton (153 µm)	2	*Protopterus aethiopicus*	4.8	2.8
Napoleon Gulf	Phytoplankton	1	*Bagrus docmac*	8.0	3.4
Lake Nkuruba	Phytoplankton	1	*Poecelia reticulata*	7.4	3.2
Lake Saka	Phytoplankton	1	*Lates niloticus*	8.9	3.6

Table 3.4 Summary of significant relationships observed between δ^{15}N, δ^{13}C and total length (L).

Lake	Species	δ^{15}N vs. L	r^2_{adj}	P	δ^{13}C vs. L	r^2_{adj}	P
Albert	*L. macrophthalamus*	+	0.96	<0.05	+	0.99	<0.01
Albert	*S. intermedius*				+	0.99	<0.05
Edward	*Haplochromis* sp.	+	0.67	<0.05	-	0.72	<0.05
Edward	*P. aethiopicus*	+	0.64	<0.01	-	0.50	<0.05
George	*O. niloticus*	+	0.24	<0.05			
Mburo	*O. niloticus*	+	0.27	<0.05			
Murchison	*O. niloticus*	+	0.22	<0.05			
Napoleon	*L. niloticus*	+	0.40	<0.01			
Nkuruba	*O. leucostictus*				-	0.73	<0.05
Saka	*O. niloticus*				+	0.35	<0.01

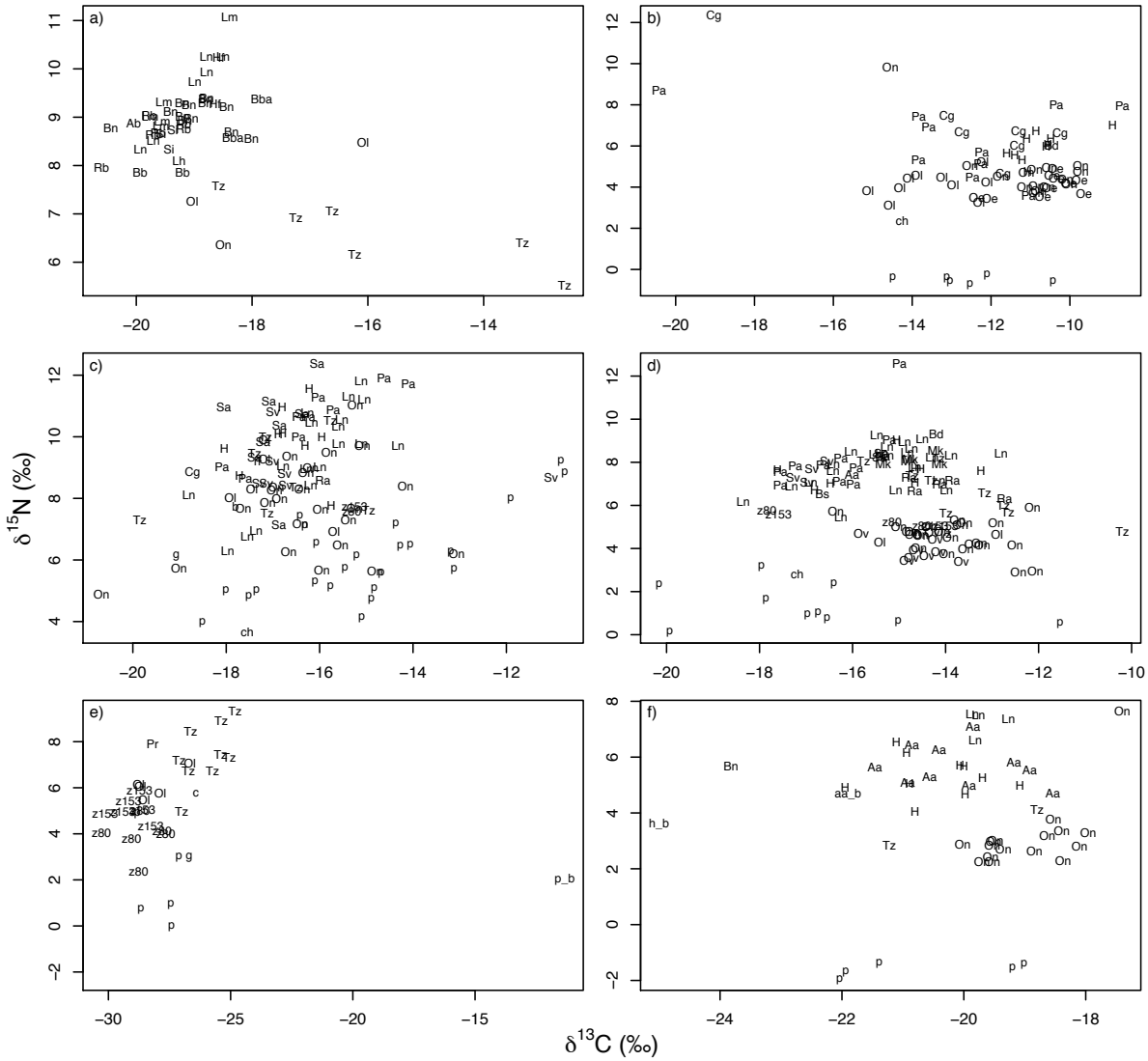

Figure 3.1 Graphical representation of food web structure using stable carbon and nitrogen isotopic ratios for a) Lake Albert, b) Lake Mburo, c) Murchison Bay, d) Napoleon Gulf, e) Lake Nkuruba, and f) Lake Saka. Codes used as labels are found in Table 2.

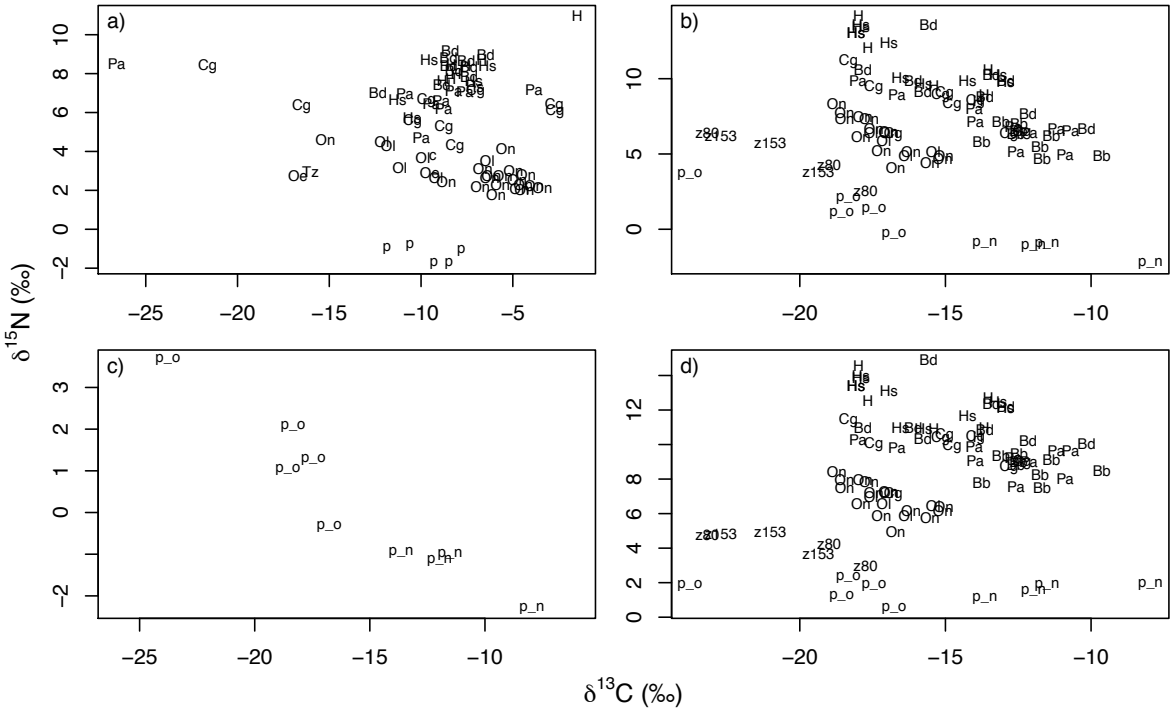

Figure 3.2 Graphical representation of food web structure using stable carbon and nitrogen isotopic ratios for a) Lake George, and b) Lake Edward (with uncorrected $\delta^{15}N$ values). Regression of $\delta^{15}N$ vs. $\delta^{13}C$ for Lake Edward phytoplankton from nearshore (p_n) and offshore (p_o) sampling sites is shown in panel c) ($r^2_{adj} = 0.92$, $P<0.001$). The slope of the regression was used to correct Lake Edward $\delta^{15}N$ values (see Equation 3.4 in text). Graphical representation of food web structure using stable carbon and nitrogen isotopic ratios for Lake Edward with corrected $\delta^{15}N$ values is shown in panel d). Codes used as labels are found in Table 3.2.

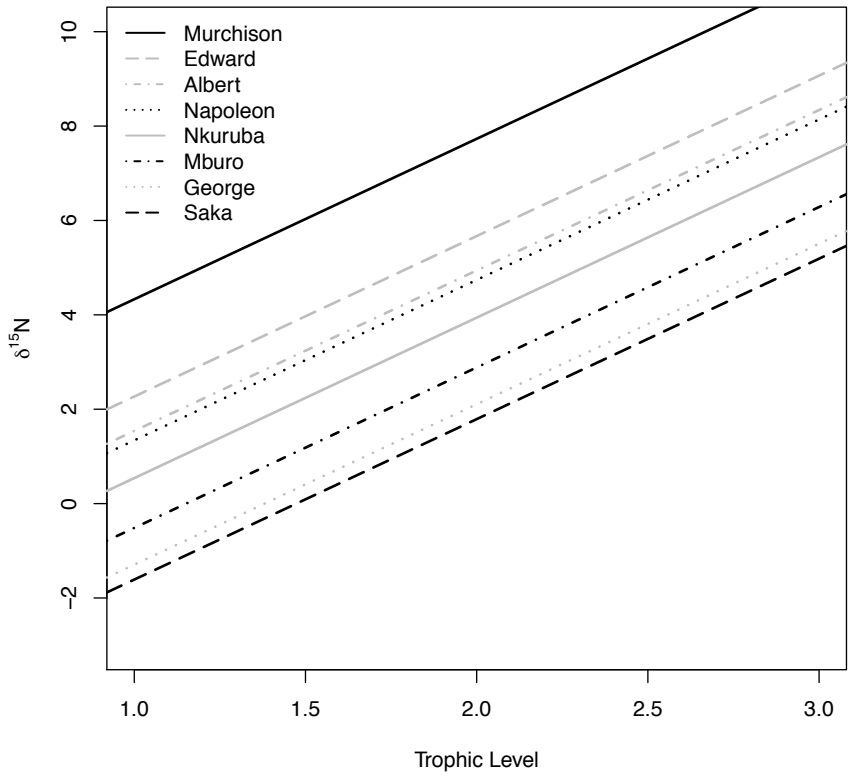

Figure 3.3 Regressions between $\delta^{15}N$ and trophic level for each study site. Differences in intercepts signify differences in baseline $\delta^{15}N$ values between sites. Since trophic level was calculated using an assumed increase in $\delta^{15}N$ of 3.4 ‰ per trophic level, the slopes of these regressions are all 3.4 ‰ / trophic level.

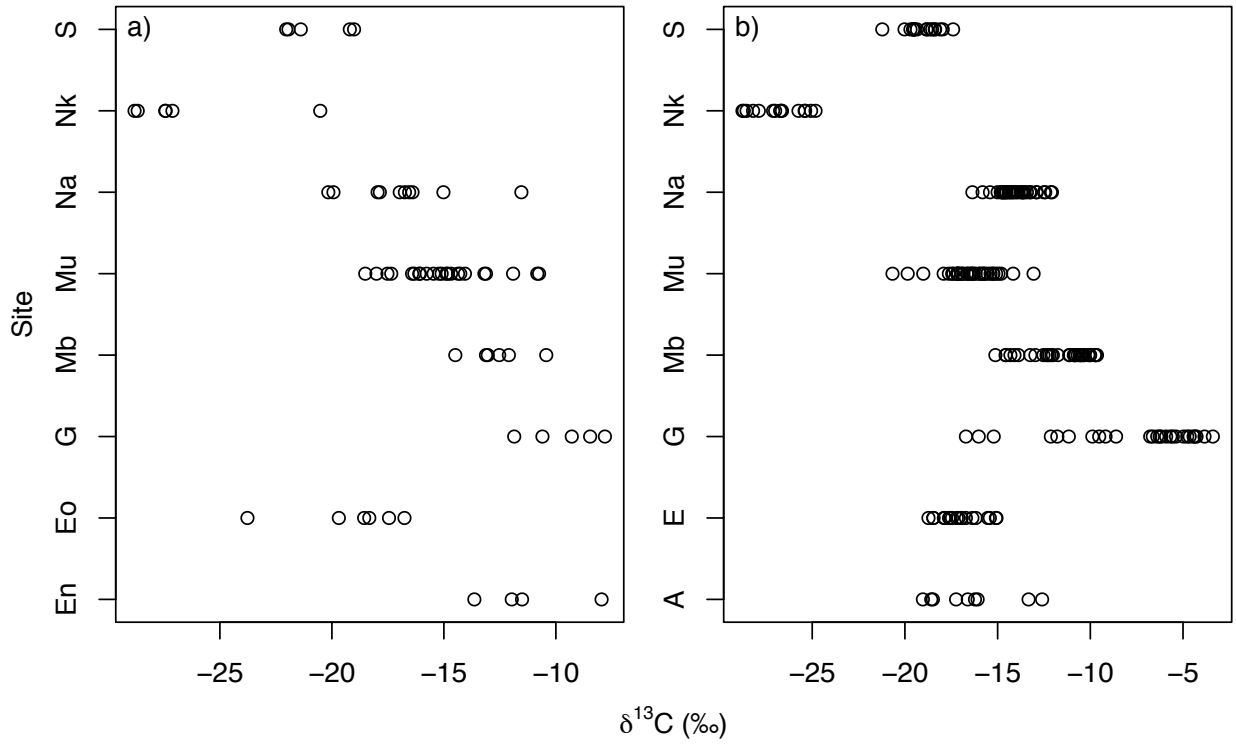

Figure 3.4 $\delta^{13}C$ values for a) phytoplankton and b) tilapiine cichlids from each study site (A=Albert, E=Edward mean (En=Edward nearshore, Eo=Edward offshore), G=George, M=Murchison Bay, Mb=Mburo, Na=Napoleon Gulf, Nk=Nkuruba, S=Saka).

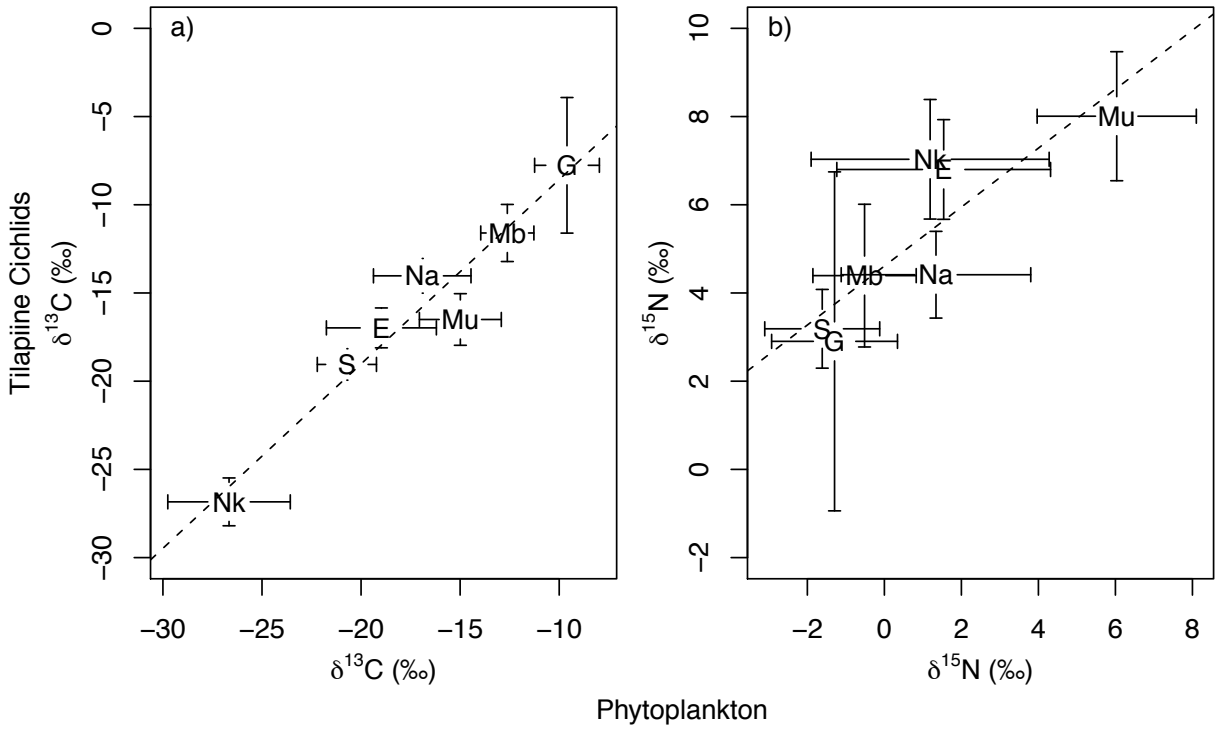

Figure 3.5 Linear regressions of a) $\delta^{13}C$ values of tilapiine cichlids against $\delta^{13}C$ values of phytoplankton (slope: 1.05, $r^2_{adj} = 0.93$, $P<0.001$); and b) $\delta^{15}N$ values of tilapiine cichlids against $\delta^{15}N$ values of phytoplankton (slope: 0.67, $r^2_{adj} = 0.68$, $P<0.05$). Labels represent sites (A=Albert, E=Edward, G=George, M=Murchison Bay, Mb=Mburo, Na=Napoleon Gulf, Nk=Nkuruba, S=Saka). The data points represent mean isotopic ratios, while the error bars represent standard deviation in isotopic ratios.

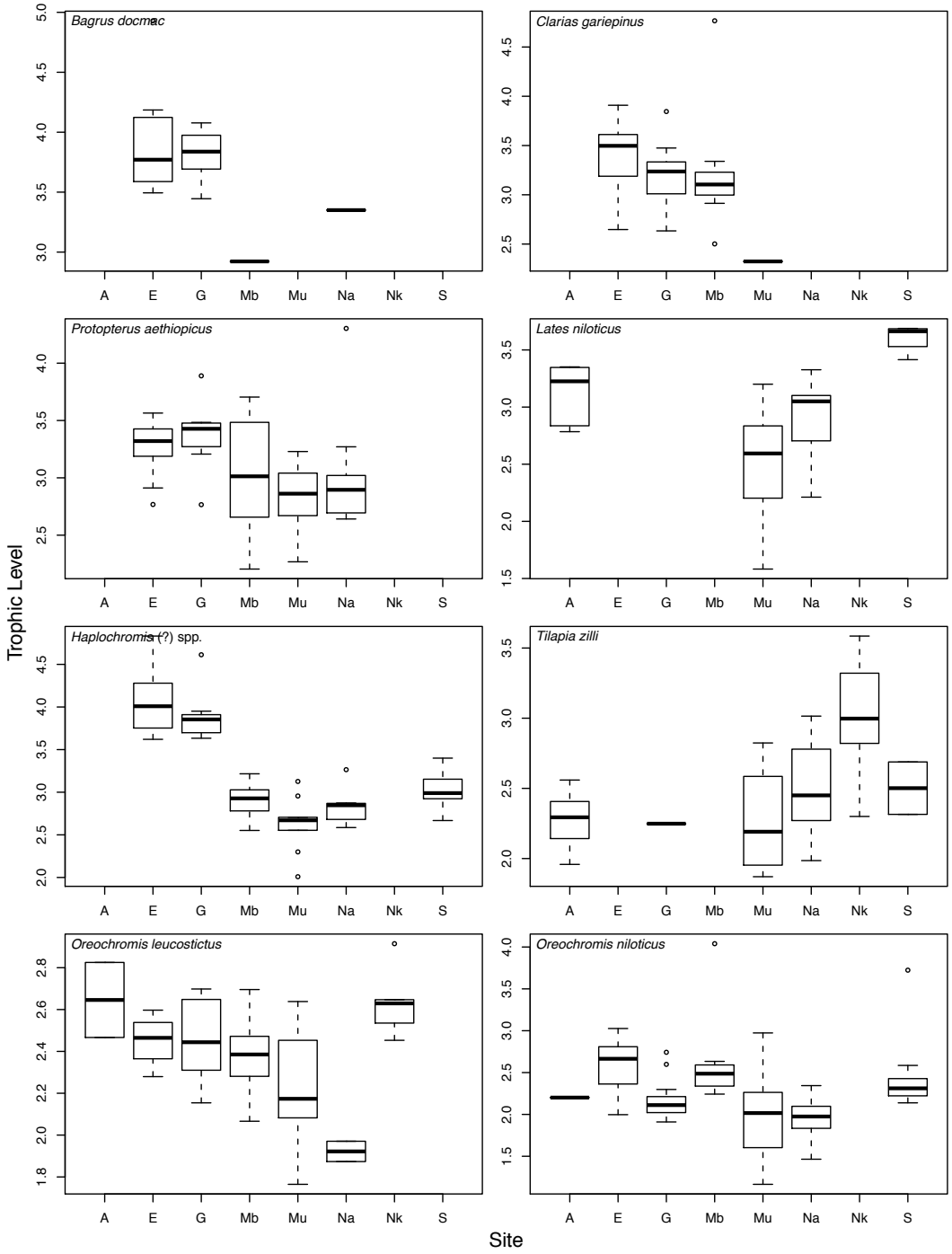

Figure 3.6 Boxplots of trophic level for selected fish species among sites (site codes are the same as in Figures 4 and 5). The centre of these boxplots represents median TL, the outside edges of the boxes represent upper and lower quartiles, and the whiskers extend to the highest and lowest values that are not outliers. Outliers are shown as open circles.

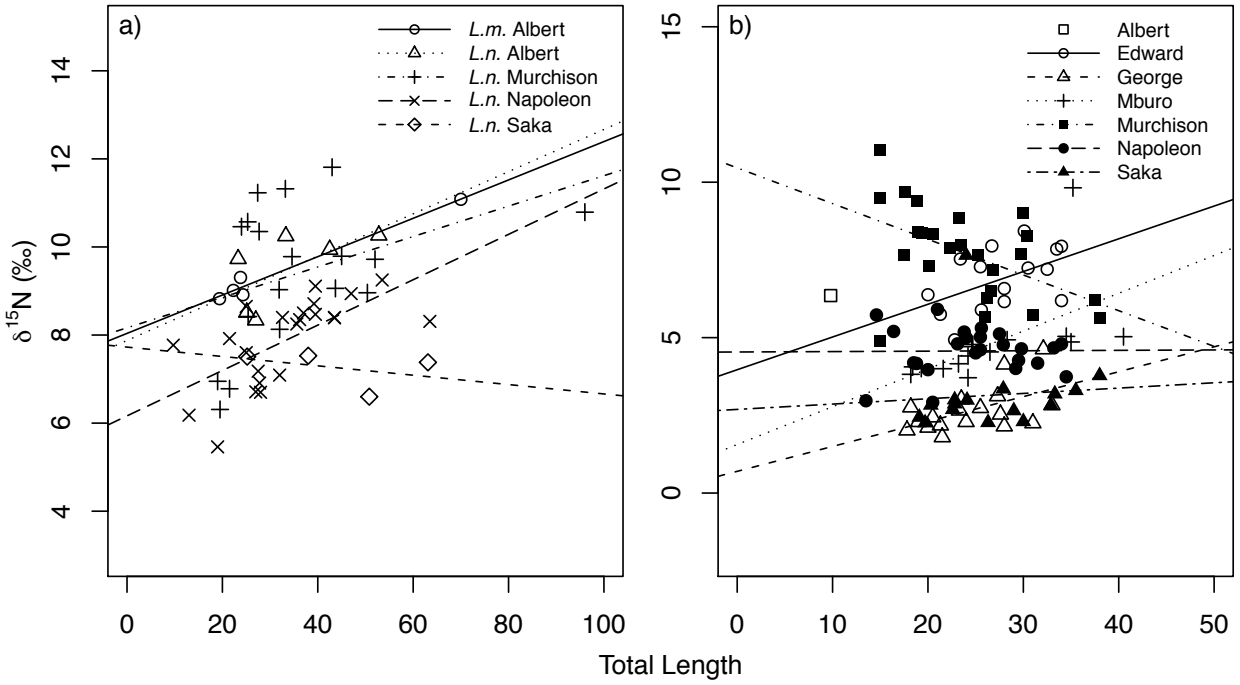

Figure 3.7 Regressions of $\delta^{15}N$ vs. total length for a) *Lates* spp. (*L.m.* is *L. macrophthalamus*, and *L.n.* is *L. niloticus*) and b) *Oreochromis niloticus* from indicated study sites.

Chapter 4

Bioaccumulation and biomagnification of mercury in several Ugandan lakes: the importance of lake trophic status

4.1 Introduction

Globally, anthropogenic activities have led to an increase in the release of mercury to the environment; and while industrial controls in Europe and North America have led to decreased emissions, on a global scale total mercury emissions continue to rise due to increasing emissions from Asia, Africa and South America (Pacyna *et al.* 2006). In East Africa, biomass burning, coal combustion, metal processing, and long-range atmospheric transport are expected to be important sources of mercury to aquatic ecosystems (UNEP 2002, Campbell *et al.* 2003a. AMAP/UNEP 2008).

Methyl mercury (MeHg) is a highly bioaccumulative neurotoxic compound that is known to biomagnify through aquatic and terrestrial food webs. The majority of the total mercury in freshwater fish muscle tissue is in the form of methyl mercury (Bloom 1992), and fish consumption is a dominant source of mercury exposure to human (WHO 1990). Mercury concentrations in fish are influenced by a number of factors, outlined in Chapter 1 of this thesis. In particular, the bioavailability of mercury in a system (and methylation rates), the food web structure, the productivity of the system, and fish growth-rate and life-span are likely to be important determinants of mercury concentrations in fish (Meili *et al.* 1991, Cabana and Rasmussen 1994, Stafford and Haines 2001, Pickhardt *et al.* 2002, Kidd *et al.* 2003, Herendeen and Hill 2004, Simoneau *et al.* 2005).

In order to understand the trophic transfer of contaminants in aquatic systems, it is critical to have a comprehensive understanding of the underlying food web. It has been demonstrated that stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios can be used to characterize food webs and trophic interactions (Peterson and Fry 1987, Fry 1991). A detailed outline of the principles behind the use of stable isotope analysis in food web studies can be found in Chapter 3 of this thesis. Stable isotope analysis has been successfully applied in food-web contaminant studies in several tropical, temperate and arctic systems (Kidd *et al.* 1995, Atwell *et al.* 1998, Campbell *et al.* 2003a, Kidd et al 2003, Campbell *et al.* 2006, Gantner 2009).

Mercury contamination has been well studied in temperate systems with low to intermediate primary productivity; however, the behaviour of this contaminant in eutrophic tropical systems lacks the same

level of understanding. This study includes several East African lakes that range from mesotrophic to hypereutrophic and in size from small crater lakes to some of East Africa's largest lakes. By including a wide range of lakes, I seek to explore the factors that influence the accumulation and biomagnification of mercury in tropical lakes, and that ultimately determine mercury concentrations in fish. In particular, the inclusion of hypereutrophic tropical lakes where phytoplankton biomass and growth is high year-round will hopefully provide insight into the potential for biomass and/or growth dilution of mercury (Meili 1991, Pickhardt *et al.* 2002, Herendeen and Hill 2004) in lakes such as these. Mercury concentrations in fish have been previously reported for three of the eight study sites (Napoleon Gulf in northern Lake Victoria, Campbell *et al.* 2003a; and the crater lakes Saka and Nkuruba, Campbell *et al.* 2006). However, this study is the first that I know of that describes mercury concentrations in fish from the remaining five study sites: the great lakes Albert and Edward, the smaller lakes George and Mburo (both of which sustain important subsistence fisheries), and Murchison Bay (an embayment in northern Lake Victoria that provides fish for export as well as water and fish for the most densely populated region of Uganda).

4.2 Methods

4.2.1 Sampling Methodology

Water and food web samples were collected from Lake Albert in April and May of 2007. Most of the fish sampled from Lake Albert were collected at Kaiso, in the central eastern portion of the lake, using gillnets set by Uganda's National Fisheries Resources Research Institute. The remaining fish were purchased directly from fishermen at Butiaba, in the northeast of the lake.

Water and food web samples from Lakes Edward, George, Mburo, Victoria (Murchison Bay and Napoleon Gulf), Nkuruba and Saka were collected between September 2008 and February 2009. Fish were purchased directly from fishermen and the general location of the catch was confirmed.

Water samples for analysis of total mercury (THg) were collected using trace-metal clean sampling methods (U.S. EPA 2004) at approximately 15 cm below surface. Certified trace-metal clean glass bottles were lowered to the sampling depth; then were opened, filled, and re-sealed at depth.

Subsamples of dorsolateral muscle tissue were taken from fish and were kept frozen until analysis in Canada. Where fish were too small to isolate dorsolateral muscle tissue, whole fillets of axial musculature were collected. Where this was not possible (generally when fish were less than 5 cm long), they were analyzed whole. At all sites, plankton samples were collected using vertical net hauls (20 µm mesh for phytoplankton; 80 µm and 153 µm for zooplankton), and samples were subsequently filtered onto

precombusted quartz-fibre filters (nominal pore size 0.7 μm). *Chaoborus* was visually detected and separated from zooplankton samples. Benthic invertebrates were collected using a Ponar grab. Plankton and invertebrate samples were kept frozen until freeze-dried in Canada. Care was taken to avoid mercury contamination during the collection and processing of all food web samples.

4.2.2 Sample Analysis

Total mercury concentrations in water samples were determined at the National Water Research Institute (Burlington, Canada) using EPA method 1631 (U.S. EPA 1999). NIST 1641c was used as a standard and was on average within 3.1 % of the expected value. Quality control acceptance criteria (U.S. EPA 1999) were met for all sample runs, and it was not necessary to discard any sample data. The precision for samples run in duplicate was on average ± 9.7 % and the mean standard deviation for replicates within study sites was ± 0.07 ng/L.

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios were determined for all fish and food web samples as described in Campbell *et al.* (2003b). Stable isotope analysis was carried out at the Environmental Isotope Laboratory at the University of Waterloo, and a detailed description of methodology is found in Chapter 3 of this thesis. Mean standard deviations from expected values for standard material are ± 0.2 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$. Mean standard deviations of samples run in duplicate were ± 0.05 ‰ for $\delta^{13}C$ and ± 0.21 ‰ for $\delta^{15}N$. Detailed quality control information for stable isotope analysis is found in Chapter 3 of this thesis.

Total mercury (THg) concentrations in biotic samples were determined according to EPA Method 7473 (U.S. EPA 1998), using a DMA-80 direct mercury analyzer at the National Water Research Institute in Burlington, ON, Canada. Fish tissue was analyzed wet (with the exception of fish from Lake Albert, where fish tissue was analyzed dry). All other biotic samples were freeze-dried prior to analysis. Zooplankton material was gently scraped off of filters and analyzed, while phytoplankton filters were run whole. Blank filters were also analyzed in order to account for the trace amounts of mercury present on filters, and a filter blank correction of 0.03 ng Hg was applied. Dry-weight THg concentrations for fish, invertebrates, and zooplankton/phytoplankton were converted to wet-weight concentrations using conversion factors of 0.31, 0.25, and 0.1 respectively (Campbell *et al.* 2003a, Evans *et al.* 1996).

For THg analysis of biotic samples, standard reference materials (SRMs) were analyzed in each run in order to determine between-run variability (SRMs included DORM-1, NIST 1556b, TORT-2, DOLT-2, and orchard leaves). Data were used where reference materials were within ± 10 % of certified value; and

where this was not the case, samples were re-analyzed. Also, approximately one in every twenty samples was run in duplicate to estimate variability within runs, and the mean coefficient of variation for duplicated samples was 3.1 ± 2.8 %.

4.2.3 Calculations and Statistical Analyses

Trophic level (TL) was calculated for all biotic samples, using a standard trophic transfer enrichment factor of 3.4 ‰, based on sample $\delta^{15}N$ values relative to a baseline organism (from the same study site) with an assumed well-defined trophic level (ideally a long-lived primary consumer with an obligate feeding behaviour; Post 2002). Meanwhile, food chain length (FCL) was calculated for all lakes based on the mean $\delta^{15}N$ value of the top predator (highest $\delta^{15}N$) in the lake relative to the mean $\delta^{15}N$ value of a baseline organism with an assumed trophic level. The equations and baseline/top predator organisms used for TL and FCL calculations are outlined in Chapter 3 of this thesis (Table 3.3).

Phytoplankton bioconcentration factors (BCFs; the concentration of a compound in an organism relative to the aqueous concentration of the compound in the environment) for THg were calculated for all sites but Albert (where I did not have phytoplankton THg data) by dividing measured THg concentrations in net phytoplankton (in ng/kg wet weight) by dissolved THg concentrations in water (in ng/L). Since whole water samples were analyzed for THg, dissolved THg concentrations in water were estimated based on whole water THg concentrations, THg concentrations in phytoplankton, and estimated phytoplankton biomass (based on microscopy, Chapter 2, this thesis).

To quantify and compare biomagnification of mercury and the bioavailability and uptake of mercury at the base of the food web in these lakes, I carried out linear regressions of log-transformed total mercury (THg) concentrations against both $\delta^{15}N$ and calculated trophic level (TL) for fish. The regression slope for log(THg) vs. $\delta^{15}N$ has been widely used as a measure of the rate of biomagnification through the food web in many studies from around the world (Kidd *et al.* 1995, Campbell *et al.* 2003a, Campbell *et al.* 2006), and is useful in the comparison of biomagnification between study sites. However, given that there are often differences in the baseline $\delta^{15}N$ values of lakes (see Chapter 3), although log(THg)-$\delta^{15}N$ regression slopes are comparable between sites, regression intercepts are not. The regression of log (THg) against calculated trophic level (TL) allows for more direct comparison of the movement of mercury through the food web (indicated by regression slope) and uptake of mercury at the base of the food web (indicated by y-intercept) between study sites, given that using calculated trophic level provides a correction for differences in baseline $\delta^{15}N$ values between lakes.

The slopes and intercepts of the log(THg)–TL regressions were compared between lakes using analysis of covariance (ANCOVA). Trophic magnification factor (TMF) was calculated for each lake as follows:

$$TMF = 10^{b}; \text{ where b is the slope of the log(THg)-TL regression for fish}$$

To determine whether biomass or growth dilution was taking place, I explored the relationship between the predicted log(THg) values at TL=1 (based on log(THg)–TL regressions) with total mercury concentrations in water as well as chlorophyll *a* concentrations. I assume that these predicted values represent time-integrated mean mercury concentrations at the first trophic level (primary producers). Since sampling was only carried out on a monthly basis over six months of the year, mean measured mercury concentrations in phytoplankton may not accurately integrate the range of values likely to occur throughout the year. Also, since log(THg)-TL regressions were calculated using only fish, where MeHg makes up most of the total mercury present (Bloom 1992), predicted phytoplankton mercury values at TL=1 may more accurately reflect MeHg rather than THg concentrations in phytoplankton, given that compared to fish much less of the mercury present in phytoplankton is in the form of methyl mercury. As such, these predicted values will be referred to as "MeHg" rather than THg. This is valuable since MeHg is more directly relevant for biomagnification than is THg. If biomass or growth dilution is taking place, water THg concentrations will positively contribute to the predicted mercury concentrations at TL =1, while chlorophyll *a* should negatively contribute to the predicted concentrations. I also explored the relationship between log(THg)-TL regression slopes and chlorophyll *a* to determine whether trophic status influenced biomagnification rate across study sites.

Linear regressions were carried out in order to determine whether there were relationships between total mercury in different fish species and $\delta^{13}C$, $\delta^{15}N$, or total length within sites. All statistical analyses were carried out using R, version 2.11.1 (R Development Core Team 2010).

4.3 Results

Mean total mercury concentrations in water from the study lakes ranged from 0.38 ng/L (in offshore Lake Edward) to 1.30 (in Murchison Bay; Table 4.1). There was a strong positive relationship between mean mercury concentrations in water and mean chlorophyll *a* concentrations ($r^2_{adj} = 0.75$, $P<0.001$, $n= 9$). Results of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic analysis are given in Table 4.2 and are described and discussed in detail in Chapter 3 of this thesis.

There was a strong negative relationship between $\delta^{15}N$ and $\delta^{13}C$ of phytoplankton from Lake Edward, primarily due to differences in stable carbon and nitrogen isotopic ratios between nearshore and offshore

phytoplankton. To account for this trend in baseline values, a correction factor (outlined in Chapter 3) was applied to $\delta^{15}N$ values for all fish and other biotic samples from Lake Edward. Corrected values were used in all subsequent plots and calculations.

Site means for total mercury concentrations in phytoplankton ranged from 1.9–3.1 ng/g (wet weight), and THg values in zooplankton overlapped with those observed for phytoplankton (1.1–4.5 ng/g wet weight) (Table 4.2). Bioconcentration factors for THg in phytoplankton ranged from 1465–8026 L/kg (on a wet weight basis), and were negatively related to chlorophyll *a* concentrations ($P<0.01$, $r^2_{adj} = 0.59$, Figure 4.1). Benthic invertebrates exhibited a wide range in THg values, with concentrations generally exceeding those observed for phytoplankton and zooplankton. Mean THg for mollusc tissue collected from Lake Albert ranged from 6.4–40.9 ng/g wet weight. In Murchison Bay, mayflies, leeches and chironomids had THg concentrations of 5.9, 10.7 and 14.3 ng/g respectively; while chironomids from Napoleon Gulf had THg concentrations of 17.1 ng/g wet weight (Table 4.2).

Total mercury concentrations in fish (Table 4.2) ranged from 0.8 ng/g (in an individual *O. niloticus* from Lake George), to a maximum of 855 ng/g (in an individual *Haplochromis squamipinnis* from Lake Edward). Individual fish with THg concentrations exceeding 200 ng/g (WHO guideline for at-risk populations) were observed in Lake Albert (*Lates macrophthalamus* and *Lates niloticus*); Lake Edward (*Bagrus docmac*, *Clarias gariepinus* and *H. squamipinnis*), and in Napoleon Gulf (*Synodontis victoriae*).

To determine overall differences in THg in fish between sites, I compared mean THg concentrations among sites on a species by species basis (using direct comparison of concentrations as well as analysis of variance). I found that concentrations tended to be highest in Lake Albert followed by Lake Edward, were often intermediate in Murchison Bay, Lake Nkuruba and Napoleon Gulf, lower in Lakes Mburo and Saka, and were generally lowest in Lake George (Figure 4.2, Table 4.3).

Within sites, several fish species exhibited increasing mercury concentrations with increasing total length (using log-transformed THg data), including all of the species indicated above as occasionally having THg concentrations that exceed 200 ng/g. These relationships are summarized in Table 4.4. Many other species of fish at all sites also showed positive, albeit not statistically significant, trends for this relationship. Based on analysis of covariance (ANCOVA), for *L. niloticus*, the slopes of the log(THg)~total length (L) regressions are not significantly different for Lake Saka, Napoleon Gulf, or Murchison Bay. Meanwhile, the log(THg)~L regression slope for *L. niloticus* from Lake Albert is higher than the three other sites where *L. niloticus* was present. Given that there were no consistent log(THg)-

total length relationships within all species or across all sites, mercury data have not been normalized to a standard length.

Log-transformed THg values in fish were significantly negatively related to $\delta^{13}C$ for fish from several of the study sites, including Lake Albert, Lake Edward, Lake George, Lake Mburo, Napoleon Gulf and Lake Saka (Figure 4.3, Table 4.5). This relationship was not observed in Lake Nkuruba or in Murchison Bay (Figure 4.3, Table 4.5).

Slopes for regressions of log-transformed total mercury concentrations against $\delta^{15}N$ ranged from 0.08 to 0.22 (Figure 4.4, Table 4.6). Regressions of log-transformed total mercury concentrations against calculated trophic level were also used to quantify the biomagnification of mercury through the food webs of the study sites. Baseline $\delta^{15}N$ values differ between lakes due to differences in anthropogenic inputs of nitrogen and atmospheric nitrogen fixation rates (see Chapter 3 for a detailed treatment of baseline $\delta^{15}N$ in these systems). In using calculated trophic level rather than $\delta^{15}N$ in these regressions, I am able to correct for these differences in baselines, allowing direct comparison of both regression slope and regression intercepts. For all sites, significant positive relationships were observed between log(THg) and calculated trophic level (Table 4.6). Trophic magnification factors (TMFs) for total mercury, calculated based on the slope of the log(THg)–TL regression for a study site, ranged from 1.89 to 5.58 (Table 4.6).

ANCOVA analyses comparing log(THg)–TL regression slopes revealed two distinct groups of sites (Figure 4.5). Slopes for Lake George, Lake Mburo, Murchison Bay and Lake Saka (Group A) did not differ significantly from one another. Meanwhile regression slopes for Lake Albert, Lake Edward, Lake Nkuruba and Napoleon Gulf (Group B) were all significantly higher than those observed for the previous group of sites and were not significantly different from one another. Across all sites, the slope of the log(THg)–TL regressions was negatively related to chlorophyll *a* concentrations at the $P<0.1$ level. The regression equation was as follows:

$$\text{log(THg) vs. trophic level slope} = 0.572 - 0.003(\text{Chlorophyll } a)$$
$$(r^2_{adj} = 0.41, P<0.1, n= 7)$$

Given that comparison of intercepts in ANCOVA requires that the slopes not be significantly different from one another, regression intercepts were compared within the two groups of sites (group A and group B). Within group A, Murchison Bay had a significantly higher y-intercept than the other two sites. Within group B, there were no significant differences in y-intercepts between sites.

In considering whether biomass dilution was taking place, I found that predicted "MeHg" values at TL = 1 (based on log(THg)–TL regressions) were not significantly related to mercury concentrations in water or to chlorophyll *a* concentrations. However, when a multiple linear regression was carried out, together, mercury concentrations in water and chlorophyll *a* concentrations explained 92.7 % of the variability in the predicted mercury concentrations in primary producers. The overall relationship was significant at the $P<0.05$ level, while the beta-coefficients for both THg in water and chlorophyll *a* were significant at the $P<0.001$ level. The resulting regression equation was as follows:

predicted "MeHg" at TL 1 =

-2.248 + 11.291(THg in water) – 0.063(Chlorophyll *a*)

($r^2_{adj} = 0.93$, $P<0.01$, $n=8$)

4.4 Discussion

4.4.1 Mercury in Water

At all study sites, total mercury concentrations in water were very low, ranging from 0.38–1.3 ng/L. In Napoleon Gulf, measured THg concentrations in water (Table 4.1) were generally lower than those previously reported for the gulf by Ramlal *et al.* (2003) and Campbell *et al.* (2003a), both of whom used comparable analytical methods. However, my sampling station was near the entry to the Buvuma Channel, where Campbell *et al.* (2003a) observed the lowest mercury concentrations (0.7 ng/L, very similar to my observed concentrations). Meanwhile, Ramlal *et al.* (2003) collected water samples from Bugaia Island, where Campbell *et al.* (2003a) also observed elevated mercury concentrations relative to many of their other sampling stations. To my knowledge, THg concentrations in water have not previously been reported for the remaining study sites.

The highest total mercury concentrations in water (Table 4.1) were observed in Murchison Bay, which is likely attributable to the region's dense urban and industrial development as well as the inflow of wastewater from Uganda's capital city, Kampala (Haande *et al.* 2010). Although Lake George is located primarily within Queen Elizabeth National Park, given the history of nearby copper mining and heavy metal contamination in Lake George (Denny *et al.* 1995, Lwanga *et al.* 2003), it is not surprising that Lake George had THg concentrations that were close to those observed in Murchison Bay. THg concentrations were lowest in offshore Lake Edward, which is surrounded by largely undeveloped protected areas and is unlikely to be strongly affected by local anthropogenic activities. Meanwhile, mercury concentrations at the nearshore Lake Edward study site (where the Kazinga Channel enters Lake

Edward from Lake George) were intermediate between those observed in Lake George and those in offshore Lake Edward as might be expected with the strong hydrological connection between Lake George and the nearshore Lake Edward site. The elevated mercury concentrations at the nearshore site relative to the offshore site suggest the possibility of mercury inputs to Lake Edward from Lake George or the Kazinga Channel. However, concentrations at all study sites were nevertheless quite low (never exceeding 2 ng/L), suggesting low levels of direct mercury contamination of water.

I observed a strong positive relationship between total mercury concentrations in water and chlorophyll *a* concentrations; however, I did not observe a correlation between phytoplankton bound THg (in ng/L, calculated from measured THg in phytoplankton and phytoplankton biomass) and THg in whole water samples, suggesting that this relationship is not a reflection of differences in the amount of phytoplankton-bound mercury included in these samples. One possibile explanation is that when anthropogenic input of nutrients and resultant algal biomass are high, there may also be increased anthropogenic input of mercury. There are several other factors that may also act to determine mercury concentrations in water, including the extent of wetlands, stratification, mixing, and site depth. In particular, shallow non-stratified sites (including Murchison Bay, Lake Mburo, Lake George and Lake Saka) are more likely to experience frequent re-suspension of sediment-borne mercury.

4.4.2 Mercury in Plankton and Benthic Invertebrates

Total mercury concentrations in phytoplankton were low, and bioconcentration factors for phytoplankton (range: 1465–8026 L/kg wet weight, or log-transformed BCF: 3.2–3.9) were generally an order of magnitude lower than other reported values for THg (Watras and Bloom 1992, Chen *et al.* 2000). This difference may be due to reduced mercury in phytoplankton due to growth dilution and/or biomass dilution in these productive tropical lakes, or differences in the proportion of THg that is in the form of methyl mercury. It is also important to note that I estimated dissolved THg concentrations based on whole water THg and phytoplankton-bound THg; if an appreciable amount of the THg pool in the whole water samples was bound to non-phytoplankton particulate matter, phytoplankton BCFs may be underestimated. The significant negative relationship that I observed between phytoplankton THg bioconcentration factors and chlorophyll *a* may reflect reduced mercury concentrations in phytoplankton relative to water due to either growth or biomass dilution. It is particularly difficult to determine whether growth or biomass dilution is primarily responsible for differences in mercury concentrations at the base of the food web, and indeed these processes are not likely to be mutually exclusive.

Total mercury concentrations in zooplankton largely overlapped with those determined for phytoplankton (Table 4.2). This overlap in concentrations may be due to the fact that for plankton samples, mass analyzed as well as mercury concentrations tended to be very low (and often approached the methodological detection limit). However, this is also likely a reflection of the high intrinsic variability (both spatial and temporal) in mercury concentrations in plankton.

In Lake Albert, molluscs had THg concentrations that were comparable to those observed for fish species with calculated trophic levels close to 2 (including *O. niloticus*, *O. leucostictus*, and *T. zilli*), which is consistent with their known trophic position as primary consumers. However, there was a great deal of variability in total mercury concentrations both within and between species for molluscs from Lake Albert (Table 4.2). This is likely due to the low number of replicates, and the lack of a representative size range within species.

In Murchison Bay, mayflies (*Povilla* sp.) had very low total mercury concentrations, consistent with their expected diet of phytoplankton and detritus (Roy and Sharma 1982). Meanwhile, leeches had low THg concentrations despite the fact that they are known to feed on benthic invertebrates and their calculated trophic level approached that of *L. niloticus* and was equal to that of *S. victoriae*, species which both had much higher THg concentrations than the leeches sampled. Low mercury concentrations in leeches relative to other benthic invertebrates have been observed in several studies from temperate systems (summarized in McNicol *et al.* 1997). Also, given that leeches were only collected from Murchison Bay on one occasion, this may be a reflection of seasonal mercury concentrations in prey (see Sarica *et al.* 2005 for an example of rapid changes in mercury concentrations in temperate leeches). Chironomids from Murchison Bay had THg concentrations that were comparable to those observed in other primary consumers from the same site (e.g. tilapiine cichlids). However, in Napoleon Gulf, THg in chironomids generally exceeded the concentrations observed in other primary consumers (including *O. leucostictus*, *O. variabilis*, and *T. zilli*). Chironomids are known to have diverse feeding habits (Pinder 1986), and these differences may reflect differences in the species collected at each site.

4.4.3 Mercury in Fish

Published total mercury concentrations in fish are available for several species of fish from Napoleon Gulf (Campbell *et al.* 2003a, Ramlal *et al.* 2003), Lake Nkuruba and Lake Saka (Campbell *et al.* 2006). To my knowledge, mercury concentrations in fish have not been reported in detail for the remainder of the study sites.

In comparing THg concentrations in fish observed in the current study with observations made by Campbell *et al.* (2003a), I find that while in both studies reported concentrations for *Rastrineobola argentea* that were very similar, for most species for which comparisons were possible, THg concentrations in the current study tended to be lower than those observed by Campbell *et al.* (2003a) (Figure 4.6). Species for which this was the case included: *Haplochromis* (?) spp., *T. zilli*, *P. aethiopicus*, *O. niloticus*, and *L. niloticus*. There are several factors that may be responsible for this difference in observed THg concentrations: mercury inputs to Napoleon Gulf may have decreased, primary productivity in Napoleon Gulf may have increased (leading to biomass dilution of mercury), or these differences may reflect differences in methodology (in Campbell *et al.* (2003a) fish were oven-dried prior to analysis and analyzed using different methodology). Also, in the case of *Haplochromis* (?) spp., it is possible that different species of haplochromine cichlids (with different dietary exposures to mercury) may have been included in the two studies. These differences do not appear to be due to differences in the size of sampled fish between studies, given that when *O. niloticus* and *L. niloticus* from the current study are divided into the same size classes used by Campbell *et al.* (2003a), across all size classes THg concentrations are still lower in the current study. Also, based on stable isotope analysis there does not appear to have been an obvious change in food web structure in Napoleon Gulf (see Chapter 3). Campbell *et al.* (2003c) observed a decline in sedimentary mercury in two sediment cores from Lake Victoria (one nearshore and one offshore) that appeared to begin in the 1980's, although the study was not able to determine whether this was due to a decline in mercury inputs to the lake, or to an increase in sedimentation rate due to eutrophication of the lake. Given that mercury concentrations in water and fish from Napoleon Gulf were lower than in samples collected in the late 1990's by Campbell *et al.* (2003a) and Ramlal *et al.* (2003), there seems to be some evidence that mercury concentrations in Lake Victoria are declining. Although reduced mercury concentrations in fish may also reflect increased biomass/growth dilution of mercury due to increased primary productivity.

Several additional species, not previously sampled for Hg analysis, were included in the current study. For example, in addition to *O. niloticus*, three other tilapiine cichlids were sampled, including the rare native tilapia *O. variabilis* as well as *O. leucostictus* and *T. zilli*. While THg concentrations were fairly similar among all four of the tilapiine cichlids sampled, the differences in THg concentrations between species were consistent with differences in calculated trophic levels for these species. *T. zilli* had both the highest calculated trophic level and the highest mercury concentrations, while *O. variabilis* and *O. leucostictus* had the lowest calculated trophic levels and mercury concentrations, and *O. niloticus* had intermediate values. Some of the highest THg concentrations observed for fish from Napoleon Gulf were

in fish that have not been included in previous studies, including *Bagrus docmac*, *Brycinus sadleri*, *Mormyrus kannume*, *Synodontis afrofischeri*, and *Synodontis victoriae.* All of these species are generally secondary consumers. With the exception of the largest specimen of *S. victoriae*, with THg of 340.9 ng/g wet weight, no fish in Napoleon Gulf exceeded the WHO guideline for at-risk populations of 200 ng/g.

THg concentrations in fish have been documented for several regions of Lake Victoria including Napoleon Gulf near the Buvuma Channel (Campbell *et al.* 2003a), Thruston Bay in Napoleon Gulf (Campbell *et al.* 2004), and Winam Gulf, in Kenya (Campbell *et al.* 2003a). However, mercury concentrations in the food web of Murchison Bay (in northern Lake Victoria) have yet to be reported, despite the high degree of anthropogenic influence on this embayment by the city of Kampala and the large commercially and locally important fishery sustained by these waters. THg concentrations in fish from Murchison Bay were very similar to the concentrations observed in Napoleon Gulf, with only *O. leucostictus* having a statistically significant difference in THg concentrations between the two embayments (on average this species had higher mercury concentrations in Murchison Bay than in Napoleon Gulf). The species composition and food web structure of Murchison Bay is fairly similar to that of Napoleon Gulf (see Chapter 3, this thesis). Given that mercury concentrations in water are higher in Murchison Bay than in Napoleon Gulf, I expected that mercury concentrations in fish would similarly be higher in Murchison Bay; however, this was not the case. This may be due to differences in the availability of MeHg in each embayment, differences in phytoplankton biomass and growth rate (Murchison Bay is hypereutrophic and Napoleon Gulf is not), or to differences in biomagnification rate (higher in Napoleon Gulf, Figure 3.5).

I found that among fish from Murchison Bay, the highest THg concentrations were observed in *L. niloticus*, *Synodontis* spp., *R. argentea*, and some individual haplochromine cichlids. These enriched mercury concentrations are consistent with the relatively high calculated trophic levels for these fish, indicating that fish feeding higher on the food web tended to have higher mercury concentrations. The one exception to this trend was *P. aethiopicus*, which despite high calculated trophic levels in both Murchison Bay and Napoleon Gulf, tended to have low mercury concentrations. One possible explanation is that *P. aethiopicus* from Lake Victoria that were included in this study were generally caught in the nearshore in mats of water hyacinth (*Eichhornia crassipes*). Campbell *et al.* (2003a) found low THg (and low proportions of MeHg) in water from a water hyacinth dominated site in Napoleon Gulf relative to other areas of Napoleon Gulf, suggesting that these macrophytes may act locally to reduce mercury concentrations in both water and food webs.

While only a few fish from Napoleon Gulf had mercury concentrations that exceeded the WHO guideline for at-risk populations of 200 ng/g, there were no fish from Murchison Bay that exceeded this limit. It should be appreciated and noted that the WHO guidelines assume a certain intensity of fish consumption that may be exceeded by individuals or even communities that rely on fish as a major portion of their dietary intake. However, Campbell *et al.* (2003a) concluded, that in general, risk of mercury intoxication from consuming Lake Victoria fish was quite low, and the results of the current study are in agreement with this assessment.

Mercury concentrations in fish and food web samples were reported for the western Ugandan crater lakes Saka and Nkuruba by Campbell *et al.* (2006). For all fish species sampled in Lake Nkuruba, the THg concentrations observed in the current study are similar to, although generally lower than, those observed previously (Figure 4.6). Meanwhile, in Lake Saka, *A. alluaudi* had very similar concentrations between studies. Meanwhile, *B. neumayerii, Haplochromis* (?) sp. and *O. niloticus* all had higher concentrations in the current study than those observed by Campbell *et al.* (2006). On the other hand, in the Campbell *et al.* (2006) study, *L. niloticus* were found to have higher THg concentrations than I observed. However, it is important to note that sample sizes for some species were very small in both studies, or differed greatly between studies, making unbiased comparisons difficult.

Although Campbell *et al.* (2005) published a detailed account of the food web of Lake Albert based on stable isotope analysis, no concentrations of mercury in fish have been published for Lake Albert to date. In the current study, mercury concentrations in fish from Lake Albert were generally higher than those observed in other lakes, with the highest concentrations observed in *L. macrophthalamus* and *L. niloticus*, consistent with their roles as top predators. Intermediate concentrations were observed in the zooplanktivorous *Neobola bredoi*, and *Brycinus nurse* as well as the invertivore *Schilbe intermedius* and the cichlid *Thoracochromis mahagiensis*. Tilapiine cichlids (including *O. leucostictus*, *O. niloticus* and *T. zilli*) along with *Hydrocynus forskahlii* had the lowest observed THg concentrations in Lake Albert. While it is not surprising that the detritivorous tilapiine cichlids exhibited low mercury concentrations, *H. forskahlii* is a known piscivore (Campbell *et al.* 2005), which would be expected to have high mercury concentrations. However, given that only two very small juveniles were sampled (n=2, total length range: 9.5–13.9 cm), these results do not accurately reflect mercury concentrations in this species. Mercury concentrations in both *Lates* species occasionally exceeded the WHO guideline for populations at risk of 200 ng/g wet weight, while one individual *L. niloticus* (total length (L) = 52.8 cm) had a THg concentration of 609 ng/g w.w, exceeding the acceptable limit for international markets of 500 ng/g.

Lake Mburo is located within a national park, and is surrounded by extensive papyrus wetlands. There is very little anthropogenic interference in the lake basin and the lake is hypereutrophic (Mbabazi *et al.* 2004). Total mercury concentrations in fish from Lake Mburo were very low (the highest concentration measured was 32.2 ng/g wet weight, in *C. gariepinus*). Higher concentrations were observed in fish that are known to feed on invertebrates and occasionally small fish (*B. docmac*, *C. gariepinus*, *Haplochromis* (?) sp., and *P. aethiopicus*; Greenwood 1966). Meanwhile, mercury concentrations in the three species of *Oreochromis* sampled, which are all known to feed heavily on phytoplankton and detritus (Greenwood 1958) were generally lower than in other species from Lake Mburo. Total mercury concentrations in fish from Lake Mburo were generally higher in organisms feeding at higher trophic levels, based on both published fish diet and results of stable isotope analysis.

Fish from Lake George also had very low mercury concentrations. The highest concentrations (18.8 ± 24.3 ng/g) were observed in the piscivorous *Haplochromis squamipinnis*, while intermediate concentrations (mean THg from 4.4–5.8 ng/g) were observed in *B. docmac*, *C. gariepinus*, *Haplochromis* (?) sp., and *P. aethiopicus*. The tilapiine cichlids (including three species of *Oreochromis* and *T. zilli*) had the lowest mercury concentrations of all fish sampled.

Despite being connected to Lake George via the Kazinga Channel, and having a very similar species assemblage and food web structure, total mercury concentrations in fish from Lake Edward were much higher than those observed in the same species in Lake George. This is in spite of the fact that THg concentrations in water from Lake George exceeded those observed in Lake Edward. In fact, the highest mercury concentrations in fish collected from all study sites were for *H. squamipinnis* from Lake Edward (mean of 188.3 ± 254.4 ng/g, range: 13.7–855.3 ng/g). Meanwhile, intermediate mercury concentrations were observed in *B. docmac*, *C. gariepinus*, and *Haplochromis* (?) sp..

H. squamipinnis, *B. docmac*, and *C. gariepinus* all occasionally exceeded the WHO guideline for populations at risk of 200 ng/g. The largest *H. squamipinnis* collected had a mercury concentration of 855.3 ng/g, which exceeds the acceptable limit for international markets. Similar to several other study sites, the lowest mercury concentrations were observed in the detritivorous *O. leucostictus* and *O. niloticus*. Unlike Lakes George or Mburo, but as in the two Lake Victoria study sites, *P. aethiopicus* in Lake Edward had very low mercury concentrations (comparable to those of primary consumers) despite having a high mean calculated trophic level.

When THg concentrations in fish were compared between sites at the within species level, fish from Lakes Albert and Edward tended to have the highest THg concentrations of all study sites. These large

lakes are not impacted by urban areas or industrial developments and are characterized by low mercury concentrations in water. Consequently, anthropogenic inputs of mercury are not likely to be the source of elevated mercury concentrations in fish. At the other end of the spectrum, Lake George has a known history of heavy metal contamination due to copper mining activities, and had the second highest observed mercury concentrations in water; however, THg concentrations in fish were the lowest of all sites. These trends may reflect differences in the diet of a species between sites, differences in the accumulation of mercury at the base of the food web (due to biomass/growth dilution and mercury bioavailability), and differences in biomagnification rate. In addition to having the lowest mercury concentrations in fish, lakes Saka, Mburo and George are also all small, shallow lakes with extensive wetlands, which may influence the availability of mercury. Given that the lakes with the highest mercury concentrations in fish tended to have lower phytoplankton biomass (as measured by chlorophyll *a*) than in hypereutrophic lakes, biomass/growth dilution of mercury may play an important role in determining mercury concentrations in fish.

4.4.4 THg vs. Fish Length

Positive relationships between total length (L) and mercury concentrations are often observed within species of fish (MacCrimmon *et al.* 1983). These relationships are attributable to two processes: some fish species experience ontogenetic dietary shifts, where they feed on progressively larger and higher trophic level organisms as they grow (Campbell *et al.* 2003a, b); and mercury is slowly depurated from muscle and therefore accumulates in fish over their life-time, with larger, older fish tending to have higher mercury concentrations than younger, smaller fish (MacCrimmon *et al.* 1983). Although positive trends were observed between total length (L) and log-transformed THg concentrations for many species of fish at all sites, many of these relationships were not particularly strong. Based on previous studies (Campbell *et al.* 2003a), I had expected that *L. niloticus* in particular would display increasing mercury concentrations with growth. I found that, although not always statistically significant, there were positive relationships between log(THg) and total length for *L. niloticus* in all study lakes where present. Additionally, based on an ANCOVA, the slopes of these relationships did not differ significantly between sites, suggesting that in my study lakes, *L. niloticus* is consistently experiencing growth related increases in mercury concentrations.

Where statistically significant relationships were observed between log(THg) and total length, there was rarely a corresponding statistically significant positive relationship between total length and $\delta^{15}N$. This suggests that among many of the species sampled, increases in mercury concentrations in fish with

increasing total length may be primarily attributable to the long-term accumulation of mercury in fish over time, rather than ontogenetic dietary shifts.

4.4.5 THg vs. $\delta^{13}C$

Benthic and pelagic primary producers tend to have divergent carbon isotopic ratios, with benthic periphyton tending to have higher $\delta^{13}C$ values than pelagic phytoplankton (Hecky and Hesslein 1995). However high growth rates during phytoplankton blooms and reduced carbon isotopic discrimination due to instantaneous carbon limitation can also lead to higher $\delta^{13}C$ values in phytoplankton (Hecky and Hesslein 1995). These differences in $\delta^{13}C$ values are passed on to consumers, giving insight into their dietary carbon sources. Since all but one of the study sites are eutrophic or hypereutrophic (Table 4.1) with high phytoplankton biomass and low transparency, substantial benthic algal contribution to the food web would not be expected since the amount of substrate with adequate light for benthic photosynthesis is often limited in eutrophic lakes (Vadeboncoeur *et al.* 2003, and see Chapter 3, this thesis). In Lake Victoria, $\delta^{13}C$ values in seston are strongly a function of mixing depth, where deeper mixing depth can lead to light limitation of phytoplankton, reducing the likelihood of carbon limitation, and allowing for increased isotopic discrimination relative to nearshore phytoplankton (Hecky *et al.* 2010). As a result, nearshore phytoplankton tends to have higher $\delta^{13}C$ values than offshore phytoplankton. Based on stable isotope analysis of phytoplankton from both nearshore and offshore, this trend is also apparent in Lake Edward (Chapter 3, this thesis); and is also likely to apply for Lake Albert.

The strong negative relationship between total mercury and $\delta^{13}C$ for fish within several of the study sites may reflect that fish that generally feed in the nearshore tend to have lower mercury concentrations than fish that feed more offshore. It is possible that fish feeding nearshore (or in more productive areas of the lake) are feeding more heavily on ^{13}C-enriched phytoplankton with high growth rates and biomass, where biomass/growth dilution of mercury may be occurring, leading to lower mercury concentrations in fish relying on this food source. However, given that this negative relationship is observed in lakes that have very different chlorophyll *a* concentrations, and lakes where there are not expected to be substantial spatial differences in phytoplankton biomass (e.g. Lakes George, Mburo and Saka), it is likely that differences in phytoplankton growth rate can account for the negative slope in most of these systems. This indicated that growth dilution rather than biomass dilution may be the dominant process acting to reduce mercury concentrations at the base of the food web; however, these processes are highly related and difficult to differentiate. The lack of a negative relationship between mercury concentrations in fish and

$\delta^{13}C$ in Murchison Bay may reflect the fact that although phytoplankton biomass, growth rate, and $\delta^{13}C$ values are expected to be higher in the inner bay, baseline mercury concentrations (from anthropogenic sources) are also likely to be higher here, and feeding in this productive environment may not reduce the mercury exposure of fish relative to fish feeding in lower productivity (but also lower mercury) regions of the lake.

4.4.6 THg vs. $\delta^{15}N$

The regression slope of log(THg) against $\delta^{15}N$ (as a measure of trophic level) has been widely used to quantify and compare the biomagnification rate of mercury among systems (see Kidd 1998). Meanwhile the intercepts of this regression can give us insight into nitrogen cycling in these lakes, with baseline $\delta^{15}N$ values being influenced by anthropogenic input of nutrients to these systems as well as by fixation of atmospheric nitrogen. Differences in baseline $\delta^{15}N$ values are treated in detail in Chapter 3 of this thesis. In the current study, these regressions revealed statistically significant positive relationships between log-transformed total mercury concentrations and the $\delta^{15}N$ values for fish for all sites, confirming the occurrence of biomagnification. In the current study, the observed slopes for these regressions (using fish only) ranged from 0.081 in Lake George to 0.220 in Lake Albert (Table 4.6). These values can be contrasted with one another and with those reported in other studies in order to gain insight into the differences and similarities in biomagnification rates between systems.

Previous studies have reported log(THg)~$\delta^{15}N$ regression slopes ranging from 0.12 to 0.26 in African lakes (Campbell *et al.* 2003a, Kidd *et al.* 2003, Campbell *et al.* 2004, Campbell *et al.* 2006, Poste *et al.* 2008, Campbell *et al.* 2008). Meanwhile, slopes ranging from 0.11 to 0.48 have been reported for freshwater and marine systems around the world (Bowles *et al.* 2001, Power *et al*. 2002, Kidd *et al.* 1995).

Campbell *et al.* (2006) reported log(THg)~$\delta^{15}N$ regression slopes of 0.13 and 0.14 for Lakes Saka and Nkuruba respectively, while my observed slopes were 0.08 for Lake Saka and 0.13 for Lake Nkuruba. In both studies Lake Nkuruba had very similar regression slopes; however, the slope for Lake Saka in the current study was much lower than that observed by Campbell *et al.* (2006). This may be due to differences between studies in both the species included as well as the number of replicates collected for each species. While Campbell *et al.* (2006) were able to sample 9 individuals of *L. niloticus*, the current study only included 4. Meanwhile, Campbell *et al.* (2006) included one *O. niloticus* in their study, the current study included 16. These differences likely had a strong influence on the regression slope since

these species are at the extreme ends of the range for the regression. Campbell *et al.* (2003a) reported a regression slope for Napoleon Gulf of 0.16, very similar to the 0.15 observed in the current study.

Among lower productivity large East African lakes, log(THg)~δ^{15}N regression slopes have been reported for Lake Malawi (0.23–0.25, Kidd *et al.* 2003), and Lake Tanganyika (0.22, Campbell *et al.* 2008). These values are very similar to the slope observed in the current study for Lake Albert (0.22) and to a lesser extent for Lake Edward (0.15) and Napoleon Gulf (0.15). This suggests that there may be common factors among these lakes that are involved in determining biomagnification rates; including primary productivity, presence of piscivores, large ecosystem size and possibly similar fish growth rates.

4.4.7 THg vs. Trophic Level

As for log(THg)~δ^{15}N regressions, the slopes of log(THg)~calculated trophic level regressions were always positive, indicating that biomagnification (increasing mercury concentrations with increasing trophic level) was taking place. Among my study lakes, I found that log(THg)~TL regression slopes (taken to indicate biomagnification rate) were significantly lower for Lake George, Lake Mburo, Murchison Bay and Lake Saka (group A) than for Lake Albert, Lake Edward, Napoleon Gulf and Lake Nkuruba (group B). These two groups can also be divided based on trophic status, with group A consisting entirely of hypereutrophic lakes (chlorophyll *a* > 25 µg/L), and group B consisting entirely of lakes where chlorophyll *a* concentrations are less than 25 µg/L (indicating mesotrophic/eutrophic conditions) (Vollenweider and Kerekes 1982). This, as well as the negative relationship observed between regression slope and chlorophyll *a* concentrations among lakes (r^2_{adj} = 0.41, P<0.1), suggests that in lakes with higher phytoplankton biomass (as measured by chlorophyll *a* concentrations), biomagnification is occurring at a lower rate. These differences are unlikely to be due to differences in food web structure, especially given that despite nearly identical fish species assemblages and food web structure (based on stable isotope analysis) Lakes Edward and George have highly divergent regression slopes. Similarly, regression slopes for Napoleon Gulf and Murchison Bay (two embayments in northern Lake Victoria with similar food web structure but different phytoplankton biomass) are significantly different from one another. These differences may be an indication that growth dilution through the consumer levels is occurring, that is to say, in higher productivity systems fish are growing more quickly, and as such, have lower mercury concentrations than otherwise expected (Kidd *et al.* 2003, Stafford and Haines 2001, Simoneau *et al.* 2005).

In the current study I calculated trophic magnification factors (TMFs) for total mercury in fish at all study sites as the antilog of the slope of log(THg)~calculated trophic level (TL) regressions. A positive

slope for log(THg)~TL regressions indicates that biomagnification is taking place, and since TMF is calculated as the antilog of this slope, TMF > 1 (where slope > 0) also indicates biomagnification. TMF values for my study lakes ranged from 1.89 in Lake George to 5.58 in Lake Albert (all higher than 1.0), confirming (once again) that biomagnification is occurring at all study sites, with higher TMF values indicating higher rates of biomagnification. Given that my log(THg)~TL regressions are based only on fish, and in fish most mercury is in the form of methyl mercury (MeHg; Bloom *et al.* 1992), my calculated TMFs for THg should be roughly comparable to TMFs for MeHg for these study sites.

Predicted mercury concentrations at TL = 1 (based on log(THg)~TL regressions) should yield a estimate of average mercury concentrations in primary producers at a site, assuming that biomagnification rate and nitrogen isotopic fractionation is consistent across the whole food web. Since the log(THg)~TL regressions included only fish muscle tissue, where most mercury is present as methyl mercury; the predicted values at TL = 1 are more likely to reflect phytoplankton MeHg rather than THg concentrations, which are often much lower than THg concentrations in phytoplankton. By using predicted values rather than actual phytoplankton THg concentrations, I gain a time-integrated estimate of mercury concentrations in phytoplankton, which is helpful since mercury concentrations in short-lived fast-growing organisms can vary greatly in both time and space (Kirkwood *et al.* 1999).

Based on these predicted values, I was able to test the influence of both mercury concentrations in water and chlorophyll *a* concentrations on mercury concentrations in phytoplankton. I observed that, alone, neither of these factors were significant predictors of estimated phytoplankton mercury concentrations; however, when combined, these factors were strong predictors of phytoplankton mercury, with total mercury concentrations in water positively contributing to phytoplankton mercury concentrations and chlorophyll *a* concentrations negatively contributing to the estimated phytoplankton mercury concentrations. This could be evidence of biomass dilution, whereby high phytoplankton biomass effectively dilutes the mercury on a per biomass basis (Pickhardt *et al.* 2002). However, phytoplankton growth rates may also be higher in high chlorophyll systems, leading to growth dilution of mercury, where rapidly dividing cells may not fully equilibrate with available methyl Hg concentrations (Herendeen and Hill 2004).

Biomass dilution and growth dilution at the first trophic level can lead to low mercury concentrations throughout the food web, even in top predators (Meili *et al.* 2001, Pickhardt *et al.* 2002). This may explain why the highest mercury concentrations in fish that I observed were in lakes where mercury concentrations in water, but also chlorophyll *a* concentrations and phytoplankton $\delta^{13}C$ values (as an

indicator of growth rate, Hecky and Hesslein 1995), tended to be lower. For example, despite having very similar food webs, and despite mercury concentrations being higher in Lake George, fish in less productive Lake Edward have much higher mercury concentrations. Similarly, Napoleon Gulf and Murchison Bay have very similar mercury concentrations in fish tissue even though mercury concentrations in water for Murchison Bay are more than 2-fold higher than those in Napoleon Gulf. Lake Albert also has very high mercury concentrations in fish despite very low concentrations in water. This was also observed by Campbell *et al.* (2006) in lakes Nkuruba and Saka, where fish in Lake Saka tended to have lower mercury concentrations despite higher concentrations in water.

Both TMF and food chain length (FCL, number of trophic levels between the base of the food web and top predators) have been found to be important factors in explaining inter-lake variability in the biomagnification of mercury (summarized in Gantner 2009), where top predators from lakes with higher TMF values and longer food chains tended to have higher mercury concentrations. However, given that FCL was highest in Lake Edward (where mercury concentrations in fish were highest) and second highest in Lake George (where mercury concentrations in fish were lowest) (Table 4.1), it is likely that while FCL may influence mercury concentrations at the top of the food web, growth/biomass dilution and biomagnification rate are likely much stronger predictors of Hg in top predators in these study lakes.

4.4.8 General Conclusions

The low total mercury concentrations in fish observed at the study sites in this study are consistent with previous reports of low mercury concentrations in fish from East African lakes including Winam and Napoleon Gulfs (L. Victoria, Campbell *et al.* 2003c), Lake Kyoga (Campbell *et al.* 2004), Lake Malawi (Kidd *et al.* 2003), Lake Tanganyika (Campbell *et al.* 2008), Lake Awassa (Desta *et al.* 2007), and in several other small lakes (Campbell *et al.* 2003b, 2006). Low THg concentrations in fish have also been observed in meso/eutrophic Chinese reservoirs, with mean THg concentrations in *Oreochromis mossambicus* of 9.0 ± 4.0 ng/g wet weight (Yan *et al.* 2010), which are similar concentrations to those that I observed for tilapiine cichlids in the current study. As in other studies in tropical systems, I found lower mercury concentrations in top predators from my study sites than have been reported for arctic or temperate lakes, despite comparable mercury concentrations in water, food chain lengths and biomagnification rates (Campbell *et al.* 2003c).

Reduced mercury concentrations in fish from tropical lakes relative to temperate and arctic lakes are likely attributable to combination of factors including: growth dilution of mercury both in primary producers and consumer trophic levels due to high growth rates (Herendeen and Hill 2004) as well as

possible biomass dilution of mercury. Many of my study lakes are hypereutrophic, and unlike temperate or arctic lakes, are continuously productive throughout the year. Also, the shorter lifespan of tropical fish relative to temperate fish can also act to reduce the maximum potential mercury concentrations in these systems (Kidd *et al.* 2003). Processes occurring at the base of the food web (growth and biomass dilution) appear to be strong determinants of mercury concentrations throughout these food webs and these results indicate that eutrophication may act to reduce the potential for high mercury concentrations in fish. Among the 8 study sites, only a very small number of fish from Lake Albert, Lake Edward and Napoleon Gulf had mercury concentrations in excess of 200 ng/g w.w. (the WHO guideline for at-risk individuals), suggesting that fish from these lakes do not pose a mercury exposure risk to consumers.

Table 4.1 Total mercury concentrations in water (just under surface), chlorophyll *a* concentrations (from Chapter 2), and food chain length (FCL; from Chapter 3).

Lake	THg in Water (ng/L)	Chlorophyll *a* (µg/L)	FCL (number of trophic levels)
Lake Albert	0.46 ± 0.11 (n = 9)	19.2 ± 3.4	~
Lake Edward (all)	0.52 (n = 3)	43.8	4.3
Edward nearshore	0.65 (n = 1)	66.3 ± 46.2	~
Edward offshore	0.38 ± 0.01 (n = 2)	21.3 ± 22.8	~
Lake George	1.09 ± 0.11 (n = 2)	138.0 ± 39.1	3.9
Lake Mburo	0.62 ± 0.04 (n = 2)	48.6 ± 10.1	3.3
Murchison Bay	1.30 ± 0.09 (n = 3)	96.5 ± 38.1	2.8
Napoleon Gulf	0.53 ± 0.07 (n = 3)	24.7 ± 18.4	3.4
Lake Nkuruba	0.45 (n = 1)	6.2 ± 2.2	3.2
Lake Saka	0.81 ± 0.07 (n = 4)	90.0 ± 36.3	3.6

Table 4.2 Total mercury concentrations, stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios, and calculated trophic levels for fish, benthic invertebrates and plankton.

Name	n	Code	$\delta^{15}N$ (‰) μ ± s.d	$\delta^{13}C$ (‰) μ ± s.d.	Trophic Level μ ± s.d.	THg (ng/g ww) μ ± s.d.	THg (ng/g ww) Range
Lake Albert							
Alestes baremose	1	Ab	8.9	-20.0	2.9	79.9	~
Barbus bynii	2	Bb	7.9–7.9	-19.9 to -19.2	2.6–2.6	38.6–40.1	38.6–40.1
Bagrus bayad	2	Bba	8.6–9.4	-18.3 to -17.8	2.8–3.1	28.4–29.5	28.4–29.5
Brycinus nurse	13	Bnu	9.1 ± 0.3	-19.0 ± 0.6	3.0 ± 0.1	81.4 ± 25.6	36.3–116.4
Hydrocynus forskahlii	2	Hf	9.3–10.2	-18.6 to -18.6	3.1–3.3	20.0 ± 10.3	12.7–27.3
Labeo horie	1	Lh	8.1	-19.3	2.7	62.7	~
Lates macrophthalamus	4	Lm	9.5 ± 1.1	-19.3 ± 0.6	3.1 ± 0.3	165.6 ± 169.3	68.7–419.3
Lates niloticus	6	Ln	9.5 ± 0.9	-19.1 ± 0.6	3.1 ± 0.3	257.5 ± 206.8	86.4–609.4
Oreochromis leucostictus	2	Ol	7.3–8.5	-19.0 to -16.1	2.5–2.8	26.6–35.6	26.6–35.6
Oreochromis niloticus	1	On	6.4	-18.5	2.2	19.9	~
Neobola bredoi	6	Rb	8.7 ± 0.4	-19.7 ± 0.5	2.9 ± 0.1	37.9 ± 10.8	23.8–55.8
Schilbe intermedius	3	Si	8.6 ± 0.2	-19.5 ± 0.1	2.9 ± 0.1	51.3 ± 7.6	44.4–59.5
Thoracochromis mahagiensis	2	Tm	~	~	~	47.3–78.0	47.3–78.0
Tilapia zilli	6	Tz	6.6 ± 0.7	-15.8 ± 2.3	2.3 ± 0.2	22.7 ± 8.5	9.6–33.7
Unknown clam	1		~	~	~	14.3	~
Bellamya sp.	3		~	~	~	14.1 ± 6.0	9.4–20.8
Byssandodonta sp.	4		~	~	~	6.7 ± 2.8	4.1–10.7
Cleopatra sp.	2		~	~	~	10.4–21.2	10.4–21.2
Gabbia sp.	2		~	~	~	31.8–49.9	31.8–49.9
Melanoides sp.	1		~	~	~	6.4	~
Lake Edward							
Barbus bynni	9	Bb	8.6 ± 0.7	-12.1 ± 1.2	3.1 ± 0.2	11.6 ± 4.0	6.4–19.8
Bagrus docmac	9	Bd	11.4 ± 1.5	-14.2 ± 2.4	3.9 ± 0.5	70.7 ± 106.0	3.5–277.1
Clarias gariepinus	9	Cg	9.7 ± 1.3	-15.2 ± 2.0	3.4 ± 0.4	53.7 ± 91.9	6.1–296.4
Haplochromis (?) spp.	6	H	12.0 ± 1.6	-15.4 ± 2.0	4.1 ± 0.5	27.3 ± 30.9	5.7–86.0
Haplochromis squamipinnis	10	Hs	12.6 ± 1.1	-16.2 ± 2.0	4.3 ± 0.3	188.3 ± 254.4	13.7–855.3
Oreochromis leucostictus	4	Ol	6.5 ± 0.4	-16.6 ± 0.9	2.5 ± 0.1	5.6 ± 0.5	5.0–6.1
Oreochromis niloticus	15	On	6.9 ± 1.0	-17.1 ± 1.2	2.6 ± 0.3	8.4 ± 2.7	3.7–13.9
Protopterus aethiopicus	10	Pa	9.2 ± 0.8	-13.3 ± 2.4	3.3 ± 0.2	5.2 ± 2.8	1.9–11.0
Nearshore phytoplankton	3	p_n	1.7 ± 0.2	-11.3 ± 2.4	1	2.9 ± 1.2	2.0–4.2
Offshore phytoplankton	2	p_o	1.2–1.8	-23.8 to -18.6	0.9–1.1	3.0–3.1	3.0–3.1
Zooplankton (80 μm net)	2	z_{80}	3.7–4.8	-22.7 to -21.0	1.6–2.0	1.3–2.3	1.3–2.3
Zooplankton (153 μm net)	2	z_{153}	3.0–4.8	-23.2 to -17.7	1.4–2.0	1.1–4.5	1.1–4.5
Lake George							
Bagrus docmac	10	Bd	8.3 ± 0.7	-8.4 ± 1.6	3.8 ± 0.2	4.9 ± 4.4	1.6–15.9
Clarias gariepinus	9	Cg	6.2 ± 1.2	-9.7 ± 6.1	3.2 ± 0.3	4.9 ± 3.7	0.9–12.3
Haplochromis (?) spp.	7	H	8.6 ± 1.1	-7.1 ± 2.5	3.9 ± 0.3	5.8 ± 2.3	3.7–9.5
Haplochromis squamipinnis	6	Hs	7.4 ± 1.1	-8.6 ± 2.1	3.6 ± 0.3	18.8 ± 24.3	3.8–67.7
Oreochromis esculentus	2	Oe	2.8–2.9	-16.7 to -9.5	2.2–2.2	2.9 ± 0.2	2.8–3.1
Oreochromis leucostictus	6	Ol	3.6 ± 0.7	-10.1 ± 2.1	2.5 ± 0.2	3.3 ± 2.2	1.9–7.1
Oreochromis niloticus	18	On	2.7 ± 0.7	-5.9 ± 2.6	2.2 ± 0.2	1.6 ± 1.4	0.8–7.1
Protopterus aethiopicus	9	Pa	6.8 ± 1.0	-10.5 ± 6.4	3.4 ± 0.3	4.4 ± 7.9	1.1–25.3

Name	n	Code	$\delta^{15}N$ (‰) μ ± s.d	$\delta^{13}C$ (‰) μ ± s.d.	Trophic Level μ ± s.d.	THg (ng/g ww) μ ± s.d.	THg (ng/g ww) Range
Tilapia zilli	1	Tz	3.0	-16.0	2.3	2.9	~
Phytoplankton	4	p	-1.3 ± 0.5	-10.1 ± 1.5	1.0 ± 0.1	2.6 ± 1.0	1.6–3.6
Chaoborus	1	c	3.8	-9.4	2.5	~	~
Lake Mburo							
Bagrus docmac	1	Bd	6.0	-10.5	2.9	7.9	~
Clarias gariepinus	7	Cg	7.2 ± 2.4	-12.8 ± 2.9	3.3 ± 0.7	10.9 ± 10.5	2.6–32.2
Haplochromis (?) spp.	10	H	6.0 ± 0.7	-10.8 ± 0.8	2.9 ± 0.2	9.1 ± 8.9	1.6–27.3
Oreochromis esculentus	10	Oe	4.1 ± 0.5	-10.6 ± 0.9	2.3 ± 0.1	3.7 ± 4.5	1.7–16.4
Oreochromis leucostictus	10	Ol	4.1 ± 0.6	-13.5 ± 1.1	2.4 ± 0.2	5.4 ± 1.8	3.2–8.8
Oreochromis niloticus	15	On	4.8 ± 1.5	-11.0 ± 1.2	2.6 ± 0.4	4.0 ± 2.2	1.5–9.9
Protopterus aethiopicus	10	Pa	6.3 ± 1.7	-12.9 ± 3.1	3.0 ± 0.5	8.4 ± 8.5	1.7–31.4
Phytoplankton	6	p	-0.5 ± 0.2	-12.6 ± 1.3	1	2.7 ± 1.4	1.5–5.5
Chironomidae	1	ch	2.4	-14.3	1.8	~	~
Lake Victoria (Murchison Bay)							
Clarias gariepinus	1	Cg	8.8	-18.7	2.3	17.3	~
Haplochromis (?) spp.	9	H	9.8 ± 1.1	-16.7 ± 0.8	2.6 ± 0.3	18.9 ± 5.3	12.9–25.9
Lates niloticus	18	Ln	9.4 ± 1.6	-16.2 ± 1.2	2.5 ± 0.5	42.0 ± 22.0	17.6–101.1
Oreochromis leucostictus	5	Ol	8.5 ± 1.1	-17.1 ± 0.8	2.2 ± 0.3	11.0 ± 5.7	5.2–19.4
Oreochromis niloticus	24	On	7.7 ± 1.5	-16.3 ± 1.5	2.0 ± 0.4	14.1 ± 18.8	3.5–91.5
Protopterus aethiopicus	9	Pa	10.5 ± 1.1	-16.1 ± 1.3	2.8 ± 0.3	7.6 ± 2.8	4.4–13.9
Rastrineobola argentea	1*	Ra	8.6	-15.9	2.3	49.1	~
Synodontis afrofischeri	8	Sa	10.2 ± 1.5	-17.0 ± 0.6	2.7 ± 0.5	40.8 ± 19.9	10.1–59.2
Synodontis victoriae	7	Sv	9.0 ± 0.8	-16.1 ± 2.3	2.4 ± 0.2	27.9 ± 21.5	6.2–69.6
Tilapia zilli	7	Tz	8.7 ± 1.3	-17.0 ± 1.5	2.3 ± 0.4	12.4 ± 6.4	7.0–25.4
Phytoplankton	6	p	5.8 ± 0.9	-13.9 ± 2.7	1.4 ± 0.3	1.9 ± 0.7	1.3–2.7
Zooplankton (80 μm net)	1	z_{80}	7.6	-15.3	2.0	~	~
Zooplankton (153 μm net)	1	z_{153}	7.7	-15.3	2.0	~	~
Mayflies (*Povilla*)	1	g	6.1	-19.1	1.5	5.9	~
Leeches	1	h	9.2	-17.3	2.4	10.7	~
Snails (*Bellamya*)	1	b	7.7	-17.8	2.0	~	~
Chironomidae	1	ch	3.7	-17.6	0.8	14.3	~
Lake Victoria (Napoleon Gulf)							
Astatoreochromis alluaudi	1	Aa	7.4	-16.0	2.8	7.3	~
Bagrus docmac	1	Bd	9.3	-14.2	3.4	27.9	~
Brycinus sadleri	1	Bs	6.5	-16.7	2.5	85.3	~
Haplochromis (?) spp.	8	H	7.6 ± 0.7	-15.4 ± 1.5	2.8 ± 0.2	30.4 ± 18.5	17.0–73.1
Lates niloticus	23	Ln	7.9 ± 1.0	-15.3 ± 1.2	2.9 ± 0.3	34.8 ± 13.0	15.3–71.3
Mormyrus kannume	5	Mk	8.1 ± 0.3	-14.6 ± 0.5	3.0 ± 0.1	31.7 ± 29.1	9.4–82.3
Oreochromis leucostictus	2	Ol	4.3–4.6	-15.4 to -12.9	1.9–2.0	3.1 ± 0.5	2.8–3.5
Oreochromis niloticus	24	On	4.6 ± 0.7	-13.8 ± 1.0	2.0 ± 0.2	12.8 ± 36.3	2.7–182.5
Oreochromis variabilis	9	Ov	4.0 ± 0.5	-14.5 ± 0.6	1.8 ± 0.2	3.2 ± 0.7	2.0–4.1
Protopterus aethiopicus	10	Pa	8.2 ± 1.7	-16.4 ± 0.9	3.0 ± 0.5	7.1 ± 5.6	1.9–20.7
Rastrineobola argentea	5*	Ra	6.9 ± 0.4	-14.0 ± 0.8	2.6 ± 0.1	17.0 ± 9.5	8.1–31.2
Synodontis afrofischeri	3	Sa	8.3 ± 0.1	-15.4 ± 0.04	3.1 ± 0.1	28.6 ± 6.1	22.7–34.9
Synodontis victoriae	4	Sv	7.5 ± 0.4	-16.9 ± 0.3	2.8 ± 0.1	111.5 ± 152.9	27.0–340.5

Name	n	Code	$\delta^{15}N$ (‰) μ ± s.d	$\delta^{13}C$ (‰) μ ± s.d.	Trophic Level μ ± s.d.	THg (ng/g ww) μ ± s.d.	THg (ng/g ww) Range
Tilapia zilli	10	Tz	6.4 ± 1.3	-13.6 ± 1.5	2.5 ± 0.4	9.3 ± 3.8	3.7–14.6
Phytoplankton	6	p	1.0 ± 0.8	-16.3 ± 2.8	0.9 ± 0.2	2.0 ± 1.0	0.6–3.1
Zooplankton (80 μm net)	3	z_{80}	5.3 ± 0.4	-15.8 ± 1.8	2.2 ± 0.1	~	~
Zooplankton (153 μm net)	3	z_{153}	5.2 ± 0.3	-15.3 ± 2.0	2.2 ± 0.1	~	~
Chironomidae	1	ch	2.8	-17.2	1.4	17.1	~
Lake Nkuruba							
Oreochromis leucostictus	5	Ol	6.1 ± 0.6	-28.1 ± 0.9	2.6 ± 0.2	10.8 ± 7.2	6.4–23.6
Poecelia reticulata	1*	Pr	7.9	-28.2	3.2	20.1	~
Tilapia zilli	9	Tz	7.5 ± 1.3	-26.0 ± 0.9	3.0 ± 0.4	11.7 ± 5.3	5.4–20.9
Phytoplankton	1	p	-0.03	-27.4	0.8	3.1	~
Epilithic Phytoplankton	1	p_b	2.0	-11.3	1.4	~	~
Zooplankton (80 μm net)	3	z_{80}	4.3 ± 0.6	-28.5 ± 0.6	2.1 ± 0.2	2.1 ± 0.4	1.6–2.5
Zooplankton (153 μm net)	5	z_{153}	5.1 ± 0.6	-29.0 ± 0.7	2.3 ± 0.2	2.1 ± 0.7	1.5–3.1
Chaoborus	1	c	5.8	-26.4	2.5	~	~
Snails	1	g	-26.7	3.0	1.7	~	~
Lake Saka							
Astatoreochromis alluaudi	10	Aa	5.7 ± 0.7	-20.1 ± 1.0	3.1 ± 0.2	9.7 ± 8.8	4.5–33.9
Barbus neumayerii	1	Bn	5.7	-23.8	3.1	49.2	~
Haplochromis (?) spp.	10	H	5.3 ± 0.7	-20.5 ± 0.8	3.0 ± 0.2	9.1 ± 9.6	2.9–31.7
Lates niloticus	4	Ln	7.3 ± 0.4	-19.7 ± 0.3	3.6 ± 0.1	8.0 ± 4.9	4.1–14.8
Oreochromis niloticus	16	On	3.1 ± 1.3	-18.9 ± 0.8	2.4 ± 0.4	7.8 ± 10.4	1.9–39.2
Tilapia zilli	2	Tz	2.9–4.1	-21.2 to -18.8	2.3–2.7	12.9 ± 10.8	5.2–20.5
Phytoplankton	4	p	-1.5 ± 0.1	-20.4 ± 1.5	1.0	1.3 ± 0.5	0.8–2.0
A. alluaudi yolk-sac brood	1	aa_b	4.7	-21.9	2.9	14.8	~
H. (?) spp. yolk-sac brood	1	h_b	3.6	-25.0	3.1	20.0	~

Table 4.3 Statistical comparisons between sites for mercury concentrations in different species. The rows show the lakes for which mercury concentrations in the given fish species are significantly lower (ANOVA, P<0.05) than for the lake indicated at the top of the column. Where no lakes are listed, there are no significant differences between the lakes. Lake codes are as follows: A=Albert, Ed=Edward, G=George, Mb=Mburo, Mu=Murchison, Na=Napoleon, Nk=Nkuruba, S=Saka.

	A	Ed	G	Mb	Mu	Na	Nk	S
B. docmac	~	G	~	~	~	~	~	~
C. gariepinus	~	G	~	~	~	~	~	~
H. (?) spp.	~	~	~	~	Mb, S	G, Mb, S	~	~
L. niloticus	Mu, Na, S	~	~	~	S	S	~	~
O. leucostictus	Ed, G, Mb, Na, Nk	~	~	~	G, Na	~	G	~
O. niloticus	G	G, Mb	~	G	G, Mb, S	G	~	G
P. aethiopicus	~	~	~	G	G	G	~	~
T. zilli	G, Na	~	~	~	~	~	~	~

Table 4.4 Summary of signficant relationships between total length (L) and log-transformed total mercury concentrations (log(THg)) in fish.

Site	Species	log(THg) vs. L	r^2_{adj}	P
Albert	*L. macrophthalamus*	+	0.99	<0.01
Albert	*L. niloticus*	+	0.97	<0.001
Edward	*B. docmac*	+	0.75	<0.01
Edward	*C. gariepinus*	+	0.25	<0.1
Edward	*Haplochromis* sp.	+	0.88	<0.01
Edward	*Haplochromis squamipinnis*	+	0.79	<0.001
Mburo	*O. niloticus*	+	0.18	<0.1
Murchison	*L. niloticus*	+	0.28	<0.05
Murchison	*S. victoriae*	+	0.80	<0.01
Napoleon	*S. victoriae*	+	0.88	<0.05
Nkuruba	*O. leucostictus*	+	0.95	<0.01
Saka	*A. alluaudi*	-	0.39	<0.05
Saka	*L. niloticus*	+	0.76	<0.1

Table 4.5 Summary of log(THg)~δ^{13}C regressions for fish from all study sites.

Lake	log(THg):δ^{13}C regression equation	r^2_{adj}	P
Lake Albert	-0.08 - 0.10(δ^{13}C)	0.15	<0.01
Lake Edward	0.21 - 0.06(δ^{13}C)	0.07	<0.05
Lake George	0.21 - 0.03(δ^{13}C)	0.09	<0.05
Lake Mburo	-0.05 - 0.06(δ^{13}C)	0.14	<0.01
Murchison Bay	1.33 + 0.01(δ^{13}C)	0	N.S.
Napoleon Gulf	-0.72 - 0.12(δ^{13}C)	0.12	<0.001
Lake Nkuruba	2.99 + 0.07(δ^{13}C)	0.16	N.S.
Lake Saka	-1.34 - 0.11(δ^{13}C)	0.09	<0.05

Table 4.6 Summary of log(THg)~δ^{15}N and log(THg)~TL (trophic level) regressions for fish, including trophic magnification factor (TMF).

Lake	log(THg):δ^{15}N regression equation	log(THg):TL regression equation	r^2_{adj}	P	TMF
Lake Albert	-0.14 + 0.22(δ^{15}N)	-0.39 + 0.75(TL)	0.43	<0.01	5.58
Lake Edward	-0.30 + 0.15(δ^{15}N)	-0.58 + 0.53(TL)	0.43	<0.01	3.35
Lake George	0.01 + 0.08(δ^{15}N)	-0.37 + 0.28(TL)	0.25	<0.01	1.89
Lake Mburo	0.20 + 0.09(δ^{15}N)	-0.14+ 0.30(TL)	0.20	<0.01	2.01
Murchison Bay	0.45 + 0.09(δ^{15}N)	0.52 + 0.29(TL)	0.14	<0.01	1.95
Napoleon Gulf	0.11 + 0.15(δ^{15}N)	-0.20 + 0.51(TL)	0.33	<0.01	3.20
Lake Nkuruba	0.13 + 0.13(δ^{15}N)	-0.23 + 0.43(TL)	0.55	<0.01	2.72
Lake Saka	0.41 + 0.08(δ^{15}N)	-0.004 + 0.28(TL)	0.11	<0.05	1.92

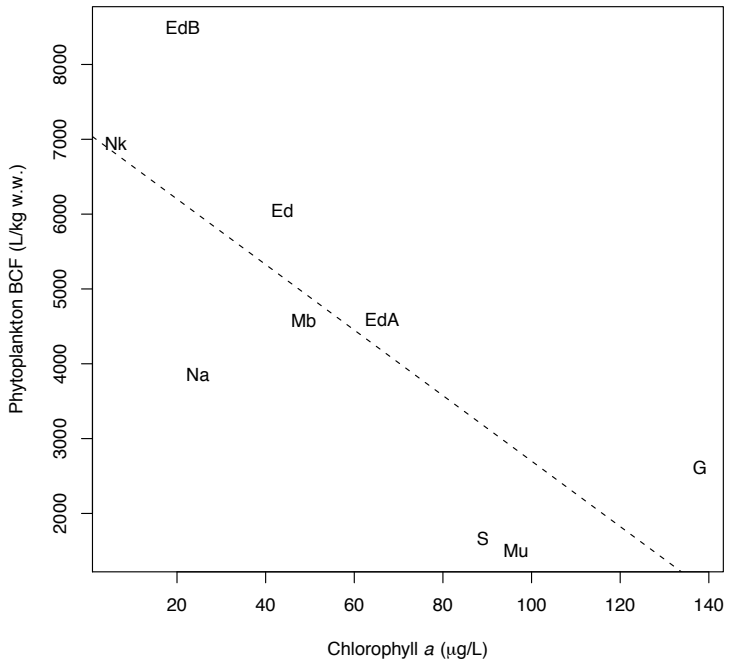

Figure 4.1 Linear regression of phytoplankton bioconcentration factors (BCFs) for THg against chlorophyll *a* concentrations (r^2_{adj} = 0.55, P<0.05). Lake codes are as follows: EdA=Edward nearshore, EdB=Edward offshore, Ed=Edward (all), G=George, Mb=Mburo, Mu=Murchison, Na=Napoleon, Nk=Nkuruba, S=Saka. BCFs were calculated by dividing phytoplankton THg concentrations (ng/kg) by estimated dissolved THg in water (ng/L).

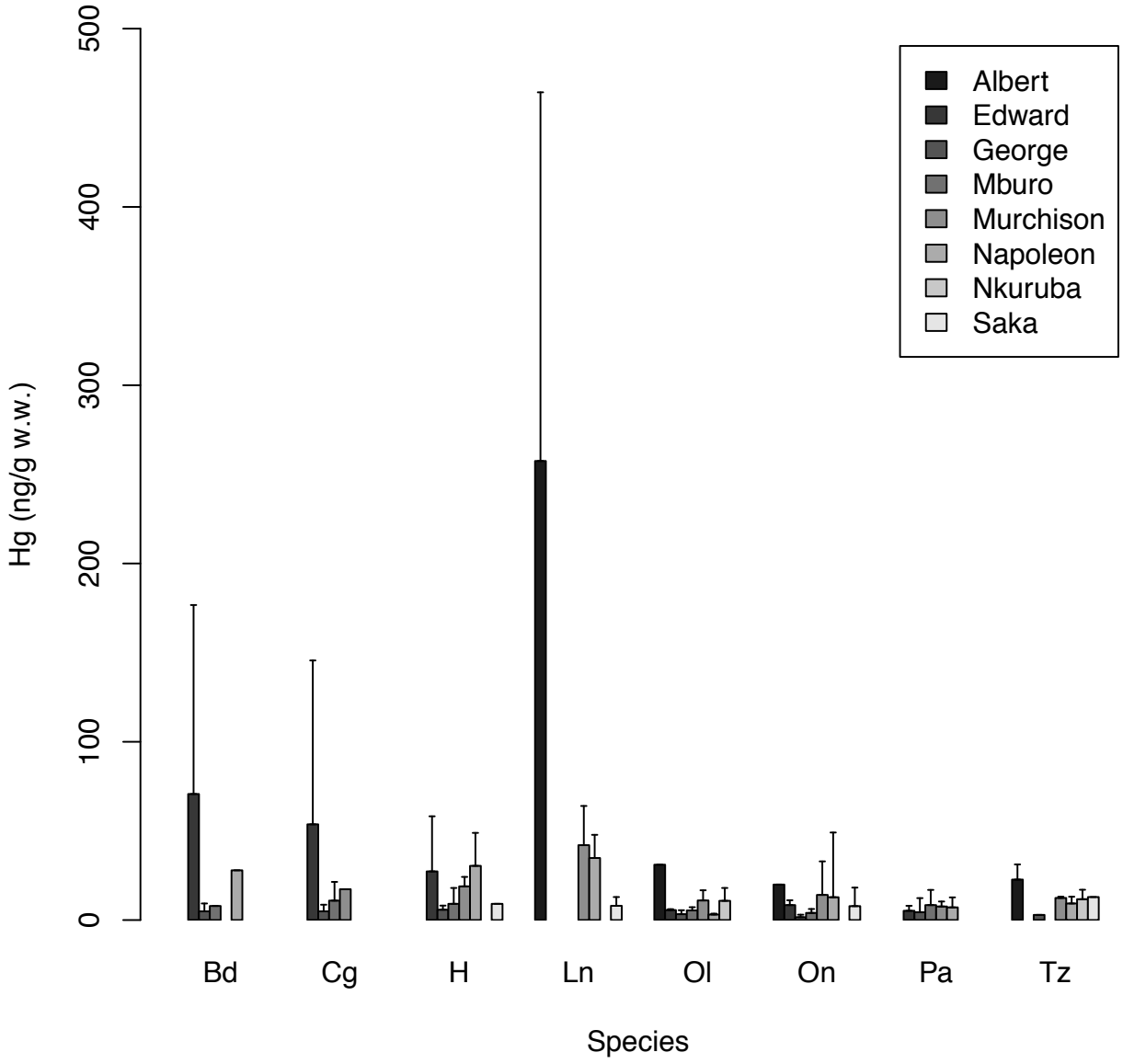

Figure 4.2 Total mercury concentrations in selected species of fish at all study sites. Bar height represents mean values, while error bars represent standard deviation. Species codes are found in Table 4.2.

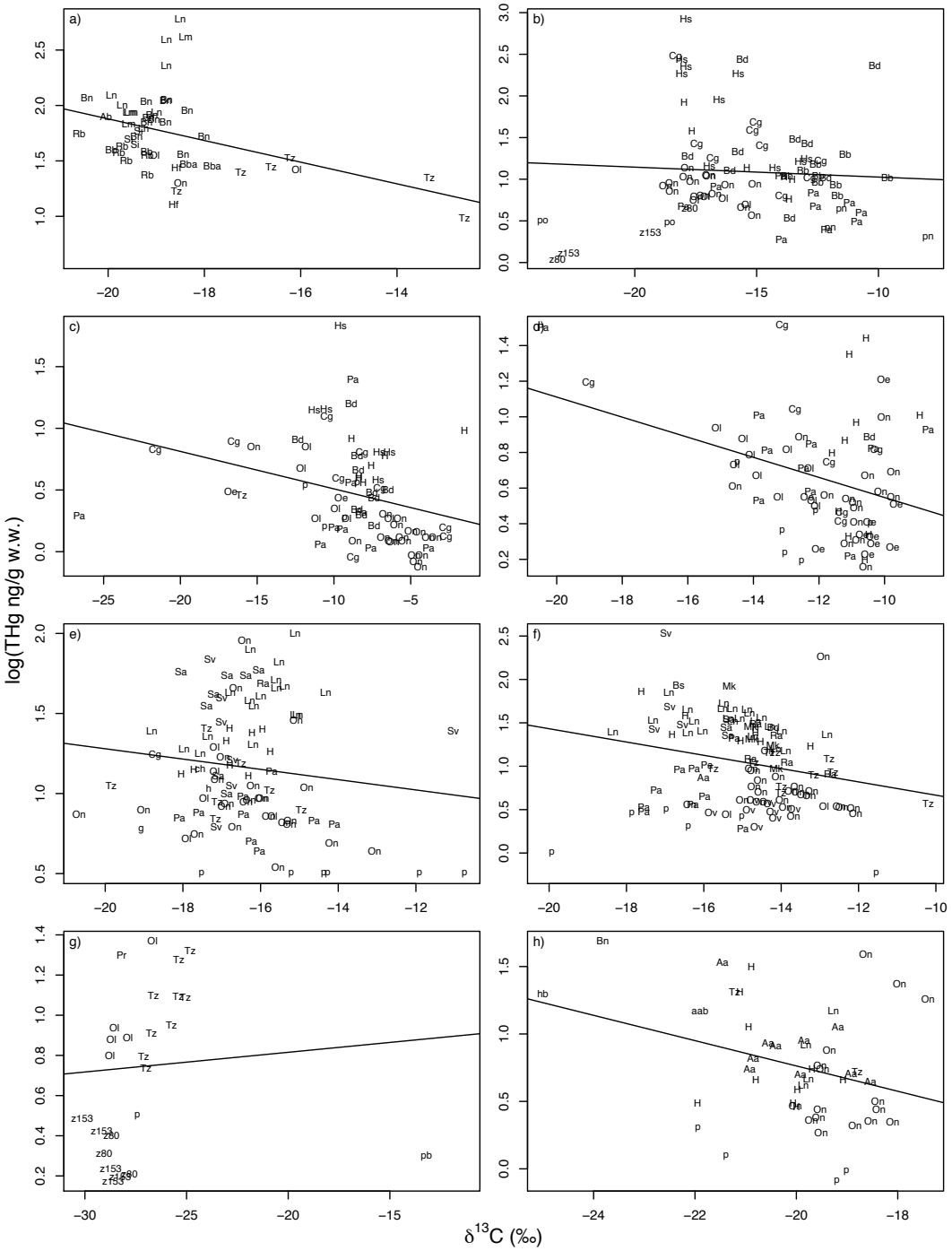

Figure 4.3 Regressions between log-transformed total mercury concentrations and stable carbon isotopic ratios ($\delta^{13}C$) for fish from a) Lake Albert, b) Lake Edward, c) Lake George, d) Lake Mburo, e) Murchison Bay, f) Napoleon Gulf, g) Lake Nkuruba, h) Lake Saka. Codes used as labels are found in Table 4.2. Detailed information about regressions is found in Table 4.5.

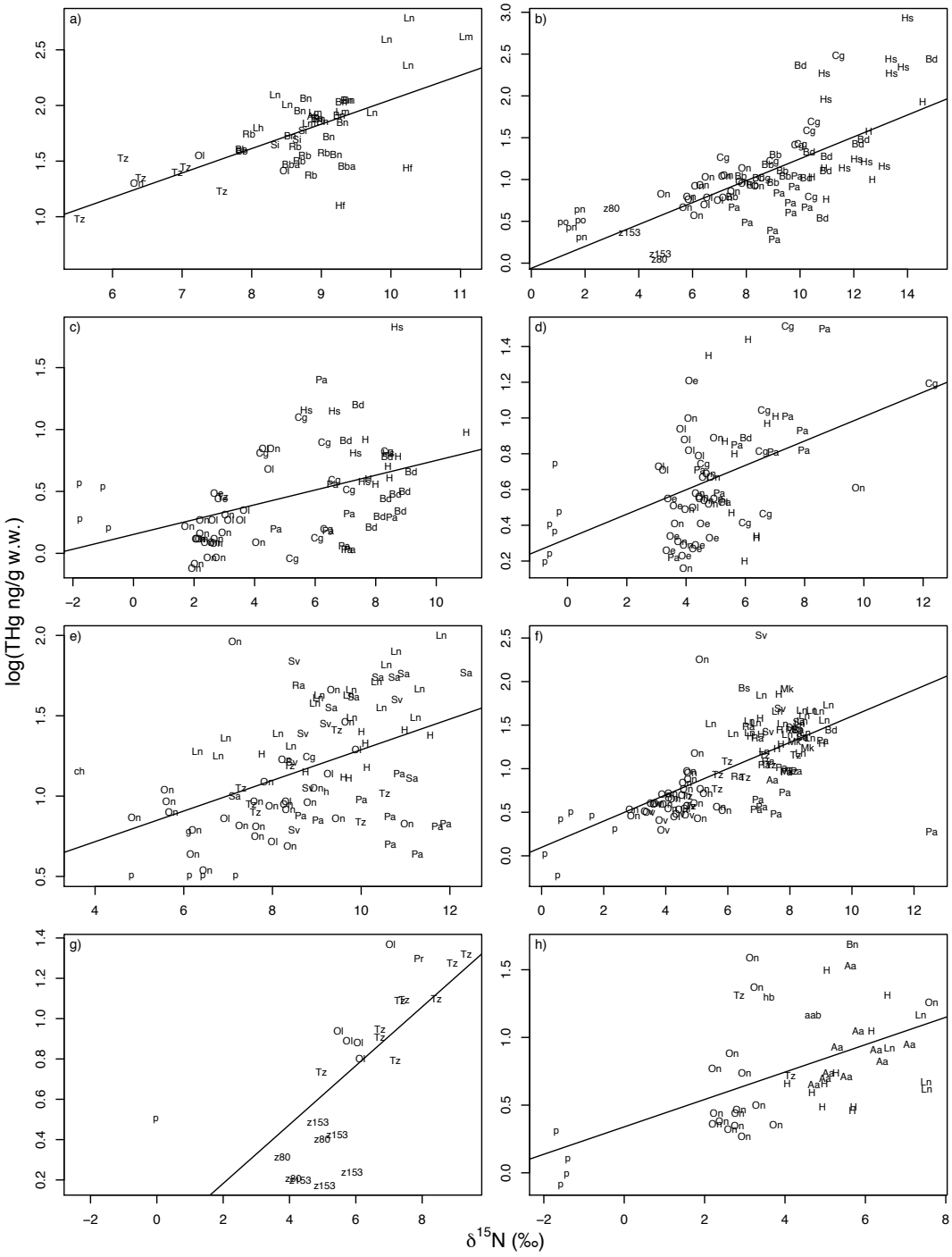

Figure 4.4 Regressions between log-transformed total mercury concentrations and calculated trophic level for fish from a) Lake Albert, b) Lake Edward, c) Lake George, d) Lake Mburo, e) Murchison Bay, f) Napoleon Gulf, g) Lake Nkuruba, h) Lake Saka. Codes used as labels are found in Table 4.2. Detailed information about regressions is found in Table 4.6.

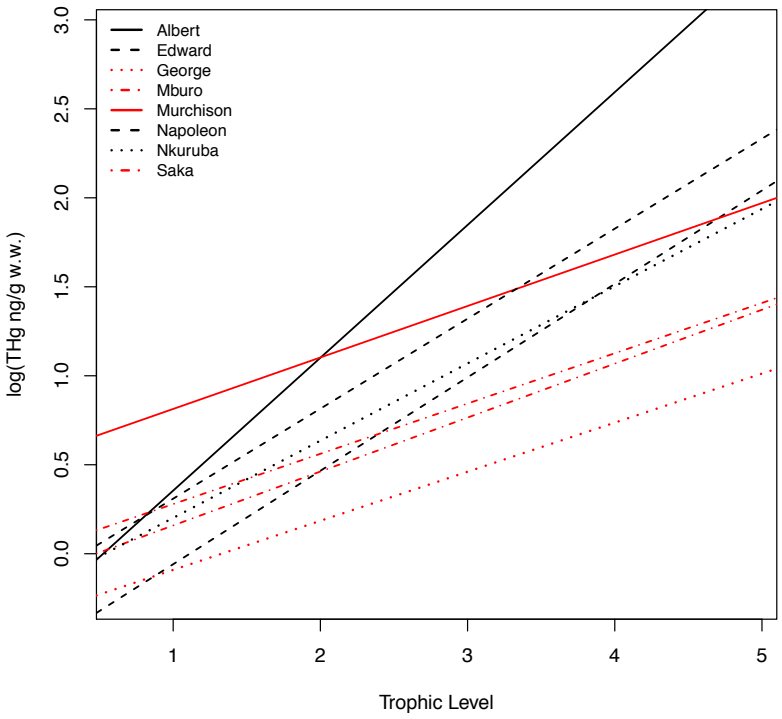

Figure 4.5 Regressions of log-transformed total mercury concentrations against calculated trophic level for fish from several study sites. Based on analysis of covariance, sites with red regression lines (group A) had significantly lower regression slopes than sites with black regression lines (group B), but within each group of sites there were no significant differences in regression slopes.

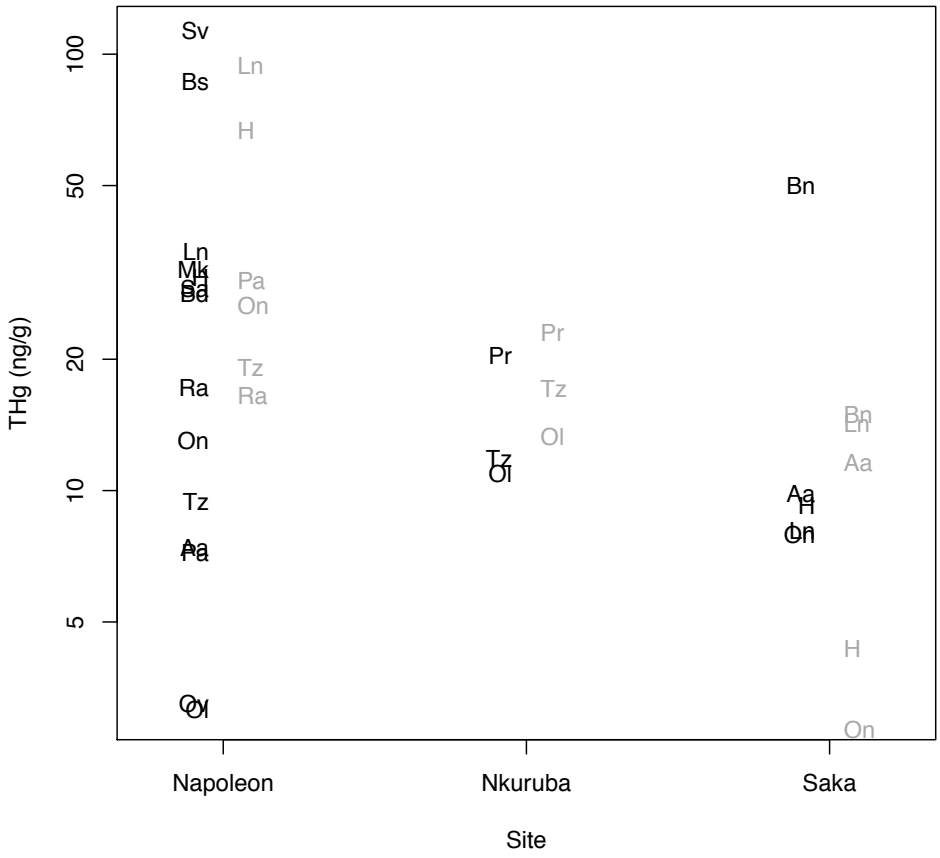

Figure 4.6 Comparisons between log-transformed mercury concentrations observed for indicated fish species in the current study (black lettering) and those previously observed (grey lettering) in both Napoleon Gulf (Campbell *et al.* 2003, samples collected in 1998) and the crater lakes Saka and Nkuruba (Campbell *et al.* 2006, samples collected in 2000). Species codes are found in Table 4.2.

Chapter 5
Accumulation, trophic transfer, and seasonality of microcystin in fish from several Ugandan lakes

5.1 Introduction

Globally, cultural eutrophication is leading to an increase in the cyanobacterial dominance of freshwater systems and in the occurrence of toxic cyanobacterial blooms (deFigueiredo *et al.* 2004). Additionally, climate warming is expected to further exacerbate the frequency and duration of these blooms (Paerl and Huisman 2008, 2009). Cyanobacterial blooms can threaten the ecological integrity and social value of freshwater systems through beach fouling, oxygen depletion, changes in food web structure and species assemblages, as well as through the production of harmful toxins.

Cyanobacteria are known to produce a wide range of toxins, however the most common cyanotoxins produced in fresh water belong to the hepatotoxic microcystin family, while in brackish waters the closely related hepatotoxin nodularin is widespread (Sivonen and Jones 1999). Several cyanobacterial taxa are known to be capable of microcystin production, including *Microcystis*, *Anabaena*, *Anabaenopsis*, *Planktothrix* (*Oscillatoria*), and *Nostoc* (Sivonen and Jones 1999).

Microcystin exerts toxic effects through the inhibition of protein phophatases 1 and 2A in animals (MacKintosh *et al.* 1990, Williams *et al.* 1997, Sivonen and Jones 1999). Although microcystin cannot passively cross the membranes of animal, plant or bacterial cells, active transport through membranes does occur, particularly in mammalian hepatocytes (deFigueiredo *et al.* 2004, Cazenave *et al.* 2005, Babica *et al.* 2006). As a result, microcystin displays selective accumulation in liver tissue, and subjects the liver to the severe toxic effects and damage (deFigueiredo *et al.* 2004, Cazenave *et al.* 2005). Additionally, there is evidence that microcystin can act as a liver tumour promoter, and chronic exposure to this toxin may pose serious health risks (Nishiwaki-Matsushima *et al.* 1992, Falconer and Humpage 1996).

Accumulation of microcystin has been observed in zooplankton, gastropods, fish, macrophytes and even terrestrial crops irrigated with contaminated water (Kotak *et al.* 1996, Prepas *et al.* 1997, Magalhaes *et al.* 2003, deFigueiredo *et al.* 2004). Ibelings and Chorus (2007) provide a comprehensive review of studies exploring the accumulation of microcystin in freshwater organisms. Fish can be exposed to microcystin through direct ingestion of toxic cyanobacteria, indirectly through the food web or by direct

uptake of dissolved microcystin through the gills or skin (Cazenave *et al.* 2005, Ibelings and Chorus 2007, Smith *et al.* 2008). However, dietary exposure to microcystin is expected to be of greater importance than direct uptake of dissolved microcystin given the relatively hydrophilic nature of this toxin (Smith *et al.* 2008). The accumulation of microcystin in fish muscle tissue has been found to begin shortly after exposure and to increase with time (Cazenave *et al.* 2005). There is also some evidence that fish are able to depurate microcystin when no longer exposed; however, depuration rates appear to differ greatly among species, and toxin concentrations can occasionally continue to increase during the early stage of depuration (Ibelings and Chorus 2007, Martins and Vasconcelos 2009). There is little evidence of biomagnification of microcystin in aquatic food webs (Kotak *et al.* 1996, Ibelings *et al.* 2005, Zhang *et al.* 2009; but see Xie *et al.* 2005), and some studies have suggested that microcystin may instead undergo biodilution, with decreasing concentrations at higher trophic levels (Karjalainen *et al.* 2005, Ibelings and Havens 2008).

The World Health Organization has set a provisional total daily intake value (TDI) based on chronic exposure of 0.04 µg/kg body weight per day (Falconer *et al.* 1999, WHO 1998). Based on this TDI, and the assumption that 80% of daily intake can be attributed to drinking water, the current WHO guideline value for microcystin in drinking water is 1 µg/L (Falconer *et al.* 1999, WHO 1998). Despite the fact that microcystin is known to accumulate in fish, very little is known about the risk of microcystin exposure posed by fish consumption, and no widely accepted guidelines have been established for microcystin concentrations in fish tissue (although some values have been suggested by Ibelings and Chorus 2007).

The factors that determine microcystin concentrations in fish remain poorly resolved, and there is no consensus about how fish diet, absorption of microcystin from the gut and depuration can interact to influence microcystin concentrations in fish. Although there have been several studies that have explored microcystin concentrations and dynamics in fish (Kotak *et al.* 1996, Xie *et al.* 2005, Chen *et al.* 2006, Smith and Haney 2006, Deblois *et al.* 2007, Amé *et al.* 2010, Semyalo *et al.* 2010; and others reviewed in Ibelings and Chorus 2007), many studies to date have focused on one (and occasionally a few) fish species, often with few replicates and few sampling dates, making it difficult to make any broad conclusions regarding the factors that determine microcystin concentrations in fish.

In particular, little is known about microcystin in water and fish from tropical African lakes. In tropical lakes, there is the potential for the year-round presence of toxic cyanobacteria at high biomasses (Oliver and Ganf 2000, Kling *et al.* 2001), increasing the potential magnitude and duration of food web exposure to microcystin. In the current study, I measured microcystin concentrations in water and several species of

fish from seven Ugandan study sites over a six-month period. The study sites included the tropical great lakes Edward and Victoria (where both Murchison Bay and Napoleon Gulf were sampled), as well as Lake George, Lake Mburo, and the crater lakes Saka and Nkuruba. All of these lakes are described in detail in the Chapter 1 of this thesis. These sites were selected in order to provide a range in trophic status as well as in the prevalence and persistence of cyanobacterial blooms. Mean microcystin and chlorophyll *a* concentrations for the study sites are shown in Table 5.1 (data taken from Chapter 2). By collecting a comprehensive set of fish and water samples from several lakes over several months, I hope to gain insight into the accumulation and possible trophic transfer of microcystin in tropical lakes.

5.2 Methods

5.2.1 Sampling methods

Fish were collected from all study sites between September 2008 and February 2009. This sampling period includes the months when cyanobacteria is expected to be particularly abundant (in the large, deep lakes sampled) due to elevated nutrient concentrations after the mixing period in June–August, and increased water column stability favouring buoyant cyanobacteria throughout September–April (Mugidde 2002). Sampling of Lakes Edward, George, Mburo, Nkuruba and Saka was carried out on a monthly basis, while samples were generally collected from Murchison Bay and Napoleon Gulf every two weeks. Fish were purchased directly from fishermen and, where possible, the general location of the catch was confirmed. Fish were chosen in order to ensure a broad set of species from all trophic levels, and a representative size range within species.

5.2.2 Sample processing

Subsamples of dorsolateral muscle tissue were taken from the fish collected. Where fish were too small to isolate dorsolateral muscle tissue, whole fillets of axial musculature were collected, and where fish were too small to fillet (generally when under 10 cm in length), they were analyzed whole. All fish samples were initially frozen and then were oven dried at 60 ºC for at least 24 hours (until weight stabilized) at the National Fisheries Resources Research Institute in Jinja, Uganda. Dried fish samples were placed in clean plastic bags, which were then placed in larger sealed bags containing dessicant for transport to Canada. Prior to analysis, dried fish samples were homogenized using a ball-mill grinder.

5.2.3 Microcystin in Fish

Microcystin in fish muscle tissue was analyzed using methanol extraction followed by competitive indirect ELISA (Fischer *et al.* 2001) based on the method described in Wilson *et al.* (2008) with some modifications. Homogenized fish muscle tissue was extracted twice, first for 2 hours using 75 % methanol, and then for 24 hours using 75 % methanol in addition to glacial acetic acid (0.002 v/v). After each extraction, samples were centrifuged and supernatant was removed and pooled. The pooled supernatant was filtered to remove particulates, subsampled, and the solvent was evaporated using a Turbovap LV. After evaporation, the remaining solids were resuspended in de-ionized water and analyzed for microcystins/nodularins using Abraxis anti-ADDA ELISA test kits (Abraxis LLC, PN 520011). This is a "congener independent" ELISA based on the detection of the Adda side-chain found in microcystins and nodularins (Fischer *et al.* 2001). The detection range for the ELISA test kits was 0.1–5.0 µg/L. Extracts for analysis were kept within the detection range by adjusting the mass of fish extracted and by diluting extracts where necessary. Measured dry-weight microcystin concentrations in fish were converted to wet-weight concentrations using a conversion factor of 0.31 (Campbell *et al.* 2003). Each standard and sample was run in duplicate in order to assess test reproducibility. Coefficients of variation (CVs) for standards were on average 7.0 ± 6.4 % (n=75), while CVs for samples were on average 6.3 ± 7.8 % (n=432). Several fish samples were extracted twice to determine the variability associated with extraction, and CVs for duplicate extractions were on average 12.4 ± 11.3 %. To determine between-run variability, extract from a control fish was included in each run; the coefficient of variation for microcystin concentrations in the extract between runs was 13.5 % (1.5 ± 0.2 µg/L). Additionally, adjusted r-squared values for standard curves always exceeded 0.98.

5.2.4 Stable Isotope Analysis

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios were determined for all fish and food web samples as described in Campbell *et al.* (2003). Stable isotope analysis was carried out at the Environmental Isotope Laboratory at the University of Waterloo, and a detailed description of methodology is found in Chapter 3 of this thesis. Corrected $\delta^{15}N$ values were used for Lake Edward (as outlined in Chapter 3). Mean standard deviations from expected values for standard material are ± 0.2 ‰ for $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{15}N$. Mean standard deviations of samples run in duplicate were ± 0.05 ‰ for $\delta^{13}C$ and ± 0.21 ‰ for $\delta^{15}N$. Trophic level was calculated based on $\delta^{15}N$ values as described in Chapter 3.

5.2.5 Calculations and statistical analyses

Whole or gutted fish are expected to have elevated microcystin concentrations relative to muscle tissue (Martins and Vasconcelos 2009). To remove this potential source of error, unless otherwise indicated, only fish muscle tissue samples were included in statistical analyses. Additionally, microcystin concentrations in fish were normalized prior to use in statistical analyses through log-transformation.

In order to explore the influence of sampling site on microcystin concentrations in fish, a one-way analysis of variance (ANOVA) was used to compare microcystin concentrations in fish from different sites on a species-by-species basis. The set of species used in this ANOVA were commonly encountered at several study sites and included *B. docmac*, *C. gariepinus*, *Haplochromis* (?) spp., *L. niloticus*, *O. leucostictus*, *O. niloticus*, *P. aethiopicus* and *T. zilli*, and will hereafter be referred to as the "common" species. Subsequently I carried out a one-way ANOVA to compare microcystin concentrations among species on a site-by-site basis (including all species sampled for each site).

Murchison Bay and Napoleon Gulf had very similar species assemblages, so I carried out a two-way ANOVA with microcystin concentrations in fish as the response variable, and both species and site as factors, using all species that were present at both study sites (including *R. argentea*, which were analyzed whole at both sites). A similar analysis was repeated for Lake Edward and Lake George.

To determine whether there were any seasonal trends in microcystin concentrations in fish, I plotted microcystin concentrations in fish by date, and compared the general patterns that emerged with the seasonality of microcystin in water at these sites (as described in Chapter 2). I carried out one-way ANOVAs between microcystin concentrations and date for *O. niloticus* on a site-by-site basis to look for significant differences in microcystin concentrations in this species among dates. *Oreochromis niloticus* was chosen for this analysis given that it was present at all sites but Lake Nkuruba, and was collected in triplicate on each sampling date.

Regressions of microcystin concentrations in fish against $\delta^{15}N$ and calculated trophic level (TL; as calculated in Chapter 3 of this thesis) were carried out in order to reveal trends in the trophic transfer of microcystin, in particular, to determine whether biomagnification or biodilution is taking place. For each site, I carried out linear regressions between log-transformed microcystin concentrations in fish (log(MC)) and both TL and $\delta^{15}N$ at several levels, including: all fish sampled, within sampling dates, within *O. niloticus* (and *L. niloticus* where present), and within *O. niloticus* (or *L. niloticus*) within sampling dates. To assess the effects of dietary carbon source on microcystin concentrations in fish, linear

regressions were done between log(MC) and $\delta^{13}C$ as outlined above for $\delta^{15}N$ and trophic level. Linear regression was also used to explore whether there were relationships between fish length and microcystin concentrations. Regressions between log(MC) and total length (L) were done within sites for each species, with a focus on species for which there were high replicates (i.e. *O. niloticus* and *L. niloticus*). These relationships were also explored within sampling dates in order to control for temporal variability in microcystin concentrations within sites.

All statistical analyses were carried out using R, version 2.11.1 (R Development Core Team 2010).

5.3 Results

Microcystin concentrations in fish from the study lakes ranged from 0.5–198 µg/kg wet weight (µg/kg w.w.) in filleted muscle tissue, 3.4–1189 µg/kg w.w. in fish with guts and heads removed, and 2.8–898.7 in whole fish (Table 5.2). Where comparison was possible, I found that whole and gutted (guts and head removed) fish often had higher microcystin concentrations than filleted muscle tissue samples. However, there were some exceptions to this general pattern (notably in *Haplochromis* (?) spp. in Murchison Bay, *O. niloticus* and *T. zilli* in Napoleon Gulf, and *A alluaudi* in Lake Saka). *R. argentea* (from Murchison Bay and Napoleon Gulf) and *P. reticulata* (from Lake Nkuruba), both small species that were analyzed whole, had much higher microcystin concentrations than fish with similar calculated trophic levels from the same study sites.

5.3.1 Influence of Site and Species on microcystin concentrations in fish

On average, microcystin concentrations in fish were higher in Murchison Bay and Lake Saka than at other sites, however, few of these differences were statistically significant. I observed a wide range of microcystin concentrations in fish at both the species and site level. Microcystin concentrations (within sites) for the set of "common" species sampled are graphically displayed in Figure 5.1. Of these "common" species, Lake Saka had the highest mean microcystin concentrations for *Haplochromis* (?) sp., *L. niloticus* and *O. niloticus* (these were the only three "common" species collected from Lake Saka). Mean microcystin concentrations in *C. gariepinus*, *Haplochromis* (?) sp., *L. niloticus*, *O. leucostictus*, *O. niloticus*, and *T. zilli* were higher in Murchison Bay than at any other site except Lake Saka; this is true all of the "common" species sampled from Murchison Bay with the exception of *P. aethiopicus*, where concentrations were highest in Lake Edward. Surprisingly, microcystin concentrations in fish from Lake Nkuruba were comparable to those observed in fish from other sites, even though microcystin concentrations in water from this lake were consistently very low or undetectable (Chapter 2, this thesis).

Analysis of variance between log-transformed microcystin concentrations and site on a species-by-species basis (using the suite of "common" species) revealed significant differences for *Haplochromis* (?) sp., which had significantly higher concentrations in Lake Saka than in Lakes Edward, George, Mburo or Napoleon Gulf. There were no other significant differences between sites for any species.

Using ANOVA to compare microcystin concentrations between species on a site-by-site basis (graphically displayed in Figure 5.2 and summarized in Table 5.3) I found that in Murchison Bay, *P. aethiopicus* had significantly lower microcystin concentrations than *Haplochromis* (?) sp., *Synodontis afrofischeri* and *O. leucostictus*. In Napoleon Gulf, *P. aethiopicus* had significantly lower concentrations than *M. kannume* or *S. afrofischeri*. Meanwhile in Lake Saka, microcystin concentrations in *Haplochromis* (?) sp. were significantly higher than those observed in *O. niloticus*, and in Lake Mburo, *P. aethiopicus* had significantly lower microcystin concentrations than *O. esculentus* or *C. gariepinus*.

Given that Napoleon Gulf and Murchison Bay have very similar species assemblages, I carried out a two-way ANOVA with microcystin concentrations as the dependent variable, and with site and species as the predicting factors. Only species that were present at both sites were included in the analysis. I found that both site ($P<0.05$) and species ($P<0.01$) were significant predictors of microcystin concentrations in fish, and there was no significant interaction between site and species. Microcystin concentrations in fish from Murchison Bay were significantly higher than in fish from Napoleon Gulf. With regard to differences between species across both sites, *R. argentea* had significantly higher microcystin concentrations than did *L. niloticus*, *O. leucostictus*, *O. niloticus*, *T. zilli*, *S. victoriae* and *P. aethiopicus*. Meanwhile, *S. afrofischeri* had significantly higher microcystin concentrations than *L. niloticus*, *O. niloticus* and *P. aethiopicus*. Lungfish (*P. aethiopicus*) in Murchison Bay and Napoleon Gulf had significantly lower microcystin concentrations than any of the other species included in the analysis with the exception of *Tilapia zilli*.

The two-way ANOVA described above was repeated for Lake George and Lake Edward. I found that while there was no significant difference in microcystin concentrations between sites, across both sites *P. aethiopicus* had significantly lower microcystin concentrations than either *O. leucostictus* or *O. niloticus* ($P<0.05$). There was no significant interaction between site and species.

5.3.2 Seasonality of microcystin in fish

Based on analysis of variance I found no significant differences in microcystin concentrations in *O. niloticus* among sampling dates for any of the study sites, with the exception of Napoleon Gulf, where concentrations were significantly higher on December 29, 2008 than on January 29, 2009 ($P<0.05$).

Although not statistically significant, there appeared to be some consistent temporal patterns in microcystin concentrations in fish within some of the study sites. This is most notable in Napoleon Gulf and Lake Edward, where microcystin concentrations in several species of fish tended to increase and decrease in concert (Figure 5.3), and also tended to loosely follow temporal changes in microcystin concentrations in water (Figure 5.4).

In Napoleon Gulf, microcystin concentrations in fish were relatively stable from September to mid-December; then, in several species, a peak in concentrations was observed between late-December and mid-January, followed by a decrease in concentrations in late-January (Figure 5.3). This peak in microcystin concentrations was also seen in water from Napoleon Gulf, where concentrations peaked from December to mid-January and then fell considerably by late-January (Figure 5.4). Some fish species experienced earlier peaks in concentration than others, with *O. niloticus* and *M. kannume* appearing to respond more quickly to increases in microcystin concentrations in water than *L. niloticus*. Through the characterization of seasonal changes in microcystin concentrations in fish from Napoleon Gulf, several previously obscured differences in microcystin concentrations between species became more readily apparent: *O. variabilis* tended to have the highest microcystin concentrations, followed by *M. kannume* and *O. niloticus,* followed by *L. niloticus* and *T. zilli*, and finally *P. aethiopicus* tended to have the lowest microcystin concentrations (Figure 5.3).

In Lake Edward, similar seasonal trends were observed across multiple species, with concentrations that are intermediate in October, fall in November, rise to a peak in December, and then fall again in January (Figure 5.3). However, this trend was not observed in *O. niloticus*. This pattern of rising and falling concentrations is similar to the rises and falls seen in microcystin concentrations in water over the study period (Figure 5.4). However, while the peaks and troughs in microcystin concentrations in fish correspond with those observed for microcystin in water from offshore Lake Edward, they are inversely related to those observed in water from near shore Lake Edward. When seasonality is taken into account, previously imperceptible patterns in the relative microcystin concentrations of species emerge, with *O. niloticus* tending to have consistently high microcystin concentrations relative to other fish, and with *C.*

gariepinus and *B. docmac* tending to have higher microcystin concentrations than *B. bynni* or *P. aethiopicus* (Figure 5.3).

In Lake George, there is no apparent seasonality in microcystin across all species of fish. In *B. docmac*, *C. gariepinus*, and *P. aethiopicus*, concentrations tended to decrease over the study period, while in *O. niloticus* there is a general increase from the beginning of the study period to the end (Figure 5.3). The temporal trend observed in *O. niloticus* has some similarities to the pattern observed for microcystin in water, where low microcystin concentrations were observed in September and October, and higher microcystin concentrations were observed from November to February, with a maximum in January (Figure 5.4). In Lake Mburo, there is a gradual increase in microcystin concentrations in water from a low in September to a high in January/February (Figure 5.4), which appears to be reflected by microcystin concentrations in *O. esculentus* and *O. leucostictus*, but is not seen for any other species of fish (Figure 5.3).

In Murchison Bay, there appear to be no consistent seasonal patterns among species, although there is a clear decrease in microcystin concentrations in *L. niloticus* throughout the whole study period (Figure 5.3). In Lake Nkuruba (not graphically displayed), the consistently low microcystin concentrations in water, the small number of species sampled, and the low number of replicates made it impractical to look for seasonal patterns in microcystin in fish from this lake. Meanwhile, in Lake Saka, microcystin concentrations in water were lowest in September and October, slightly higher in November and early-December, and very high in late-December through February (Figure 5.4). However, among fish, this increasing trend was only shared by *A. alluaudi*, although in *O. niloticus* microcystin concentrations appear to be slightly higher in November through February as compared to September/October (Figure 5.3).

5.3.3 Trophic level and microcystin concentrations

Within site regressions of log-transformed microcystin concentrations in fish against calculated trophic level (and $\delta^{15}N$) revealed weak negative trends (not statistically significant) between these variables at all sites but Lake Saka, where a weak positive trend (not statistically significant) was observed (Figure 5.5). When the relationship between microcystin in fish and trophic level was explored within dates, as for the previous regressions, no statistically significant relationships emerged, and the log(MC)~trophic level relationship within dates was not always negative, with positive relationships observed on several sampling dates within sites.

Aggregating all sampling dates, for *L. niloticus* from Murchison Bay, there was a significant ($P<0.05$) negative relationship between microcystin concentrations and calculated trophic level (Figure 5.6), and weak (not statistically significant) positive trend between these variables for *O. niloticus*. In Napoleon Gulf, a weak positive relationship was observed for *O. niloticus* and a weak negative relationship was observed for *L. niloticus*, neither of which was statistically significant (Figure 5.6). Within *O. niloticus* from Lakes Edward, George, Mburo and Saka, there were non-significant negative trends between log-transformed microcystin concentrations and calculated trophic level.

When the relationship between microcystin concentrations and trophic level within species were considered on a date-by-date basis the relationships that emerged did not tend to be statistically significant. For *O. niloticus*, at most sites these relationships were generally negative, with the exception of Murchison Bay, where on half of all sampling dates this relationship was positive, and Napoleon Gulf, where this relationship was positive on all sampling dates. For *L. niloticus* this relationship was generally negative in Murchison Bay, and negative on half of all sampling dates in Napoleon Gulf (with weak positive relationships on the remainder of the sampling dates).

5.3.4 Dietary carbon source and microcystin concentrations

Based on linear regression, $\delta^{13}C$ (as an indicator of dietary carbon source) was not a significant predictor of microcystin concentrations in fish at either the within site level, or within both site and sampling date. However, at the within site level, there were weak (not statistically significant) negative relationships between log-transformed microcystin concentrations and $\delta^{13}C$ at all sites but Lakes George and Nkuruba, where there was a non-significant positive trend between these variables (Figure 5.7). When this relationship is explored within both site and date, this relationship is weakly positive on some occasions and weakly negative on others.

Also, there were no statistically significant relationships observed between microcystin concentrations in fish and $\delta^{13}C$ within *O. niloticus* or *L. niloticus* at any site. For *O. niloticus*, weak positive relationships were observed for all sites but George and Saka. Meanwhile, for *L. niloticus* weak negative relationships were observed between microcystin concentrations in fish and $\delta^{13}C$ values at both Murchison Bay and Napoleon Gulf (Figure 5.8).

5.3.5 Fish length and microcystin concentrations

When regressions of log transformed microcystin concentrations in fish tissue against total length were carried out for each species within sites, some statistically significant relationships emerged: in Lake

Edward, there was a negative relationship between log(MC) and total length for *C. gariepinus* ($P<0.05$). Meanwhile in Lake George, microcystin concentrations were negatively related to fish length from both *O. niloticus* and *H. squamipinnis*. Finally, in Napoleon Gulf this relationship was significant and negative for *O. niloticus* and *O. variabilis*. Additionally, although there were no other statistically significant relationships between microcystin concentrations in fish and total length, the direction of this relationship was generally negative. These relationships are summarized in Table 5.4.

In order to account for seasonal variability, these regressions were also carried out on a date-by-date basis for *O. niloticus* (at sites where present), and for *L. niloticus* from Murchison Bay and Napoleon Gulf. For both of these species at all sites, there were generally weak (not statistically significant) negative relationships between microcystin concentrations and total length, although for *O. niloticus* from Lake Saka, this relationship was only negative for half of all sampling dates.

5.4 Discussion

Microcystin concentrations in fish muscle tissue in the current study (range: 0.5–198 µg/kg w.w.) were comparable to concentrations reported for fish muscle in other studies from around the world (reviewed in Ibelings and Chorus 2007). To date, the only reported microcystin concentrations in fish from East African lakes are from *O. niloticus* from Murchison Bay and Lake Mburo (Nyakairu *et al.* 2009, Semyalo *et al.* 2010) and in *L. niloticus* from Murchison Bay (Nyakairu *et al.* 2009), all based on samples collected in 2004–2005. The microcystin concentrations for fish from Murchison Bay reported by these two studies fell within the ranges (but below the mean values) observed in the current study. However, both Nyakairu *et al.* (2009) and Semyalo *et al.* (2010) occasionally found concentrations in *O. niloticus* from Lake Mburo that exceeded those observed in the current study, demonstrating the inter-annual variability inherent in microcystin concentrations in both water and fish.

Other reported microcystin concentrations from tropical study sites include data from *O. niloticus* from an Egyptian fish farm (Mohamed and Carmichael 2003), where values were generally found to be higher (45.7–102 µg/kg) than those observed for *O. niloticus* in the current study. There have also been some South American studies that have characterized microcystin concentrations in fish. In a Brazilian coastal lagoon a maximum microcystin concentration in fish of 39.6 µg/kg was observed (Magalhaes *et al.* 2003). Meanwhile, in two Brazilian hydroelectric reservoirs Deblois *et al*. (2008) reported microcystin concentrations ranging from 0.9–12.0 µg/kg w.w. in tilapiine cichlids (*O. niloticus* and *Tilapia rendalli*). Also, in a shallow Argentinian lake, *Odontesthes bonariensis* was found to have a mean microcystin concentration in muscle of 2.2 µg/kg w.w. (Amé *et al.* 2010). These values fall within the range of values

observed in the present study, however, this study often observed much higher microcystin concentrations in fish muscle tissue than did the South American studies.

Microcystin concentrations in fish have also been reported for several Chinese lakes. In Lake Chaohu, microcystin concentrations were reported for 8 species of fish, which had maximum microcystin concentrations ranging from 26–497µg/kg (Xie *et al.* 2005). Microcystin concentrations in six species of fish were characterized in Lake Taihu (Zhang *et al.* 2009), *Carassium auratus* had the highest mean microcystin concentration (20.8 µg/kg), while the other five species had much lower microcystin concentrations. While the values reported for these two Chinese lakes overlap with the range of concentrations observed in the present study, observed concentrations were generally lower than those observed in Lake Chaohu (Xie *et al.* 2005), but higher than those reported for Lake Taihu (Zhang *et al.* 2009).

In the temperate Laurentian Great Lakes, my own data from Lake Ontario (Bay of Quinte, range: 0.5–25.8 µg/kg w.w.) and Lake Erie (Maumee Bay, range: 1.5–43.6 µg/kg w.w.) fall within the range of observed values for several Ugandan study sites. Meanwhile, microcystin concentrations in *Perca flavescens* (yellow perch) collected in 2006 from the western basin of Lake Erie were found to range from 0.04–1.25 µg/kg w.w. (Wilson *et al.* 2008), concentrations that are generally below the range of concentrations observed in my Ugandan study lakes.

The elevated microcystin concentrations that I often observed in whole and gutted fish relative to muscle tissue in the current study were consistent with results from a broad range of studies that have found higher microcystin concentrations in fish livers, guts, blood and bile as compared to fish muscle tissue (Xie *et al.* 2005, Zhang *et al.* 2009, Amé *et al.* 2010). This is largely a reflection of the fact that once absorbed by the digestive tract, microcystin is rapidly distributed throughout the fish by the blood stream, with the liver and other highly vascularized organs experiencing particularly high levels of exposure to microcystin (Martins and Vasconcelos 2009). Particularly high microcystin concentrations were observed in fish that were analyzed whole (notably *R. argentea*, *P. reticulata* and some haplochromine cichlids). In addition to the presence of internal organs with high microcystin concentrations, these fish may also have appreciable amounts of cyanobacteria present in their guts or in their slime, which would act to further increase measurable microcystin in these fish. However, given that these small fish are generally eaten whole, the concentrations measured through the analysis of whole fish most accurately reflect the exposure risk posed through consumption of these fish. The remainder of the discussion focuses on microcystin concentrations in fish muscle tissue tissue unless otherwise indicated.

5.4.1 Comparing sites

Generally, microcystin concentrations in fish were highest in the lakes where microcystin concentrations in water were also highest (Table 5.1). In particular, fish from Lake Saka (and to a lesser extent, Murchison Bay) tended to have higher microcystin concentrations than fish from other sites. This indicates that high microcystin concentrations in water can lead to elevated microcystin concentrations in fish.

When microcystin concentrations in fish from Murchison Bay and Napoleon Gulf (both embayments in northern Lake Victoria) were compared on a species-by-species basis, fish from Murchison Bay had higher microcystin concentrations than fish from Napoleon Gulf. This is consistent with the higher microcystin concentrations in water observed in Murchison Bay relative to Napoleon Gulf (Table 5.1). Given that these study sites have very similar species assemblages and food web structure (Chapter 3, this thesis), the reduction of the variability associated with differences in the species present and their trophic relationships facilitates the interpretation of comparisons between these two sites.

Lake Edward and Lake George are connected via the Kazinga Channel and have nearly identical species assemblages and trophic structure (Chapter 3, this thesis). Despite these similarities, there are substantial differences between these two lakes with respect to size, depth and trophic status, and microcystin concentrations in Lake George are much higher than those observed in offshore Lake Edward (Table 5.1). However, contrary to what was observed for Napoleon Gulf and Murchison Bay, the differences in microcystin concentrations in water between Lake Edward (offshore) and Lake George did not lead to different microcystin concentrations in fish from these lakes. There was no significant difference in microcystin concentrations in fish between the two sites. One possible explanation for this unexpected result is that cell quota of microcystin in *Microcystis* spp. in Lake Edward is much higher than cell quota in Lake George (Chapter 2), suggesting that organisms consuming (either directly or indirectly) comparable amounts of toxin-producing cyanobacteria will ingest much more microcystin in Lake Edward than in Lake George. Alternatively, the fish sampled in Lake Edward may more closely reflect microcystin concentrations in near-shore Lake Edward (and the Kazinga Channel), which are similar to those observed in Lake George, and much higher than the concentrations routinely encountered in offshore Lake Edward.

Microcystin concentrations in fish from Lake Nkuruba were comparable to, and occasionally higher than, the concentrations observed at other study sites. This was unexpected, given that microcystin concentrations in epilimnetic water from Lake Nkuruba were consistently very low or undetectable.

Additionally, during the study period, *Microcystis* spp. (the cyanobacterial genus that is primarily responsible for microcystin production in the other study lakes, see Chapter 2) was not observed in Lake Nkuruba, suggesting that other cyanobacterial taxa may be responsible for microcystin production in this lake. One possibility is that high stability in the water column (see Chapter 2) of Lake Nkuruba allows for the congregation of microcystin producing cyanobacteria at discrete depths in the water column, which I may have missed in my sampling. Several species of *Planktothrix* spp. are known to be capable of forming dense metalimnetic blooms (Mur *et al.* 1999), which have been found to have the potential to be extremely toxic (Fastner *et al.* 1999). However, although not included in the current study, I also carried out discrete sampling at several depths, including at the thermocline, but did not find any obvious differences in microcystin concentrations between depths, nor did I find any peaks in biomass of potential microcystin producers (Poste, *unpublished data*). Alternatively, benthic cyanobacteria may be responsible for toxin production in this lake, which would explain the low microcystin concentrations observed in the water column (especially given that samples were collected from the middle of the lake). Microcystin production by benthic cyanobacteria (generally either *Oscillatoria* or *Phormidium* species) is known to occur, particularly in lakes with increased water clarity (Mez *et al.* 1997, Mur *et al.* 1999). I observed that periphyton is extensive along the rocky margins of Lake Nkuruba. However, stable carbon isotopic ratios for fish from Lake Nkuruba suggest a food web based primarily on phytoplankton rather than benthic "algal" sources. As such, further study is needed to determine the source of microcystin in this lake.

When microcystin concentrations in fish were compared between sites on a species by species basis, only *Haplochromis* (?) sp. had significantly different microcystin concentrations at different sites, with higher concentrations in Lake Saka and Murchison Bay. It is difficult to identify whether this is primarily due to differences in microcystin exposure between sites (with *Haplochromis* (?) sp. from Lake Saka and Murchison Bay tending to have higher dietary microcystin exposure), or whether this difference is a reflection of differences in fish diet or even fish species between the sites. I observed that *Haplochromis* (?) sp. from Lakes Edward and George were significantly larger and at significantly higher trophic levels than *Haplochromis* (?) sp. from any other site, and that *Haplochromis* (?) sp. from Lake Saka were smaller than those sampled from all sites but Napoleon Gulf (this is described in detail in Chapter 3). These differences in both size and trophic level suggest that *Haplochromis* (?) sp. from different sites (and possibly within sites) could represent different species, with different dietary habits and consequent microcystin exposure, making it difficult to draw any conclusions about differences in microcystin concentrations in fish between sites based on differences observed in *Haplochromis* (?) sp. The lack of significant differences between sites (within species) despite differences in microcystin concentrations in

water is likely a result of several sources of variability. These may include seasonal variability in exposure to and depuration of microcystin (Smith and Haney 2006), dietary variability within species, differences in the diet of a species between sites, and differences in the exposure history of fish due to migratory feeding behavior.

5.4.2 Comparing species

This study included a wide range of fish species from all sites ranging from phytoplanktivores up to top piscivores. These species should also experience a wide range in dietary exposure to microcystin, with fish feeding at lower trophic levels (particularly those capable of feeding on cyanobacteria) more likely to experience high microcystin exposure than those that feed at higher trophic levels especially considering lack of biomagnification observed in this study.

When microcystin concentrations were compared between species on a site-by-site basis, there was a great deal of overlap between species within the study sites, and few statistically significant differences emerged. However, there were some general trends that were observed at several study sites: where present, *S. afrofischeri*, *M. kannume*, *Haplochromis* (?) sp. and tilapiine cichlids (notably *O. leucostictus*, *O. esculentus* and *O. variabilis*) tended to have relatively high microcystin concentrations, while *P. aethiopicus* tended to have particularly low microcystin concentrations. Also, as previously mentioned, *R. argentea* and *P. reticulata* tended to have much higher microcystin concentrations than other fish from the same study sites, likely due in part to their planktivorous diet, as well as to the fact that they were analyzed whole.

Meanwhile, the other species for which microcystin concentrations tended to be high included tilapiine cichlids known to feed primarily on phytoplankton and detritus, known molluscivores (*M. kannume* and *S. afrofischeri*), and *Haplochromis* (?) sp., which are often facultative feeders (Greenwood 1958). In particular, phytoplanktivorous, detritivorous and molluscivorous fish (especially those feeding primarily on bivalves) would be expected to have diets that increase the risk of exposure to pelagic cyanobacteria. Meanwhile, the lungfish (*P. aethiopicus)* is known to have an omnivorous diet that includes insects, small fish and gastropods (Greenwood 1958). This diet may result in low microcystin exposure due to reliance on benthic primary production, as gastropods are generally expected to rely more on benthic rather than pelagic sources of phytoplankton (Post 2002). Additionally, *P. aethiopicus* are often associated with near-shore wetlands, where benthic food sources are expected to be more widely available for both lungfish and their prey. However, given that stable isotope analysis suggests that fish in these lakes are relying to a

high degree on pelagic carbon sources, it is possible that *P. aethiopicus* may not readily absorb microcystin from the gut, or may have an enhanced ability to depurate microcystin.

Although there were few statistically significant differences in microcystin concentrations between species, this does not necessarily imply that these fish are experiencing similar microcystin exposure or accumulation. It is known that fish differ in their responses to microcystin exposure, with some species more capable at detoxifying and depurating microcystin than others (Tencalla and Dietrich 1997, Xie *et al.* 2004, Smith and Haney 2006). These competing influences (dietary exposure, accumulation and depuration) as well as strong seasonal variability in dietary exposure greatly complicated between-species comparisons of microcystin concentrations in fish.

5.4.3 Seasonality of microcystin in fish

In temperate lakes, strong seasonal changes in temperature, light and mixing allow for cyanobacterial dominance (and associated toxin production) primarily in the late summer and early fall (Munawar and Munawar 1986). Meanwhile, in tropical lakes, the conditions are such that there is the potential for the year-round presence of toxic cyanobacteria at high biomasses (Oliver and Ganf 2000, Kling *et al.* 2001). Despite the relatively stable conditions experienced in Ugandan lakes throughout the year, there are seasonal patterns in stratification, mixing, nutrient concentrations, phytoplankton community composition and biomass (Talling 1966, 1986, Beadle 1981, Mugidde 2002). At my study sites, I observed temporal variation in the biomass of toxin-producing cyanobacteria as well as in microcystin concentrations in both water and fish. Generally, it would be expected that the seasonality of microcystin in fish should reflect the seasonality of microcystin in water; however, the time that it takes for concentrations in fish to respond to the concentrations in water, as well as the capacity for detoxification and depuration of microcystin by fish are also expected to affect the temporal trajectory of microcystin concentrations in fish throughout the study period.

In examining the seasonality of microcystin in fish, there were three general patterns observed: 1) consistent seasonal patterns in microcystin concentrations were seen across several species as well as in water (observed in Lake Edward and Napoleon Gulf), 2) microcystin concentrations in a small number of species appeared to follow microcystin concentrations in water (observed in Lakes Mburo and George), and 3) no obvious seasonal patterns in microcystin in fish (observed in Murchison Bay, Lake Nkuruba, and Lake Saka).

In Napoleon Gulf, based on similarities in the seasonality of microcystin in both water and fish, microcystin concentrations in fish appear to be responding quickly to changes in microcystin concentrations in water. This is particularly evident in the peak in microcystin concentrations (in both water and fish) observed in late-December through mid-January, as well as in the substantial drop in microcystin concentrations in water between mid- and late-January, when concentrations in fish also fall quickly. This pattern was observed in fish from all trophic levels, although *L. niloticus* (a secondary consumer) experienced a later peak in microcystin concentrations than *O. niloticus* (a primary consumer), indicating that there may have been a slight lag in the accumulation of microcystin in higher trophic level organisms, given that their exposure would be expected to be via their prey rather than through direct consumption of toxin-producing cyanobacteria. The fact that microcystin concentrations in fish declined in concert with concentrations in water indicates that these fish depurated microcystin effectively, lowering microcystin concentrations in muscle tissue to pre-peak levels.

In Lake Edward, microcystin in several species of fish followed a seasonal trajectory that was very similar to that observed for microcystin in water from offshore Lake Edward, although it is also possible that these fish are responding to changes in microcystin concentrations in water from near-shore Lake Edward with a time-lag. However, this is not the case for *O. niloticus*, which has consistent (and relatively high) microcystin concentrations throughout the whole study period. The lack of seasonality in microcystin concentrations in *O. niloticus* may reflect a dietary source of microcystin with more consistent concentrations than pelagic cyanobacteria (possibly detritus), or may be due to opportunistic feeding on cyanobacterial blooms where they occur (with fish migrating between near-shore Lake Edward, and the Kazinga Channel). Regardless of whether the rises and falls in microcystin concentrations in fish are reflective of concentrations in offshore Lake Edward, or of concentrations in near-shore Lake Edward (with a time lag), the fact that concentrations in fish show distinct increases and decreases suggests that these fish do respond to changes in microcystin concentrations in water, and that reduced exposure combined with depuration can lead to decreases in microcystin concentrations in fish in Lake Edward.

In Lake George, only *O. niloticus* shared a general seasonal trend in microcystin concentrations with concentrations in water. Meanwhile in Lake Mburo, *O. esculentus* and *O. leucostictus* followed a trend similar to that observed in water. These tilapiine cichclids are known to consume both live and detrital cyanobacteria (Greenwood 1958, Trewavas 1983, Nagayi-Yawe *et al.* 2006, Semyalo *et al.* 2010), which may explain the faster reponse of microcystin concentrations in these fish to changes in microcystin

concentrations in the water. The lack of similarity between the seasonality of microcystin concentrations in water and most species of fish at these sites may be due to the monthly sampling frequency; short-lived toxic cyanobacterial blooms may occur between sampling dates, and while evidence of these blooms may no longer be present in the water column, there may not have been enough time for significant depuration of microcystin in fish to occur. Meanwhile, in Lakes Saka and Nkuruba, small sample sizes made it difficult to ascertain seasonal patterns in microcystin concentrations in fish.

In Murchison Bay, there were no obvious trends that were present over several species, or that matched the seasonal trends observed for microcystin in water. This may in part be due to the difficulty of knowing whether fish had been feeding primarily within inner Murchison Bay, where water samples were collected, or outside of the inner bay, which tends to have very different characteristics (Haande *et al.* 2010). Additionally, confirming catch location for fish was difficult given the nature of the landing site (a hectic urban fish market with a large number of fishermen).

Some of the variability observed in microcystin concentrations within species within the study sites does appear to be related to seasonal changes in microcystin concentrations in fish; however, the interpretation of these seasonal patterns must consider the dynamics of microcystin accumulation and depuration in fish as well as the spatial and temporal heterogeneity of toxic cyanobacterial blooms.

5.4.4 Trophic level and microcystin concentrations

Biomagnification is the process by which a compound is concentrated as it moves up the food web, such that concentrations in organisms at higher trophic levels exceed those at lower trophic levels (Mackay and Fraser 2000). Biomagnification is commonly observed for lipophilic compounds (such as PCBs), but is generally not expected to occur for hydrophilic compounds such as microcystin (Ibelings and Havens 2008). However, it is important to note that some microcystin congeners are more lipophilic than others, and their toxicity and propensity to accumulate in aquatic organisms may differ (Dietrich and Hoeger 2005, Amé *et al.* 2010). There is little evidence of biomagnification of microcystin in laboratory or field studies (Ibelings *et al.* 2005, Zhang *et al.* 2009, Kotak *et al.* 1996, but see Xie *et al.* 2005), and Ibelings and Havens (2008) and Karjalainen *et al.* (2005) suggest that microcystin may undergo biodilution as is moves through the food chain, where concentrations decrease at each successive trophic level due to the metabolization and excretion of microcystin. Additionally, fish at higher trophic levels would also be expected to experience lower dietary microcystin exposure than those feeding at lower trophic levels.

Log-transformed contaminant concentrations are often regressed against $\delta^{15}N$ or calculated trophic level in order to explore whether a compound is undergoing biomagnification or biodilution in food webs. I carried out similar regressions for log-transformed microcystin concentrations (log(MC)) in fish at my study sites and found non-significant weak negative slopes for all study sites with the exception of Lake Saka. These results indicate that biodilution may be occurring at these study sites, albeit only weakly. These results are in sharp contrast to the results for regressions between log-transformed total mercury and calculated trophic level for these same fish, where statistically significant biomagnification was observed at all sites (see Chapter 4).

On a within species level, for both *L. niloticus* and *O. niloticus*, microcystin concentrations had negative (but not statistically significant) relationships with trophic level at all sites where they were present, with the exception of *O. niloticus* from Napoleon Gulf and Murchison Bay, where positive relationships were observed between log(MC) and trophic level. The negative relationships observed support the possibility that fish feeding on lower trophic levels will have higher dietary exposure to microcystin, leading to higher microcystin concentrations. However, the elevated microcystin concentrations observed in *O. niloticus* with higher $\delta^{15}N$ values in both Napoleon Gulf and Murchison Bay may indicate that these individual fish were relying on food sources based on phytoplankton supported by anthropogenic nitrogen (human waste tends to have enriched $\delta^{15}N$ values, Harrington 1998).

Although there was no strong evidence of biodilution in the food webs examined, these food webs considered only fish. The inclusion of phytoplankton, zooplankton and other primary consumers such as bivalves and gastropods would likely reveal much stronger biodilution patterns across the whole food web than across fish alone (see Ibelings *et al.* 2005).

5.4.5 Dietary carbon source and microcystin concentrations

Stable carbon isotopic analysis is often used to indicate dietary carbon source, with higher $\delta^{13}C$ values tending to indicate benthic or rapidly growing ^{12}C-limited phytoplanktonic carbon sources (Hecky and Hesslein 1995, and see Chapter 3). The $\delta^{13}C$ values in fish from all study sites were consistent with the range of $\delta^{13}C$ observed in phytoplankton, suggesting that the food webs sampled were all highly reliant on pelagic food sources (see Chapter 3). Given that *Microcystis* (the main microcystin producer in these study lakes, see Chapter 2) is a pelagic cyanobacterium, the strongly pelagic diet of fish in these lakes may lead to elevated dietary microcystin exposure (and accumulation).

With the exception of Lakes George and Nkuruba, relationships between log-transformed microcystin concentrations and $\delta^{13}C$ values within the study sites were negative (although generally not statistically significant). It is possible that this reflects the fact that fish feeding primarily on pelagic sources of carbon are likely to be exposed to higher levels of microcystin than those that feed primarily on benthic carbon sources. However, low transparency in these highly productive lakes is not expected to allow for substantial benthic production (Vadeboncoeur *et al.* 2003) and based on $\delta^{13}C$ values the food webs in these lakes are fully supportable by phytoplankton.

In Lake Nkuruba, where *Microcystis* spp. was absent and microcystin concentrations in the water column were often undetectable (see Chapter 2), the positive relationship observed between microcystin and $\delta^{13}C$ may support the possibility that microcystin production in this lake was attributable to either benthic cyanobacteria or a dense ^{12}C-limited metalimnetic bloom of toxin-producing cyanobacteria, given that fish with enriched $\delta^{13}C$ values (possibly due to the incorporation of benthic carbon) tended to have higher microcystin concentrations. However, given that only two species were included in the analysis and the narrow range in $\delta^{13}C$ values represented by these species, these data are inconclusive.

In Lake George, the positive relationship between microcystin and $\delta^{13}C$ could reflect that fish with higher microcystin concentrations have a history of feeding opportunistically on heavy blooms of phytoplankton where reduced isotopic discrimination led to high $\delta^{13}C$ values. However, given that *O. niloticus* (the lowest trophic level species in Lake George) had the highest $\delta^{13}C$ values of any species in the lake, this positive relationship is likely to be mostly attributable to high dietary microcystin exposure in a low trophic level species with high $\delta^{13}C$ values.

5.4.6 Pairing stable isotope analysis with microcystin determination

To understand the trophic transfer of a compound, it is critical to have an understanding of the underlying food web. Stable carbon and nitrogen isotope analysis can provide insight into the dietary relationships between organisms, with $\delta^{15}N$ acting as an indicator of realized (rather than assumed) trophic level, and $\delta^{13}C$ acting as an indicator of dietary carbon source. Such an approach has not previously been used for microcystin in food webs; however, by using stable isotope analysis as an indicator of fish diet, I can examine relationships between fish diet and microcystin concentrations in fish tissue, and can characterize the trophic transfer of microcystin among many species without needing to make restrictive assumptions about fish diet and trophic level.

Stable isotope analysis provides a time-averaged representation of both dietary habits and changes in baseline stable isotopic ratios that can range from days (in primary producers) to years (in slow-growing fish with long tissue-turnover times) (Hesslein 1993, O'Reilly and Hecky 2002). However, microcystin concentrations in fish are likely to represent only recent exposure to this toxin, especially in tropical lakes, where temperature dependent depuration (Ibelings and Havens 2008) can act to quickly reduce concentrations in fish when no longer exposed.

5.4.7 Fish length and microcystin concentrations

Where fish experience ontogenetic dietary shifts, feeding on progressively higher trophic level organisms as they grow, dietary exposure to microcystin would be expected to be lower in larger fish. In the western basin of Lake Erie, Wilson *et al.* (2008) found that microcystin concentrations in yellow perch muscle tissue were negatively related to fish length, as would be expected based on the fact that yellow perch are known to experience dietary shifts with growth.

Generally, I found that within species there tended to be weakly negative (although rarely statistically significant) relationships between log-transformed microcystin concentrations and total length within sites. Due to the potential confounding influence of seasonal variability in microcystin concentrations in fish, I also evaluated the relationship between log(MC) and total length for *L. niloticus* and *O. niloticus* on a date-by-date basis (within sites). In Napoleon Gulf and Murchison Bay, on most sampling dates there were weakly negative relationships between log(MC) and total length for *L. niloticus*. Meanwhile, for *O. niloticus*, on a date-by-date basis (within sites) the relationship between log(MC) and total length was generally negative at all sites, although in Lake Saka, Napoleon Gulf and Murchison Bay, occasionally this relationship was positive. However, when *L. niloticus* from both Murchison Bay and Napoleon Gulf are divided into two size classes (TL<25 cm and TL>25 cm), the relationship between fish length and microcystin concentrations for this species becomes clearer. Small (<25 cm) *L. niloticus* in Napoleon Gulf had mean microcystin concentrations of 15.7 µg/kg w.w., while large (>25 cm) *L. niloticus* from the same site had mean microcystin concentrations of 5.9 µg/kg w.w. Meanwhile, in Murchison Bay, small and large *L. niloticus* had mean microcystin concentrations of 27.7 and 9.4 µg/kg w.w. respectively. These results suggest that generally, smaller fish tended to have higher microcystin concentrations. This is of importance given that smaller fish tend to be less commercially marketable and as such, are more likely to be consumed by subsistence fishers and their families, increasing their microcystin exposure.

5.4.8 General conclusions and risk evaluation

There are several factors that appear to have an effect on microcystin concentrations in fish, including: microcystin concentrations in water (and associated seasonality), fish species, fish diet (trophic level and relative importance of pelagic food sources), fish size, as well as physiological interactions with microcystin (including accumulation, detoxification and depuration rates). This complex set of factors can have interacting and often competing effects on microcystin concentrations in fish, making it particularly difficult to predict microcystin concentrations in fish based only on microcystin concentrations in water, highlighting the importance of monitoring microcystin concentrations in fish in order to gain an understanding of the microcystin exposure risks posed by fish consumption.

Based on the current WHO TDI for microcystin of 0.04 µg/kg body weight, fish where microcystin concentrations exceed 24 µg/kg w.w. would cause a consumer (weighing 60 kg and eating 100 g of fish) to exceed this guideline value. Several fish from all study sites (including many species from a wide range of trophic levels) exceeded this reference concentration (see Table 5.2). This suggests that although microcystin is not biomagnifying in these lakes, trophic transfer and accumulation of microcystin occurs throughout the entire food web, and in tropical lakes there is the potential for year-round exposure of aquatic food webs to microcystin. Furthermore, microcystin in fish can be an important source of microcystin exposure for humans, particularly in riparian, and especially fishing, communities where fish is consumed regularly.

Table 5.1 Mean microcystin and chlorophyll *a* concentrations in epilimnetic water (from Chapter 2, this thesis).

Lake	MC in Water (μg/L)	Chlorophyll *a* (μg/L)
Lake Edward (all)	3.61 ± 4.90	43.8
Edward nearshore	5.81 ± 5.86	66.3 ± 46.2
Edward offshore	0.97 ± 1.10	21.3 ± 22.8
Lake George	8.54 ± 6.36	138.0 ± 39.1
Lake Mburo	2.48 ± 0.96	48.6 ± 10.1
Murchison Bay	7.26 ± 5.73	96.5 ± 38.1
Napoleon Gulf	1.75 ± 1.26	24.7 ± 18.4
Lake Nkuruba	0.24 ± 0.06	6.2 ± 2.2
Lake Saka	61.2 ± 73.4	90.0 ± 36.3

Table 5.2 Mean (± s.d.) microcystin concentrations, total length, stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios, and calculated trophic levels for fish from all study lakes. Only those fish for which microcystin concentrations were analyzed are included, as such, $\delta^{13}C$ and $\delta^{15}N$ values may differ from those reported in Chapter 3. Sample type (whole, filleted or gutted (heads and viscera removed)) is indicated. Species codes in this table are used on subsequent figures. Asterisks indicate that each replicate represents 10 pooled individual fish. †Of the three individual *Haplochromis* (?) spp. from Napoleon Gulf, one did not yield enough material for both microcystin and stable isotope analysis, so $\delta^{15}N$ and $\delta^{13}C$ values are for 2 samples and MC values are for 3.

Name	n	Code	Total Length (cm) min–max	$\delta^{15}N$ (‰) μ ± s.d	$\delta^{13}C$ (‰) μ ± s.d.	Trophic Level μ ± s.d.	MC (μg/kg) μ ± s.d.
Lake Edward							
Bagrus docmac	5	Bd	26.0–65.0	11.7 ± 2.0	-14.6 ± 2.9	4.0 ± 0.6	6.2 ± 3.0
Barbus bynni	4	Bb	30.2–38.0	8.5 ± 0.8	-12.3 ± 0.7	3.0 ± 0.2	5.3 ± 3.1
Clarias gariepinus	5	Cg	36.0–78.0	9.6 ± 1.6	-15.5 ± 2.2	3.4 ± 0.5	8.6 ± 7.5
Haplochromis spp.	5	H	14.7–23.0	12.2 ± 1.6	-15.7 ± 2.1	4.1 ± 0.5	10.0 ± 3.2
Haplochromis squamipinnis	5	Hs	16.0–26.8	12.2 ± 1.2	-14.8 ± 2.1	4.1 ± 0.3	8.6 ± 3.4
Oreochromis leucostictus	4	Ol	23.5–26.5	6.5 ± 0.4	-16.6 ± 0.9	2.5 ± 0.1	21.9 ± 30.7
Oreochromis niloticus	15	On	20.0–34.0	6.9 ± 1.0	-17.1 ± 1.2	2.6 ± 0.3	8.0 ± 4.5
Protopterus aethiopicus	5	Pa	51.0–83.0	9.3 ± 1.0	-14.1 ± 3.1	3.3 ± 0.3	5.3 ± 4.9
Lake George							
Bagrus docmac	9	Bd	26.0–60.0	8.4 ± 0.6	-7.9 ± 0.8	3.9 ± 0.2	9.1 ± 4.9
Clarias gariepinus	5	Cg	35.8–52.0	6.0 ± 0.6	-9.6 ± 4.9	3.2 ± 0.2	6.9 ± 2.0
Haplochromis squamipinnis (filleted)	4	Hs	14.6–21.7	7.9 ± 0.9	-8.6 ± 2.3	3.7 ± 0.3	6.7 ± 3.5
Haplochromis squamipinnis (gutted)	1	Hs	8.0	5.7	-10.5	3.1	11.8
Oreochromis esculentus	2	Oe	13.6–17.5	2.8–2.9	-16.7 to -9.5	2.2–2.2	6.3–11.4
Oreochromis leucostictus	5	Ol	13.2–21.0	3.5 ± 0.7	-9.8 ± 2.2	2.4 ± 0.2	21.2 ± 32.3
Oreochromis niloticus	18	On	17.8–33.0	2.7 ± 0.7	-5.9 ± 2.6	2.2 ± 0.2	10.2 ± 8.6
Protopterus aethiopicus	5	Pa	47.0–82.5	6.4 ± 1.0	-7.9 ± 2.4	3.3 ± 0.3	2.4 ± 1.2
Tilapia zilli	1	Tz	19.0	3.0	-16.0	2.3	2.0
Lake Mburo							
Bagrus docmac	1	Bd	57.0	6.0	-10.5	2.9	13.4
Clarias gariepinus	5	Cg	17.9–67.0	7.5 ± 2.9	-13.6 ± 3.1	3.4 ± 0.8	20.6 ± 19.5
Haplochromis spp. (filleted)	2	H	17.7–19.2	5.3–7.0	-11.2 to -8.9	2.7–3.2	2.5–5.6
Haplochromis spp. (gutted/head removed)	2	H	9.0–13.4	4.8–6.7	-11.1 to -10.9	2.6–3.1	5.4–11.8
Haplochromis spp. (whole)	1	H	11.6	6.1	-10.6	2.9	12.1
Oreochromis esculentus	5	Oe	19.7–27.0	4.3 ± 0.5	-10.0 ± 0.4	2.4 ± 0.1	17.9 ± 12.1
Oreochromis leucostictus	5	Ol	18.3–24.6	4.4 ± 0.5	-13.1 ± 1.0	2.5 ± 0.1	8.4 ± 6.7
Oreochromis niloticus	15	On	18.2–40.5	4.8 ± 1.5	-11.0 ± 1.2	2.6 ± 0.4	7.4 ± 7.6
Protopterus aethiopicus	5	Pa	53.0–102.0	5.9 ± 2.0	-11.3 ± 2.0	2.9 ± 0.6	2.5 ± 2.1

Name	n	Code	Total Length (cm) min–max	$\delta^{15}N$ (‰) μ ± s.d	$\delta^{13}C$ (‰) μ ± s.d.	Trophic Level μ ± s.d.	MC (μg/kg) μ ± s.d.
Lake Victoria (Murchison Bay)							
Clarias gariepinus	1	Cg	57.5	8.8	-18.7	2.4	23.9
Haplochromis spp. (filleted)	4	H	12.4–14.8	10.3 ± 0.8	-16.3 ± 0.4	2.8 ± 0.2	35.6 ± 36.3
Haplochromis spp. (whole)	1	H	6.0	7.8	-15.8	2.0	19.9
Lates niloticus	18	Ln	19.0–96.0	9.4 ± 1.6	-16.2 ± 1.2	2.5 ± 0.5	13.5 ± 11.6
Oreochromis leucostictus	5	Ol	15.2–25.5	8.5 ± 1.2	-17.1 ± 0.8	2.2 ± 0.3	30.3 ± 18.1
Oreochromis niloticus	24	On	15.0–38.0	7.7 ± 1.5	-16.3 ± 1.5	2.0 ± 0.4	13.7 ± 12.5
Protopterus aethiopicus	5	Pa	37.7–79.0	10.9 ± 0.7	-15.9 ± 0.8	2.9 ± 0.2	4.1 ± 2.4
Rastrineobola argentea (whole/dry from market)	2*	Ra	apx. 3.5	6.1–8.6	-15.9 to -13.9	1.5–2.3	36.2–41.2
Synodontis afrofischeri	5	Sa	14.7–20.1	9.8 ± 1.6	-17.3 ± 0.5	2.6 ± 0.5	28.8 ± 21.9
Synodontis victoriae	5	Sv	17.1–24.5	8.7 ± 0.3	-15.8 ± 2.7	2.3 ± 0.1	16.7 ± 9.4
Tilapia zilli	5	Tz	15.3–26.5	8.6 ± 1.1	-16.6 ± 1.0	2.3 ± 0.3	15.5 ± 10.8
Lake Victoria (Napoleon Gulf)							
Astatoreochromis alluaudi	1	Aa	19.5	7.4	-16.0	2.8	6.2
Bagrus docmac	1	Bd	26.5	9.3	-14.2	3.4	15.1
Brycinus sadleri	1	Bs	12.7	6.5	-16.7	2.5	24.6
Haplochromis spp. (filleted)	2	H	14.7–17.2	6.7–7.7	-16.8 to -14.7	2.6–2.9	13.0–17.1
Haplochromis spp. † (whole)	3	H	3.8–11.0	7.0–7.1	-16.5 to -14.7	2.7–2.7	15.3 ± 11.1
Lates niloticus (filleted)	22	Ln	13.0–63.5	7.9 ± 1.0	-15.3 ± 1.3	2.9 ± 0.3	7.3 ± 6.1
Lates niloticus (gutted/head removed)	1	Ln	9.7	7.8	-14.7	2.9	12.9
Mormyrus kannume	5	Mk	16.3–31.0	8.1 ± 0.3	-14.6 ± 0.5	3.0 ± 0.1	21.1 ± 7.1
Oreochromis leucostictus	2	Ol	21.1–25.3	4.3–4.6	-15.4 to -12.9	1.9–2.0	3.2–4.3
Oreochromis niloticus (filleted)	23	On	14.6–34.5	4.6 ± 0.7	-13.9 ± 1.0	2.0 ± 0.2	9.8 ± 7.8
Oreochromis niloticus (gutted/head removed)	1	On	13.5	3.0	-12.1	1.5	6.1
Oreochromis variabilis	5	Ov	17.8–22.1	3.8 ± 0.5	-14.7 ± 0.8	1.7 ± 0.2	30.1 ± 34.2
Protopterus aethiopicus	5	Pa	50.0–100.0	8.5 ± 2.3	-16.3 ± 1.0	3.1 ± 0.7	2.8 ± 1.3
Rastrineobola argentea (whole/wet)	7*	Ra	4.0	7.0 ± 1.0	-14.6 ± 1.4	2.7 ± 0.3	83.7 ± 37.9
Rastrineobola argentea (whole/dry from market)	1*	Ra	3.5	7.2	-13.9	2.7	61.4
Synodontis afrofischeri	3	Sa	13.0–15.4	8.3 ± 0.1	-15.4 ± 0.04	3.1 ± 0.02	31.0 ± 19.9
Synodontis victoriae	4	Sv	17.1–23.5	7.5 ± 0.4	-16.9 ± 0.3	2.8 ± 0.1	16.7 ± 8.3
Tilapia zilli (filleted)	4	Tz	13.3–23.5	6.0 ± 1.1	-13.9 ± 0.5	2.4 ± 0.3	8.4 ± 3.9
Tilapia zilli (gutted)	1	Tz	14.5	4.8	-10.2	2.0	3.4
Lake Nkuruba							

Name	n	Code	Total Length (cm) min–max	$\delta^{15}N$ (‰) μ ± s.d	$\delta^{13}C$ (‰) μ ± s.d.	Trophic Level μ ± s.d.	MC (μg/kg) μ ± s.d.
Oreochromis leucostictus (filleted)	4	Ol	18.6–20.0	5.9 ± 0.3	-28.5 ± 0.4	2.6 ± 0.1	8.3 ± 4.3
Oreochromis leucostictus (gutted/head removed)	1	Ol	9.2	7.1	-26.7	2.9	17.2
Poecelia reticulata (whole)	2*	Pr	3.8	7.3–7.9	-28.2 to -26.5	3.0–3.2	4.5–73.3
Tilapia zilli (filleted)	4	Tz	9.0–19.0	6.6 ± 1.1	-26.3 ± 0.9	2.8 ± 0.3	11.7 ± 3.3
Tilapia zilli (whole)	1	Tz	5.2	6.7	-26.7	2.8	42.5
Lake Saka							
Astatoreochromis alluaudi (filleted)	3	Aa	8.8–10.6	5.8 ± 1.1	-20.4 ± 0.5	3.2 ± 0.3	71.3 ± 109.5
Astatoreochromis alluaudi (gutted/head removed)	1	Aa	11.4	5.5	-18.9	3.1	10.5
Astatoreochromis alluaudi (whole)	1	Aa	6.4	5.6	-21.5	3.1	32.5
Barbus neumayerii (gutted/head removed)	1	Bn	7.0	5.7	-23.8	3.1	9.5
Haplochromis spp. (filleted)	1	H	10.1	5.3	-19.7	3.0	52.1
Haplochromis spp. (gutted/head removed)	2	H	7.2–8.8	5.0–6.2	-20.9 to -20.9	3.0–3.3	23.2–1189.3
Haplochromis spp. (whole)	2	H	2.5–3.5	4.1–4.7	-20.8 to -20.0	2.7–2.8	21.3–215.2
Lates niloticus	4	Ln	25.2–63.1	7.3 ± 0.4	-19.7 ± 0.3	3.6 ± 0.1	16.4 ± 18.3
Oreochromis niloticus	16	On	19.1–38.0	3.1 ± 1.3	-18.9 ± 0.8	2.4 ± 0.4	17.0 ± 16.7
Tilapia zilli (filleted)	1	Tz	24.6	4.1	-18.8	2.7	4.9
Tilapia zilli (whole)	1	Tz	6.8	2.9	-21.2	2.3	898.7

Table 5.3 Summary of significant differences in microcystin concentrations (in muscle tissue) between the "common" fish species within each study sites. Species codes used are found in Table 5.2.

Site	Differences Between Species (P<0.05)
Edward	~
George	~
Mburo	Pa < Cg, Oe
Murchison	Pa < H, Ol, Sa
Napoleon	Pa < Mk, Sa
Nkuruba	~
Saka	On < H

Table 5.4 Summary of significant (P<0.05) relationships between log-transformed microcystin (log(MC)) concentrations in fish muscle tissue and total length (L).

Site	Species	log(MC) vs. L	r^2_{adj}	P
Edward	*Clarias gariepinus*	-	0.88	<0.05
George	*Oreochromis niloticus*	-	0.29	<0.05
George	*Haplochromis squamipinnis*	-	0.95	<0.05
Napoleon	*Oreochromis niloticus*	-	0.19	<0.05
Napoleon	*Oreochromis variabilis*	-	0.75	<0.05

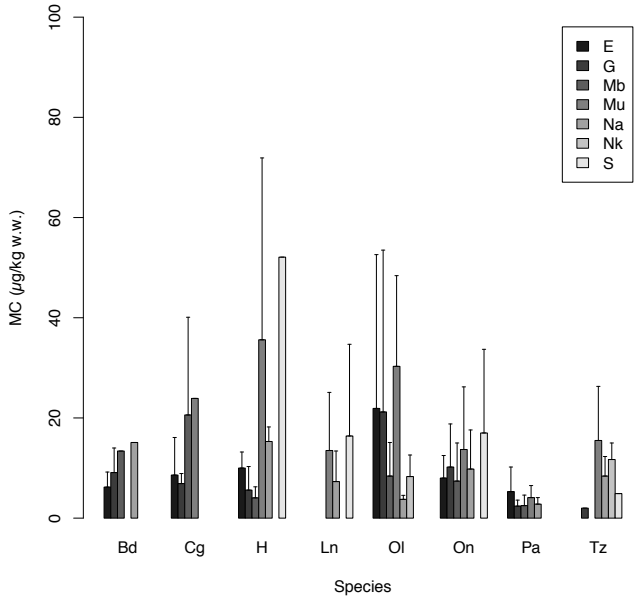

Figure 5.1 Microcystin concentrations in selected species of fish at all study sites. Data are grouped by species. Bar height represents mean values, while error bars represent standard deviation. Species codes are found in Table 5.2.

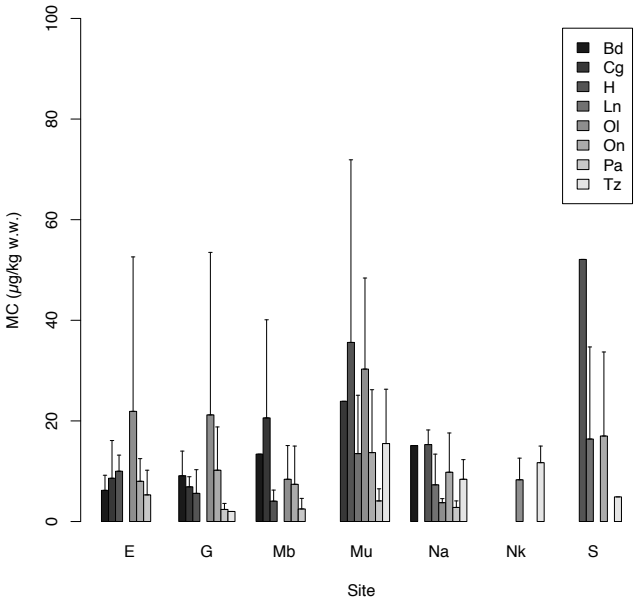

Figure 5.2 As in Figure 5.1 with data grouped by site (not species). Bar height represents mean values, and error bars represent standard deviation. Species codes are found in Table 5.2.

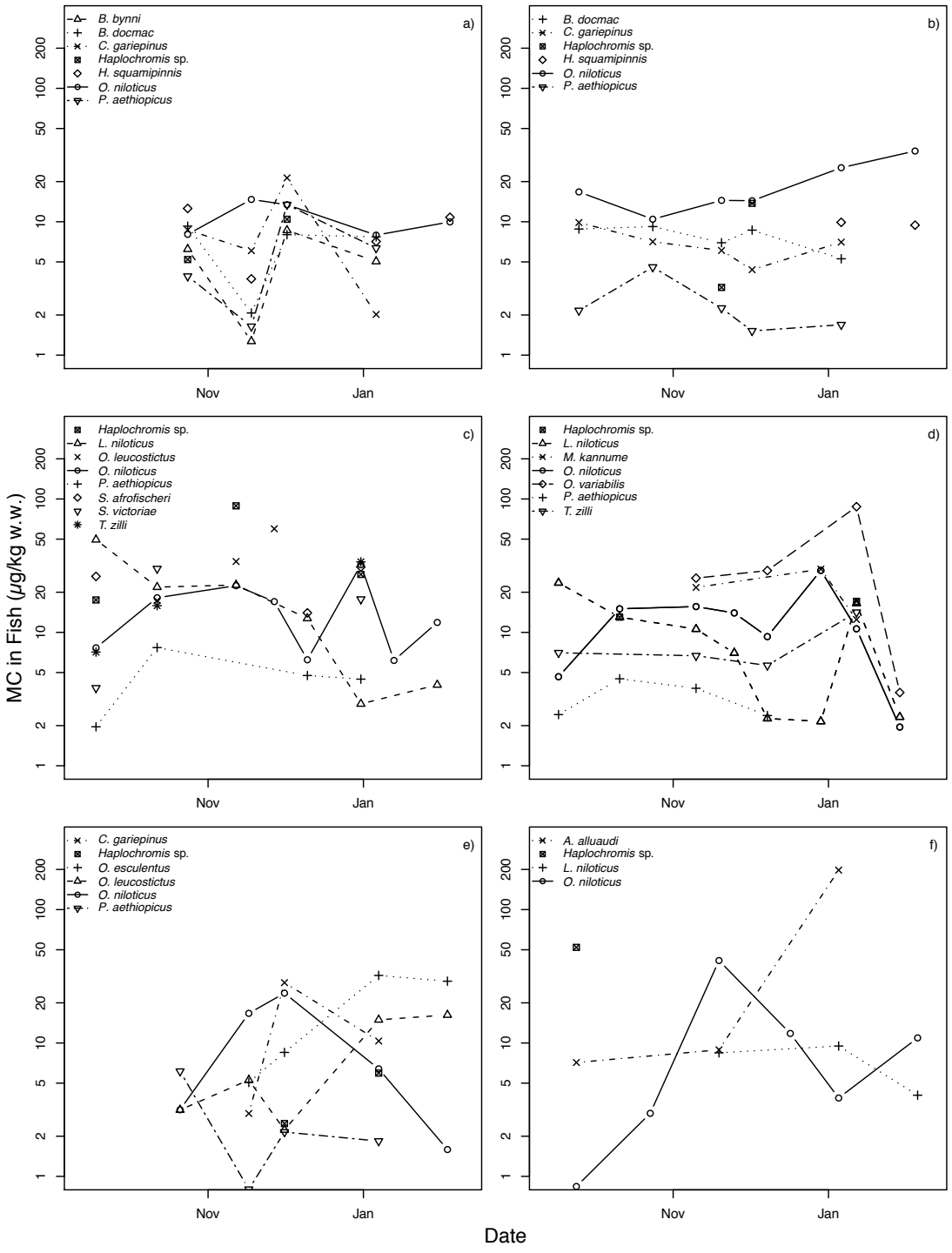

Figure 5.3 Seasonal patterns in microcystin concentrations in indicated fish species for a) Lake Edward, b) Lake George, c) Murchison Bay, d) Napoleon Gulf, e) Lake Mburo and f) Lake Saka (Lake Nkuruba is not displayed).

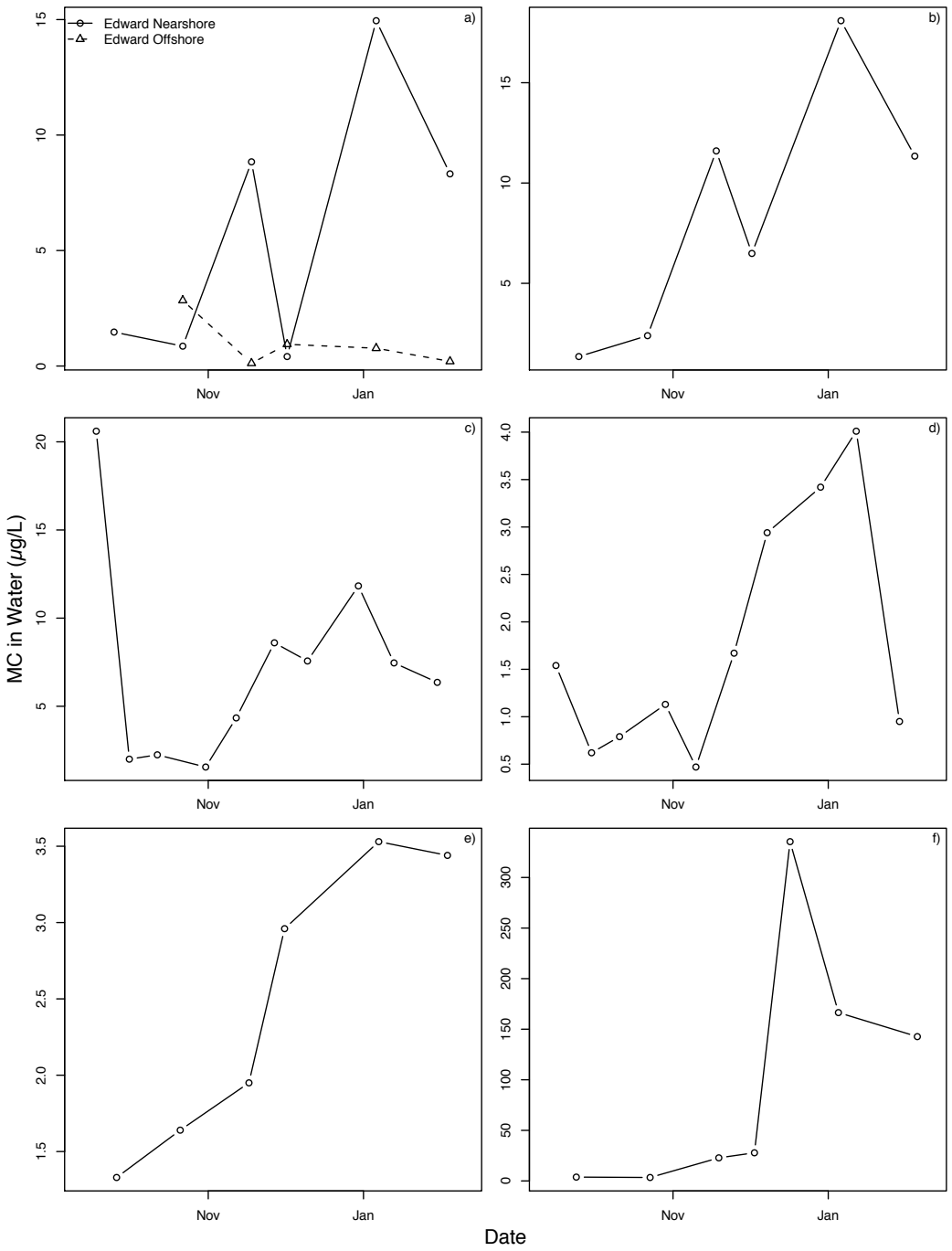

Figure 5.4 Seasonal patterns in microcystin concentrations in water for a) Lake Edward, b) Lake George, c) Murchison Bay, d) Napoleon Gulf, e) Lake Mburo and f) Lake Saka (Lake Nkuruba is not displayed). Note that the y-axis scales differ between sites.

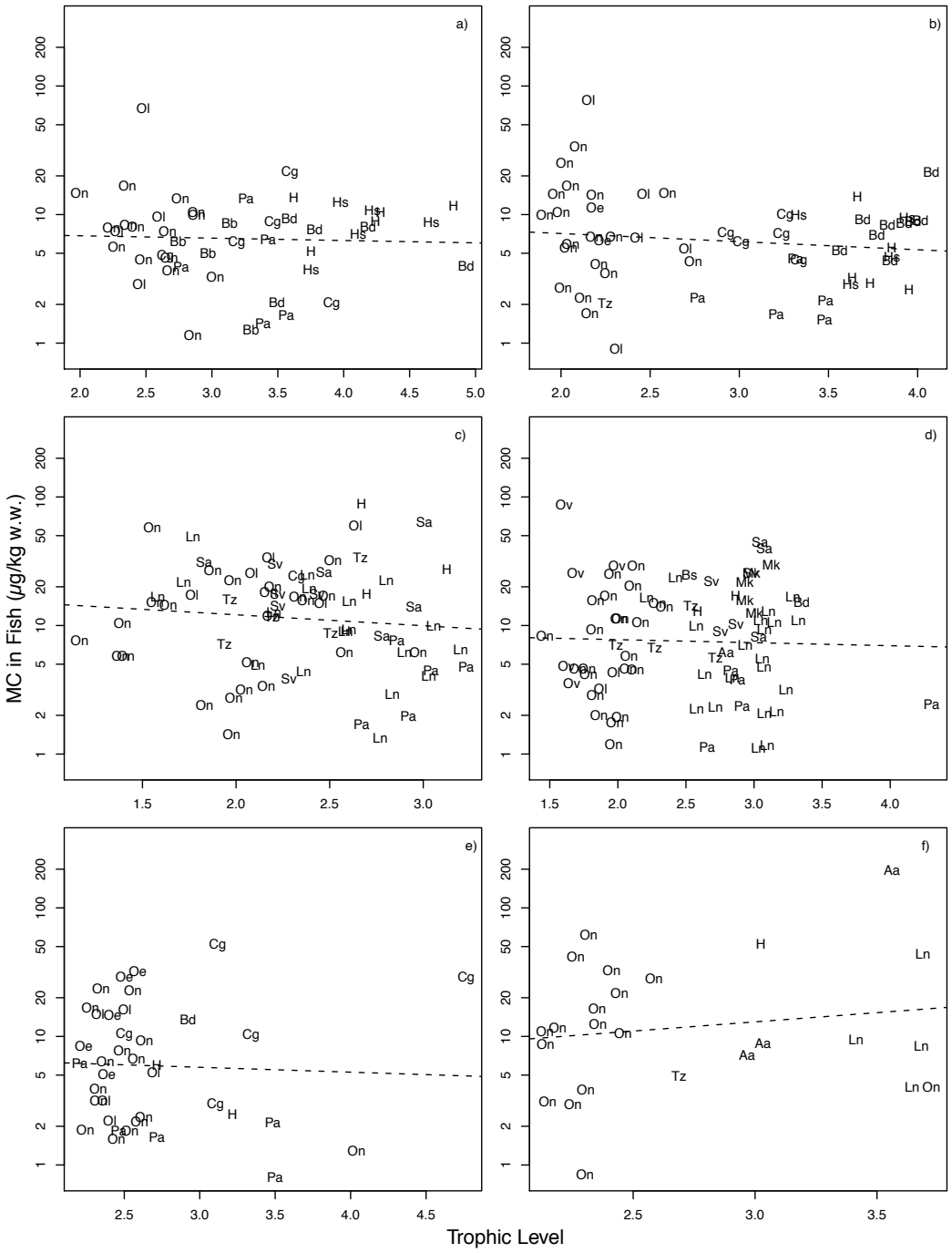

Figure 5.5 Regressions between log-transformed microcystin concentrations in fish and trophic level for a) Lake Edward, b) Lake George, c) Lake Mburo, d) Murchison Bay, e) Napoleon Gulf and f) Lake Saka (Lake Nkuruba is not displayed). Species codes are found in Table 2. Note that none of these regressions are statistically significant. Note that x-axis scales differ.

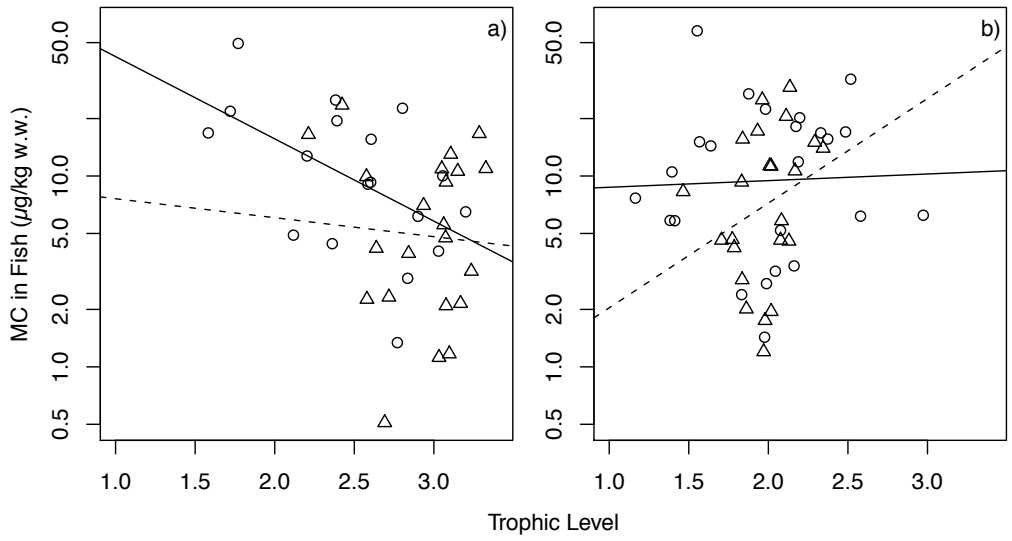

Figure 5.6 Regressions between log-transformed microcystin concentrations in fish and trophic level for a) *L. niloticus* and b) *O. niloticus* from both Murchison Bay (circles and solid lines) and Napoleon Gulf (triangles and dashed lines). With the exception of *L. niloticus* from Murchison Bay, these regression lines are not statistically significant.

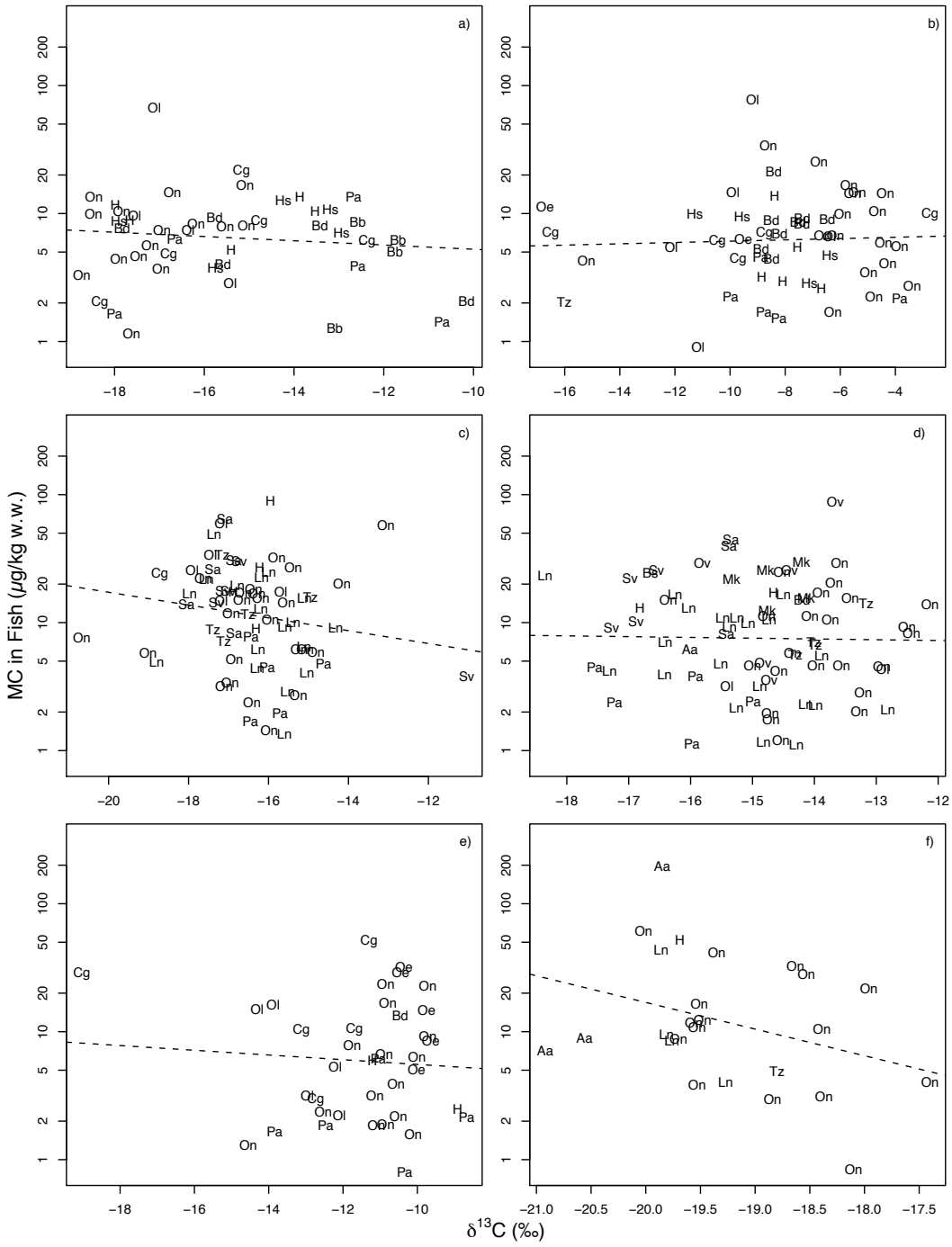

Figure 5.7 Regressions between log-transformed microcystin concentrations in fish and $\delta^{13}C$ for a) Lake Edward, b) Lake George, c) Lake Mburo, d) Murchison Bay, e) Napoleon Gulf and f) Lake Saka (Lake Nkuruba is not graphically displayed). Species codes are found in Table 2. Note that none of these regressions are statistically significant. Note that x-axis scales differ.

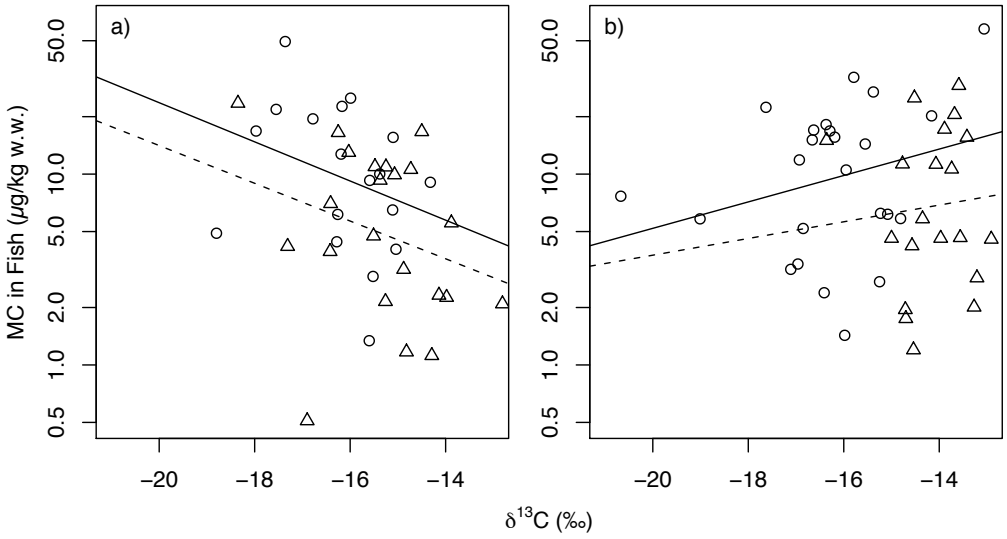

Figure 5.8 Regressions between log-transformed microcystin concentrations in fish and $\delta^{13}C$ for a) *L. niloticus* and b) *O. niloticus* from both Murchison Bay (circles and solid lines) and Napoleon Gulf (triangles and dashed lines). Note that none of these regressions are statistically significant.

Chapter 6
Evaluation of microcystin exposure risk through fish consumption

6.1 Introduction

As anthropogenic input of nutrients to freshwater systems continues to alter the total biomass and the composition of algal communities, there are a number of ecological and public health concerns that emerge. Globally, cyanobacterial dominance of freshwater phytoplankton is increasing, as is the occurrence of hazardous blooms of toxic cyanobacteria (de Figueiredo *et al.* 2004); and it is expected that a warming climate will further exacerbate the frequency and duration of such blooms (Paerl and Huisman 2009). The cyanotoxin microcystin is present in a broad range of aquatic systems (Sivonen and Jones 1999) and is a potent hepatotoxin and a potential tumour promotor (de Figueiredo *et al.* 2004, Falconer and Humpage 2006, Chen *et al.* 2009).

The World Health Organization has set a provisional total daily intake value (TDI) for microcystin of 0.04 µg/kg body weight (WHO 1998, Falconer *et al.* 1999), and has set a guideline value for microcystin in drinking water of 1 µg/L, based on the assumption that 80 percent of exposure is attributable to water consumption (WHO 1998, Falconer *et al.* 1999). Although accumulation of microcystin in fish and other aquatic organisms is known to occur (de Figeuiredo *et al.* 2004, Ibelings and Chorus 2007, Kotak *et al.* 1996, Magalhães *et al.* 2003), no widely accepted guidelines have been established for microcystin concentrations in fish tissue, and most microcystin exposure scenarios do not consider potential exposure through fish consumption.

I conducted a survey of microcystin in water and fish in two temperate great lakes (Erie and Ontario), three tropical great lakes (Victoria, Albert and Edward) and four other smaller Ugandan lakes (George, Mburo, Nkuruba, Saka). The large lakes sampled all support substantial commercially important fisheries, including the largest temperate (Lake Erie) and tropical (Lake Victoria) lake fisheries in the world. The smaller Ugandan lakes support subsistence fisheries that provide a critically important source of protein and income for riparian communities. These lakes provided a continuum of trophic status, and the fish sampled (491 fish from 33 species) were representative of several trophic levels ranging from planktivores to top predators.

6.2 Methods

Integrated epilimnetic water samples (see Chapter 2 for a detailed description of water sampling methodology) and fish were collected from all Ugandan study sites in April–May of 2007, and then again on a monthly basis between September 2008 and February 2009 for all sites but Lake Albert. The sites sampled included two embayments in northern Lake Victoria (Murchison Bay and Napoleon Gulf), Lake Albert, Lake Edward (nearshore and offshore), Lake George, Lake Mburo, and two crater lakes, Saka and Nkuruba. Water was collected from Maumee Bay (Lake Erie) and the Bay of Quinte (Lake Ontario) during the summers (May–September) of 2006 and 2007. Fish from these sites were caught during research trawls during July– September of 2006 and 2007.

Water was analyzed for chlorophyll *a* and nutrient concentrations as in Stainton *et al*. (1977). Microcystin in water was measured using indirect competitive ELISA (Abraxis LLC, Microcystins-ADDA ELISA kits, PN 520011). This is a congener-independent ELISA based on the detection of the Adda side-chain found in microcystins and nodularins (Fischer *et al.* 2001). Whole water samples were prepared for use in ELISA assays through chemical lysis (using the Abraxis LLC QuikLyse™ method, Loftin *et al.* 2008). These methods are described in detail in Chapter 2 of this thesis.

Several species of fish from different trophic levels were collected from each lake, with care taken to ensure a representative size range within species. Dorso-lateral fish muscle tissue was dried and homogenized. Where fish were very small and typically consumed whole (including *Rastrineobola argentea*, *Poecelia reticulata*, and fish less than approximately 10 cm in total length), whole dried fish were homogenized. Microcystin in fish muscle tissue was analyzed using methanol extraction followed by ELISA based on the method described in Wilson *et al*. (2008) with slight modifications (extracts were dried using a Turbovap LV rather than vacuum evaporation, and Abraxis Microcystins-ADDA ELISA kits were used to measure microcystin). This method is described in detail in Chapter 5 of this thesis.

It should be noted that the water chemistry results, and microcystin concentrations in fish in the current chapter will differ from those reported for the same study sites in Chapters 3 and 5 of this thesis because the current chapter includes water and fish samples collected in 2007 in addition to the 2008–2009 sampling period reported in the other chapters. Additionally, because the objective of the current chapter is to evaluate potential exposure to microcystin for human consumers, microcystin concentrations in whole small fish (generally <10 cm) are included in overall mean values for fish given that these fish would typically be consumed whole.

6.3 Results and Discussion

Secchi depth, total phosphorus and chlorophyll *a* concentrations are often used to indicate lake trophic status (Vollenweider and Kerekes 1982). Based on the physicochemical observations for the study sites (Table 6.1) Lake Nkuruba, Lake Albert, and the Bay of Quinte were found to be meso/eutrophic, while Maumee Bay, Napoleon Gulf, and offshore Lake Edward were eutrophic. The remaining sites (Lake George, Lake Mburo, Murchison Bay, Lake Saka, and nearshore Lake Edward) were hypereutrophic.

Microcystin concentrations in water consistently exceeded the WHO recommended guideline for microcystin in drinking water at several of the study sites (Table 6.1), including Lake Victoria, which provides drinking water for more than ten million people (Mugidde *et al.* 2003). While the meso-eutrophic lakes had the lowest observed microcystin concentrations, the highest microcystin concentrations were observed in the hypereutrophic lakes. Across all study sites (both tropical and temperate), significant positive relationships were observed between microcystin concentrations and chlorophyll *a* concentrations (log transformed data, $r^2_{adj} = 0.50$, n = 72, $P<0.001$) as well as microcystin concentrations and total phosphorus concentrations (Figure 6.1, log transformed data, $r^2_{adj} = 0.40$, n = 72, $P<0.001$). Also, chlorophyll *a* showed a strong positive relationship with total phosphorus (log transformed data, $r^2_{adj} = 0.69$, n = 73, $P<0.001$). These results confirm that microcystin concentrations were higher where algal biomass and total phosphorus concentrations were high, suggesting that eutrophication can increase the prevalence and magnitude of microcystin producing cyanobacterial blooms.

Microcystin was found to have accumulated in muscle tissue from fish at all of the study sites, and observed concentrations ranged from 0.5–1917 µg/kg of wet weight (Table 6.2, Figure 6.2). Microcystin concentrations in fish tended to be higher in lakes where microcystin concentrations in water were also high. Within lakes, microcystin concentrations in fish exhibited a great deal of variability. This variability is largely attributable to the seasonal variability in microcystin in water, the differences in diet between the species sampled, and the wide range in size within species (see Chapter 5).

In the Ugandan lakes, the highest microcystin concentrations were observed in *Rastrineobola argentea*, a small zooplanktivorous cyprinid that now dominates landings in Lake Victoria (Kolding *et al.* 2008). Microcystin concentrations in these fish ranged from 36.2–41.2 µg/kg w.w. in Murchison Bay, and from 39.0–129 µg/kg w.w. in Napoleon Gulf (Table 6.2, Figure 6.2). These concentrations would cause a consumer (weighing 60 kg and consuming 100 g of fish daily) to exceed the WHO TDI for microcystin by a factor of 1.5–5.4. *R. argentea* is typically consumed whole, and as such, I analyzed whole fish for

microcystin because this most accurately represents the exposure risk to consumers. Microcystin concentrations were likely high due to cyanobacteria present in the gut of these small fish. High microcystin concentrations were also observed in the muscle tissue of small (<25 cm) *Lates niloticus* (Nile perch), *Haplochromis* spp., and to a lesser extent, some tilapiine cichlids (*Oreochromis* spp. and *Tilapia zilli*) (Table 6.2, Figure 6.2). These trends are of importance because the fish with the highest observed microcystin concentrations represent the less commercially marketable and less profitable fish that tend to be consumed by low-income local residents and those living in fishing communities. Large *Lates niloticus*, which are economically important fish for export (Kolding *et al.* 2008), pose no risk to consumers given the low microcystin concentrations observed in fish exceeding a total length of 25 cm (mean concentrations of 8.0, 5.9, and 6.7 µg/kg w.w. in Murchison Bay, Napoleon Gulf and Lake Albert respectively).

Microcystin concentrations in fish collected in summer from embayments on the North American great lakes experiencing seasonal cyanobacterial blooms (see Watson *et al.* 2008) can reach levels similar to those observed at the Ugandan study sites (Table 6.2, Figure 6.2). In the western basin of Lake Erie, the highest microcystin concentrations were observed in walleye (5.3–41.2 µg/kg w.w.), white bass (4.2–27.1 µg/kg w.w.) and smallmouth bass (1.5–43.6 µg/kg w.w.). In the Bay of Quinte (Lake Ontario), the zooplanktivorous alewives had the highest microcystin concentrations (20.0–37.5 µg/kg w.w.), followed by northern pike (1.6–25.8 µg/kg w.w.). Many of these fish are important species for both sport and commercial fisheries, and, if eaten, several of the fish sampled would cause a consumer to exceed the WHO TDI for microcystin.

Figure 6.3 displays estimates of potential daily microcystin exposure based on an individual (weighing 60 kg) consuming water (2 L) and fish (100 g) from each of the study lakes. The fish used in the exposure estimates were those with the highest mean microcystin concentrations, as this provides a worst-case scenario of potential daily exposure. At all sites but Lake Albert, such daily exposure estimates exceeded the WHO TDI for chronic exposure.

At the majority of study sites, potential exposure from water exceeded potential exposure from fish. So where people are getting drinking water and fish from the same lake, water tends to be the main source of exposure. However, fish can represent a significant and sometimes dominant source of microcystin to consumers, particularly where people are consuming species with higher concentrations. Based on the scenario outlined in Figure 6.3, fish were the dominant exposure source in Lake Albert, Napoleon Gulf (Lake Victoria), Lake Nkuruba, and the Bay of Quinte (Lake Ontario), indicating that even where

chlorophyll and microcystin concentrations in the water are relatively low, exposure from fish may increase total daily exposure to potentially detrimental levels. Given the high potential exposure from fish at several of the study sites, avoiding or treating drinking water would not eliminate the risk of microcystin exposure. For example, in Napoleon Gulf, an individual eating 100 g of *R. argentea* would be exposed to 8.1 µg of microcystin, which is high enough to greatly exceed the recommended WHO TDI before even considering potential exposure from drinking water. Avoiding the consumption of some fish species (such as *R. argentea* and *Haplochromis* spp.) may be necessary to reduce significant exposure risk; however, in many households this may not be an option. Also, it is of importance to note that because microcystin is a heat stable compound, neither boiling water nor cooking fish prior to consumption can reduce the risk of microcystin exposure (Harada 1996, Zhang *et al.* 2010).

From a chronic exposure standpoint, year round presence of microcystin in the water and food webs of the Ugandan lakes is a likely scenario, given that algal blooms can occur year-round in tropical lakes (Kling *et al.* 2001, Oliver and Ganf 2000), indicating the potential for persistent exposure of fish (and their human consumers) to possibly harmful levels of microcystin. However, in temperate lakes, where algal biomass is much lower during winter periods (Munawar and Munawar 1986), year-round chronic exposure of aquatic food webs and human consumers to microcystin would not be expected. Although observed microcystin concentrations and potential daily microcystin exposure values for Maumee Bay and the Bay of Quinte sometimes exceeded the WHO TDI for chronic exposure to microcystin, these values were based on samples collected in the summer and early fall, which is likely to represent the highest microcystin concentrations experienced throughout the year. Because fish are able to depurate microcystin when no longer exposed (Tencalla and Deitrich 1997, Xie *et al.* 2004), microcystin concentrations in fish are likely to decline in concert with microcystin in water as the winter season approaches. Also, in the Ugandan lakes, the likelihood that an individual is consuming both water and fish daily from the same lake is much higher than for the North American study sites, where individuals often have alternative sources of drinking water and food. These differences between the tropical and temperate study sites suggest that while the daily exposure scenario in Figure 6.3 is realistic for Ugandan consumers, North American consumers are unlikely to experience chronic exposure to microcystin that exceeds WHO TDI guidelines.

These results demonstrate the broad prevalence of microcystin in water and fish from temperate and tropical lakes that support important commercial, sport, and subsistence fisheries as well as being critical sources of drinking water. My observed microcystin concentrations in fish and water fall within the range

of concentrations reported in other studies from around the world, including previously reported data from East Africa, North America, Egypt, Brazil, Argentina, and China (Magalhães *et al.* 2003, Mohamed *et al.* 2003 Sekadende *et al.* 2005, Xie *et al.* 2005, Deblois *et al.* 2008, Okello *et al.* 2009, Zhang *et al.* 2009, Amé *et al.* 2010, Semyalo *et al.* 2010), confirming that accumulation of microcystin in fish is of global concern. As such, current guidelines for quantifying risk may not adequately reflect the potential for fish to make up a considerable proportion of microcystin exposure. Of particular concern are riparian fishing communities consuming water and small fish from the tropical study sites, where there is risk of chronic year-round exposure to microcystin, and potential detrimental health effects.

Table 6.1 Summary of select physicochemical properties of study lakes, including microcystin concentrations in water. Values are reported as mean ± s.d., while n is the sample size. Trophic status was determined based on Vollenweider and Kerekes (1982), and reported as: H (hypereutrophic), E (eutrophic), and M/E (mesotrophic/eutrophic; in these lakes some trophic status indicators suggest eutrophic conditions while others suggest mesotrophic conditions).

Lake	n	Site Depth (m)	Secchi Depth (m)	Chl *a* (µg/L)	TP (µg/L)	Trophic Status	Microcystin in Water (µg/L)
Albert	2	12.0	1.6 ± 0.3	19.2 ± 3.5	32.5 ± 2.2	M/E	0.1 ± 0.02
Edward							
Nearshore	7	3.5	0.5 ± 0.2	67.7 ± 42.3	131.5 ± 50.4	H	5.0 ± 5.7
Offshore	5	7.3	1.1 ± 0.3	23.5 ± 27.5	58.9 ± 9.2	E	1.0 ± 1.1
George	7	2.8	0.4 ± 0.1	124.7 ± 40.7	188.7 ± 24.6	H	7.3 ± 6.6
Mburo	7	3.2	0.5 ± 0.1	68.5 ± 39.0	121.3 ± 39.7	H	2.2 ± 1.1
Victoria							
Murchison Bay	10	5.2	0.7 ± 0.1	101.8 ± 48.3	106.2 ± 28.0	H	7.3 ± 5.7
Napoleon Gulf	12	17.5	1.4 ± 0.2	24.0 ± 18.0	58.8 ± 14.6	E	1.5 ± 1.3
Nkuruba	7	33.4	1.7 ± 0.4	7.9 ± 3.5	34.4 ± 8.5	M	0.2 ± 0.1
Saka	7	3.2	0.4 ± 0.1	133.8 ± 84.5	182.0 ± 34.7	H	57.1 ± 67.9
Bay of Quinte	4	3.9	2.1 ± 0.8	11.2 ± 8.4	24.1 ± 6.4	M/E	0.9 ± 1.0
Maumee Bay	4	3.5	1.5 ± 0.7	9.5 ± 7.0	46.0 ± 24.7	E	1.3 ± 2.3

Table 6.2 Microcystin concentrations in fish from all study sites. Asterisks beside n values indicate that at least one specimen was analyzed whole. For *R. argentea* and *P. reticulata* all specimens were analyzed whole.

Name	Microcystin (µg/kg wet weight)		
	n	mean ± s.d.	range
Lake Albert			
Lates niloticus (> 25 cm)	5	6.7 ± 3.5	3.9–11.6
Tilapia zilli	5	4.0 ± 1.3	2.7–6.2
Lake Edward			
Bagrus docmac	5	6.2 ± 3.0	2.1–9.3
Barbus bynni	5	5.5 ± 2.7	1.3–8.6
Clarias gariepinus	5	8.6 ± 7.5	2.0–21.3
Haplochromis spp.	5	10.0 ± 3.2	5.2–13.6
Haplochromis squamipinnis	5	8.6 ± 3.4	3.7–12.6
Oreochromis leucostictus	4	21.9 ± 30.7	2.9–67.7
Oreochromis niloticus	20	6.7 ± 4.5	1.1–16.7
Protopterus aethiopicus	5	5.3 ± 4.9	1.4–13.4
Lake George			
Bagrus docmac	5	9.4 ± 6.9	4.4–21.2
Clarias gariepinus	5	6.9 ± 2.0	4.4–9.9
Haplochromis spp.	5*	5.6 ± 4.7	2.6–13.7
Haplochromis squamipinnis	5	7.7 ± 3.8	2.9–11.8
Oreochromis esculentus	4	13.8 ± 6.5	6.3–21.5
Oreochromis leucostictus	5	21.2 ± 32.3	0.9–78.4
Oreochromis niloticus	18	10.2 ± 8.6	1.7–33.9
Protopterus aethiopicus	5	2.4 ± 1.2	1.5–4.6
Tilapia zilli	1	2.0	~
Lake Mburo			
Bagrus docmac	1	13.4	~
Clarias gariepinus	5	20.6 ± 19.5	3.0–51.3
Haplochromis spp.	5	7.6 ± 4.2	2.5–12.1
Oreochromis esculentus	6	23.9 ± 18.4	1.3–54.2
Oreochromis leucostictus	5	8.4 ± 6.7	2.2–16.2
Oreochromis niloticus	15	7.4 ± 7.6	1.3–23.6
Protopterus aethiopicus	5	2.3 ± 2.1	0.8–6.1
Lake Victoria (Murchison Bay)			
Clarias gariepinus	1	23.9	~
Haplochromis spp.	5*	32.5 ± 21.1	9.0–88.9
Lates niloticus (< 25 cm)	7	21.2 ± 14.8	3.1–49.5
Late niloticus (> 25 cm)	17	8.0 ± 6.8	1.3–25.0
Oreochromis leucostictus	5	30.3 ± 18.1	14.9–59.8
Oreochromis niloticus	28	12.8 ± 11.9	1.4–57.7
Protopterus aethiopicus	5	4.1 ± 2.4	1.7–7.7
Rastrineobola argentea	2*	38.7 ± 3.5	36.2–41.2
Synodontis spp.	10	22.8 ± 17.1	3.8–64.4
Tilapia zilli	5	15.4 ± 10.8	7.1–33.8
Lake Victoria (Napoleon Gulf)			

Name	Microcystin (µg/kg wet weight)		
	n	mean ± s.d.	range
Astatoreochromis alluaudi	1	6.2	~
Bagrus docmac	1	15.1	~
Brycinus sadleri	1	24.6	~
Haplochromis spp.	5*	15.2 ± 8.0	2.8–24.2
Lates niloticus (< 25 cm)	6	12.4 ± 7.0	3.9–23.5
Lates niloticus (> 25 cm)	17	5.9 ± 4.9	0.5–16.7
Mormyrus kannume	5	21.1 ± 7.1	12.5–29.8
Oreochromis leucostictus	2	3.8 ± 0.8	3.2–4.3
Oreochromis niloticus	24	9.7 ± 7.6	1.2–29.1
Oreochromis variabilis	5	30.1 ± 34.1	3.5–87.6
Protopterus aethiopicus	5	2.8 ± 1.3	1.1–4.5
Rastrineobola argentea	8*	80.9 ± 36.0	39.0–128.5
Synodontis spp.	7	22.8 ± 15.0	8.2–44.9
Tilapia zilli	5	7.4 ± 4.0	3.4–14.1
Lake Nkuruba			
Oreochromis leucostictus	10	7.2 ± 5.1	1.6–17.2
Poecelia reticulata	2*	38.9 ± 48.6	4.5–73.3
Tilapia zilli	12*	19.8 ± 18.9	2.1–62.3
Lake Saka			
Astatoreochromis alluaudi	5*	51.4 ± 82.5	7.1–197.7
Barbus neumayerii	1	215.2	~
Haplochromis spp.	10*	600.5 ± 750.1	21.3–1917
Lates niloticus (> 25 cm)	4	16.4 ± 18.3	4.1–43.7
Oreochromis niloticus	19	19.3 ± 19.4	0.8–63.42
Tilapia zilli	2*	451.8 ± 632.0	4.9–898.7
Lake Ontario (Bay of Quinte)			
Alosa pseudoharengus (alewife)	3	25.9 ± 10.1	20.0–37.5
Ameiurus nebulosus (brown bullhead)	6	4.4 ± 0.6	3.3–5.0
Aplodinotus grunniens (freshwater drum)	3	0.8 ± 0.3	0.5–1.1
Esox lucius (northern pike)	8	10.2 ± 7.6	1.6–25.8
Lepomis gibbosus (pumpkinseed)	4	1.9 ± 1.0	0.7–2.9
Lepomis macrochirus (bluegill)	1	4.8	~
Morone americana (white perch)	9	4.5 ± 4.1	0.7–14.8
Perca flavescens (yellow perch)	7	3.1 ± 2.0	0.5–5.6
Pomoxis nigromaculatus (black crappie)	2	1.7 ± 0.3	1.5–1.9
Stizostedion vitreum (walleye)	14	2.1 ± 1.6	0.5–6.1
Lake Erie (Western Basin)			
Aplodinotus grunniens (freshwater drum)	2	2.4 ± 6.0	1.7–10.1
Coregonus clupeaformis (whitefish)	5	4.1 ± 1.0	2.9–5.4
Micropterus dolomieu (smallmouth bass)	5	13.4 ± 17.8	1.5–43.6
Morone americana (white perch)	6	5.6 ± 4.9	1.9–15.0
Morone chrysops (white bass)	5	18.3 ± 8.7	4.2–27.1
Perca flavescens (yellow perch)	4	5.0 ± 1.4	3.6–7.0
Stizostedion vitreum (walleye)	5	23.9 ± 17.2	5.3–41.2

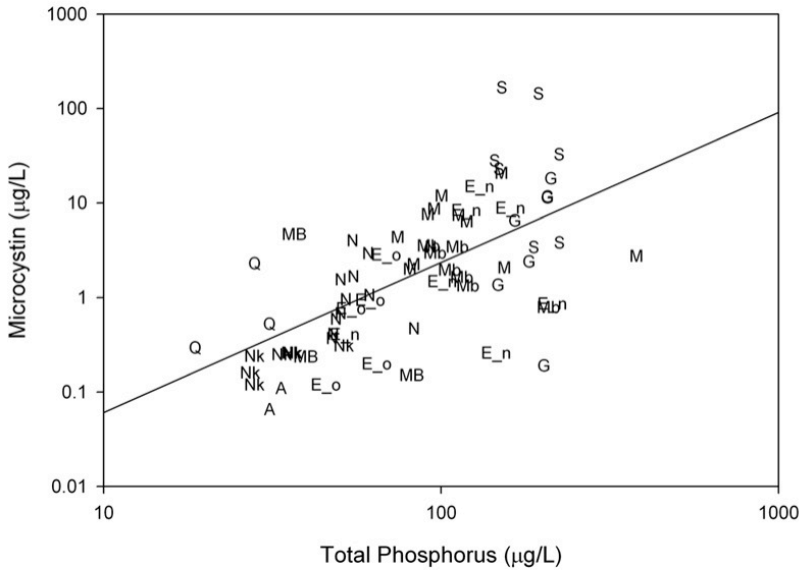

Figure 6.1 Regression of microcystin concentrations against total phosphorus concentrations from all study sites (r^2_{adj} : 0.40, n=72, P<0.001). Site labels in Figure 1 are indicated as follows: A (Lake Albert), E_n (Lake Edward nearshore), E_o (Lake Edward offshore), G (Lake George), Mb (Lake Mburo), M (Murchison Bay, Lake Victoria), N (Napoleon Gulf, Lake Victoria), Nk (Lake Nkuruba), S (Lake Saka), MB (Maumee Bay, Lake Erie), Q (Bay of Quinte, Lake Ontario).

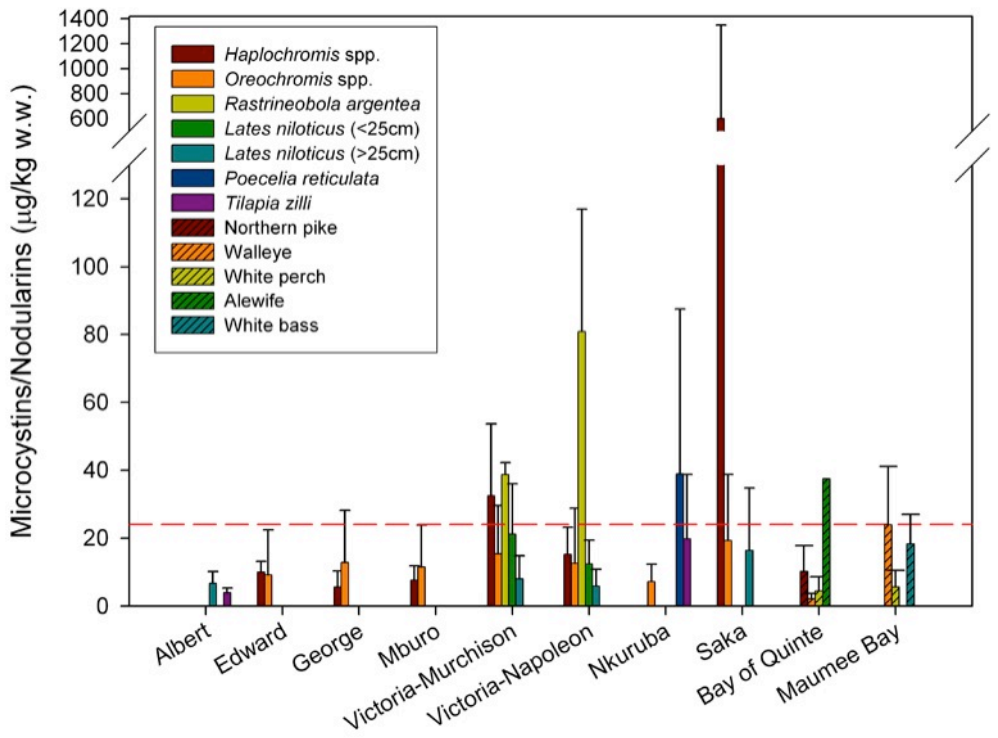

Figure 6.2 Microcystin concentrations in several species of fish. The red line represents the concentration of microcystin in fish that would cause a consumer to exceed total daily intake (TDI) values recommended by the WHO for chronic exposure (0.04µg/kg body weight/day; which yields a threshold concentration of microcystin in fish of 24 µg/kg wet weight for an individual weighing 60 kg and consuming 100 g of fish/day).

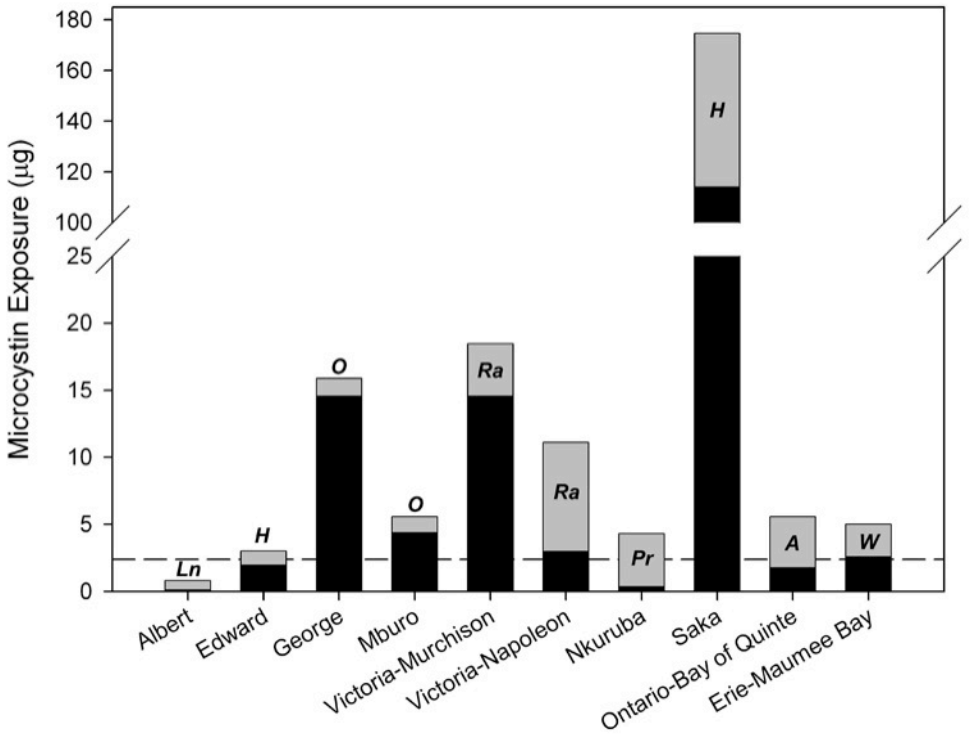

Figure 6.3 Potential daily microcystin exposure for individuals consuming water and fish from the study lakes. The reference line indicates the threshold value at which a 60 kg consumer would exceed the WHO TDI. Exposure from water is indicated in black, and is based on a daily consumption of 2 L. Exposure from fish is indicated in grey, and is based on a daily consumption of 100 g of the fish species with the highest mean microcystin concentrations at the given study site. The species used in calculating exposure from fish are indicated as follows: *Ln* (*Lates niloticus*), *H* (*Haplochromis* spp.), *O* (*Oreochromis* spp.), *Ra* (*Rastrineobola argentea*), *Pr* (*Poecelia reticulata*), *A* (alewife), *W* (walleye).

Chapter 7
General Conclusions and Recommendations

7.1 Summary and general conclusions

As eutrophication of freshwater systems continues to alter the composition and total biomass of phytoplankton communities, there are a number of public health concerns that emerge. Of particular concern is the occurrence of the cyanobacterial toxin microcystin, a potent hepatotoxin that is common in freshwaters worldwide. This thesis reports the results of an extensive survey of microcystin concentrations in water and fish from several Ugandan lakes (as well as some data from two temperate eutrophic embayments), including lakes that support some of the largest freshwater fisheries in the world and are critical sources of drinking water. My results yield important insight into the factors that determine microcystin concentrations in both water and fish.

Microcystin concentrations in nearly all of the Ugandan study lakes consistently exceeded the WHO recommended guideline for microcystin in drinking water of 1.0 μg/L (Chapter 2). Microcystin in these lakes was primarily produced by *Microcystis* spp., and as such, microcystin concentrations were strongly related to *Microcystis* biomass and growth dynamics. *Microcystis* biomass was highest at the shallowest study sites, where high nutrient concentrations and shallow depth can sustain much higher phytoplankton biomass as compared to deeper sites with comparable nutrient concentrations where self-shading would be expected to lead to light limitation at lower phytoplankton biomass. However, the importance of nutrient concentrations in addition to light in controlling *Microcystis* biomass was most evident in mesotrophic Lake Nkuruba, where *Microcystis* was not present and where low nutrient concentrations were likely limiting total and cyanobacterial biomass, since estimated mean mixed layer light intensity did not indicate the potential for light limitation. Due to the influence of both nutrient concentrations and light on *Microcystis* biomass, microcystin concentrations were highest at the shallow hypereutrophic study sites.

To explore the trophic transfer of microcystin, the underlying food webs in these study lakes were described using stable isotope analysis (Chapter 3). The food webs in these tropical East African lakes were characterized by a high degree of omnivory, and the $\delta^{13}C$ values observed in fish from these lakes were generally consistent with strong reliance on pelagic primary organic carbon sources, with little evidence of strong contribution of benthic carbon to these food webs. Given that these lakes are all eutrophic and hypereutrophic (with the exception of Lake Nkuruba), this is consistent with previous

observations in the literature of reduced availability and importance of benthic food sources in lakes with high phytoplankton biomass (Vadeboncoeur *et al.* 2003). This has particularly important implications with respect to potential food web exposure to microcystin, since microcystin in these lakes is predominantly produced by the pelagic *Microcystis*. The dominance of pelagic trophic pathways in these lakes suggests that fish at all trophic levels may be susceptible to exposure to and accumulation of microcystin.

The distribution and trophic transfer of mercury in the Ugandan study lakes was also described (Chapter 4), both to increase the body of knowledge on mercury trophodynamics in tropical eutrophic lakes (which are not as well-studied as temperate and arctic systems), and to provide a valuable comparison for microcystin trophodynamics in these systems. By characterizing the movement of a relatively well-studied compound (mercury) through the food webs of these lakes, I was able to directly contrast the accumulation and trophic transfer (and potential for biomagnification) of microcystin in these same lakes (using the same fish). Total mercury concentrations in fish from all of the Ugandan study sites were low, and would not likely pose a risk to even the most frequent fish consumers. Mercury concentrations in fish appeared to be more strongly determined by processes at the base of the food web than by mercury concentrations in water. My results suggest that year-round high phytoplankton biomass and growth rates in eutrophic tropical lakes may act to mitigate the potential for high mercury concentrations in fish; whereby growth and possibly biomass dilution can act to reduce mercury concentrations at the base of the food web, and growth dilution of mercury at consumer trophic levels can act to reduce the realized biomagnification rate of mercury. Indeed the highest mercury concentrations were observed in the lakes with the lowest phytoplankton biomass (and often the lowest mercury concentrations in water). These results have many implications regarding the relationship between lake trophic status and trophodynamics of mercury, and it would be useful to explore the extent to which these processes extend to higher-latitude lakes.

In contrast to mercury, microcystin did not biomagnify in the fish food webs of the Ugandan study lakes, and strong biodilution of microcystin was not observed either (Chapter 5; also Figure 7.1). Microcystin accumulated in fish from all trophic levels, suggesting that although biomagnification of microcystin does not occur, trophic transfer of microcystin can be substantial. Although there was no strong evidence for biodilution of microcystin through the fish food webs, the inclusion of phytoplankton (and zooplankton and other primary consumers such as mollusks) in this analysis would likely reveal much stronger biodilution patterns. Figure 7.1 highlights the fact that although eating fish from lower on

the food web will reduce exposure to biomagnifying compounds such as mercury (or other persistent organic pollutants), this strategy will not reduce exposure to microcystin. Microcystin in fish was also observed to be highly seasonally variable, and in some lakes, concentrations in fish closely mirrored seasonal changes in microcystin concentrations in water, highlighting the ability of fish to respond quickly to increases and decreases in microcystin concentrations in water. Rapid response to changing microcystin concentrations in water also explains why few strong relationships were observed between microcystin concentrations in fish and fish diet as characterized by stable isotope analysis, since integrated long-term dietary information may not reflect the recent diet (and associated microcystin exposure) of these omnivorous fish with dietary plasticity.

Microcystin exposure estimates for individuals consuming water and fish from the Ugandan study lakes as well as from the two temperate eutrophic embayments (Maumee Bay and Napoleon Gulf) indicate that at nearly all of the study sites, there is the potential for chronic exposure to microcystin exceeding the WHO recommended tolerable daily intake (Chapter 6). Microcystin was detected in all fish sampled (491 fish representing 33 species), establishing that microcystin accumulation in fish is pervasive at these study sites. My results also indicate that fish can be an important and sometimes dominant source of microcystin exposure to human consumers. I found that in many lakes, fish consumption alone has the potential to expose consumers to levels of microcystin that may be detrimental to human health. This challenges the current paradigm that water is the primary source of microcystin exposure to humans, and highlights the need to consider potential exposure to microcystin through fish consumption in order to adequately assess human exposure and risk. In particular, risk of chronic microcystin exposure is high in riparian fishing communities regularly consuming water and fish from the tropical study sites, where year-round microcystin exposure of both aquatic food webs and human consumers is likely.

In general, the Ugandan study sites can be divided into two groups with distinct characteristics. The first group of sites (Lake Albert, Lake Edward offshore, Napoleon Gulf and Lake Nkuruba) tended to have deeper mixing depths, lower chlorophyll *a* (due to a combination of lower nutrient concentrations and higher potential for light limitation at lower phytoplankton biomass), lower *Microcystis* biomass, and lower microcystin concentrations. These sites also had significantly higher mercury biomagnification rates that at the other study sites, and mercury concentrations in fish from these sites were often higher than at more productive sites with comparable or higher mercury concentrations in water. The sites in the second group (Lake Edward nearshore, Lake George, Lake Mburo and Lake Saka) were shallower, and had higher chlorophyll *a* (due to a combination of nutrient and light availability), *Microcystis* biomass

and microcystin concentrations. Meanwhile, mercury biomagnification rates and mercury concentrations in fish from these sites tended to be low, even where mercury concentrations in water were elevated relative to other sites. Although the highest microcystin concentrations in fish were observed in hypereutrophic Murchison Bay and Lake Saka, the consistent detection of microcystin in fish from all study sites highlights the potential for fish to be a source of microcystin exposure to human consumers even where microcystin concentrations in water are low.

7.2 Future Research

1. Microcystin concentrations in fish from the current study could also be measured using HPLC for confirmation of and comparison with ELISA results. These alternative detection methods would also allow for an examination of the microcystin congeners present in fish muscle tissue.

2. It is very likely that other cyanotoxins are being produced in these systems, and these toxins should also be characterized in both water and fish.

3. The relationship between phytoplankton biomass and mercury accumulation/biomagnification should be explored further. It would be of particular interest to examine whether these relationships are also observed in temperate systems, where mercury concentrations in fish are often higher.

4. Microcystin concentrations should be characterized in all levels of these food webs in order to gain further insight into the trophic transfer of microcystin (particularly through the analysis of non-fish food web components).

5. Gut content analysis of fish should be carried out in order to explore whether recent fish diet (as determined through examination of gut contents) is related to microcystin concentrations in muscle tissue. This may further our understanding of how fish diet influences microcystin exposure and accumulation.

6. It would be useful to conduct high frequency sampling with many replicates in a single system in order to examine accumulation and depuration of microcystin in fish, and to characterize short- and long-term responses to changing microcystin concentrations in water.

7.3 Recommendations for Public Health and Management

Microcystin in both water and fish should be monitored, particularly in lakes where microcystin is expected to be persistently present. Also, riparian communities should be educated about both the risks posed by cyanotoxins, as well as simple and cost-effective measures for risk-reduction (e.g. cloth filtration of water prior to boiling in order to remove cell bound microcystin). Also, it may be necessary to

discourage consumption of fish species known to have consistently high microcystin concentrations (e.g. *Rastrineobola argentea*). However, this may not be economically feasible for many families, and the benefits of regular protein-intake may outweigh the risks posed by chronic exposure to microcystin. Finally, the development of risk assessment frameworks and guideline values for microcystin exposure should take into account the potential for microcystin exposure through fish consumption, which, based on my results, can be considerable.

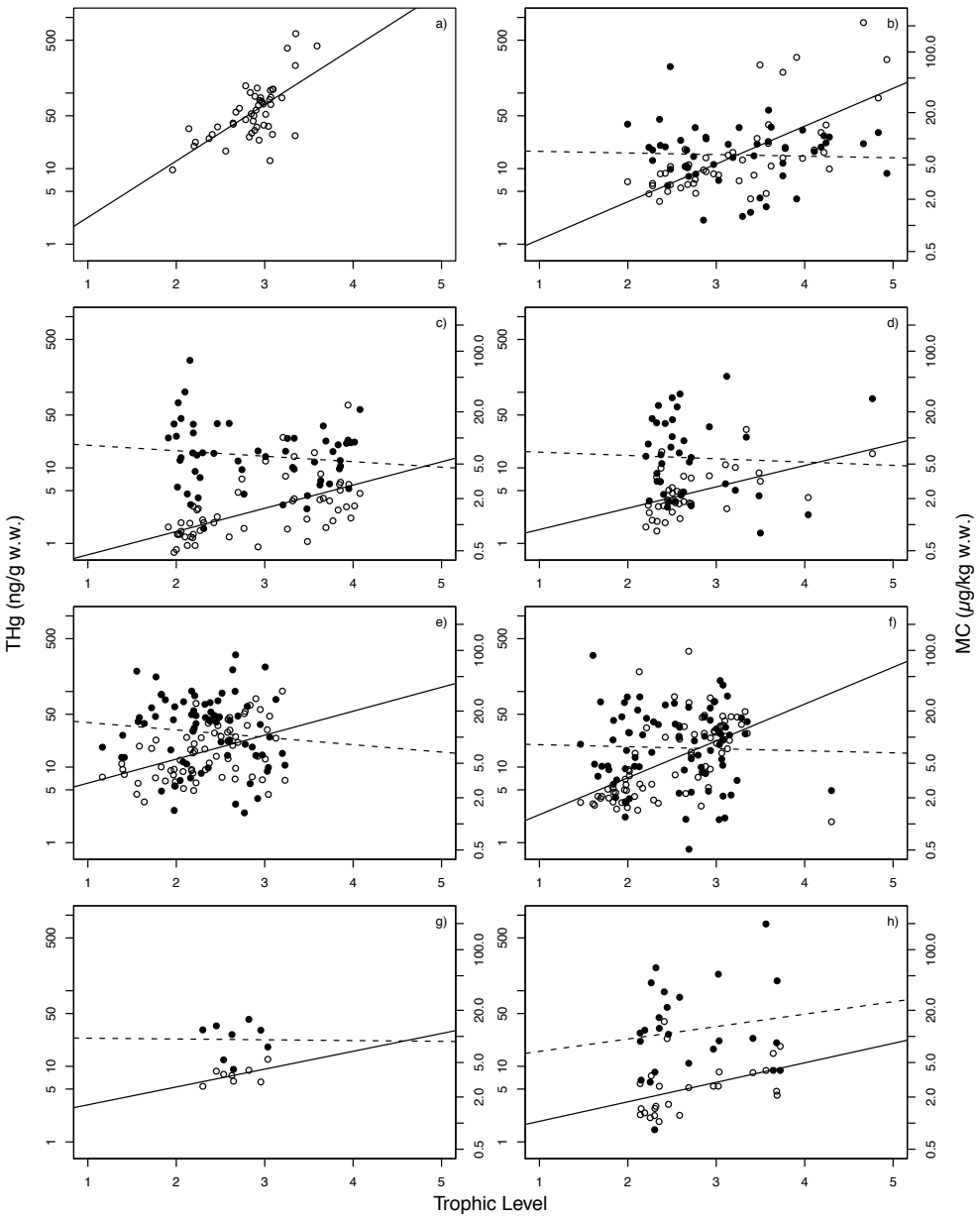

Figure 7.1 Regressions of log-transformed total mercury vs. calculated trophic level (open circles, solid regression lines) and log-transformed microcystin concentrations vs. calculated trophic level (solid black circles, dashed regression lines) for fish muscle samples from a) Lake Albert, b) Lake Edward, c) Lake George, d) Lake Mburo, e) Murchison Bay, f) Napoleon Gulf, g) Lake Saka and h) Lake Nkuruba. Data for this figure are taken from Chapters 4, 5 and 6. The fish shown are those for which data were available for THg, MC and $\delta^{15}N$.

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