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Assessing the potential health risk of cyanobacteria harmful algal blooms and cyanotoxins in Lake Naivasha, Kenya

Melissa H. Raffoul
The University of Western Ontario

Supervisor
Dr. Charles G. Trick
The University of Western Ontario

Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

ASSESSING THE POTENTIAL HEALTH RISK OF CYANOBACTERIA HARMFUL ALGAL BLOOMS AND CYANOTOXINS IN LAKE NAIVASHA, KENYA

(Spine title: Health risk of cyanoHABs in Lake Naivasha, Kenya)

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by

Melissa H. Raffoul

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THE UNIVERSITY OF WESTERN ONTARIO
SCHOOL OF GRADUATE AND POSTDOCTORAL STUDIES

CERTIFICATE OF EXAMINATION

Supervisor

_____
Dr. Charles G. Trick

Supervisory Committee

_____
Dr. Jack Bend

_____
Dr. Irena Creed

_____
Dr. Yolanda Morbey

Examiners

_____
Dr. Yolanda Morbey

_____
Dr. Denis Maxwell

_____
Dr. Graeme Taylor

The thesis by

Melissa Helen Raffoul

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Assessing the potential health risk of cyanobacteria harmful algal blooms and cyanotoxins in Lake Naivasha, Kenya

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Abstract

Cyanobacteria harmful algal blooms (cyanoHABs) pose a threat to water quality and human health. The Lake Naivasha, Kenya community expressed concern about these events which prompted this study of influences, characteristics and health impacts of cyanoHABs. While eutrophication of Lake Naivasha was caused by years of nutrient loading from agricultural activities, land use changes and improper sewage treatment, results suggest that lake-wide cyanoHAB formation was controlled by shifts from drought to flood conditions, followed by relative stability. Particulate concentrations of the liver toxin microcystin were less than provisional guidelines; however, our limited knowledge on long-term microcystin impacts is limited and future cyanoHABs could have higher levels. CyanoHABs may play a role in dissolved oxygen dynamics and in turn fish health, but the extent of this is unclear without further study. Better management practices of Lake Naivasha's ecosystem services will lead to enhanced health and well-being of the lake and community.

Keywords: cyanobacteria harmful algal blooms, cyanotoxins, microcystin, freshwater, ecosystem services, Lake Naivasha, Kenya

Muhtasari

Cyanobacteria harmful algal blooms (cyanoHABs) (cyanobakteria yenye madhara na inayonawiri zaidi) ni tisho kwa usafi na ubora wa maji na afya ya binadamu. Wakaazi wa Ziwa Naivasha walielezea wasi wasi yao kutokana na matukio ambayo yalichangia utafiti wa kiiini, tabia na adhari za afya za cyanoHABs. Ijapokuwa kumekuwa na mabadiliko kwa hali ya maji katika Ziwa Naivasha kuwa yenye madini mengi hali ambayo imechangiwa pakubwa na mbolea nyingi kuoshwa kutoka kwenye mashamba ya kukuza mimea na kuelekezwa kwenye ziwa, mabadiliko ya utumizi wa ardhi na pia ukosefu wa usafishaji bora wa maji taka, matokeo ya utafiti yana onyesha kwamba kusambaa kwa cyanoHABs kwa ziwa lote kwa ujumla ina tegemea zaidi mabadiliko kutoka kwa msimu wa kiangizi na kuingia kwa msimu wa mvua na mafuriko ikifuatiwa na wakati wa utulivu katika Ziwa. Viwango vya sumu ya maini ijulikanayo kama microcystin ndani ya miili ya viini vya cyanoHABs ilikuwa ndogo ikilinganishwa na viwango vinavyopendekezwa ili kuepuka madhara ya kiafya, lakini kuna upungufu wa maarifa kuhusu adhari ambazo zinaweza kutokea baada ya muda mrefu kutokana na viwango hivi vidogo vya microcystin na kuna uwezekano kwamba kiwango kidogo bado kinaweza kuadhiri afya. CyanoHABs zinaweza kuchangia kwa upungufu wa hewa (oksijeni) ambayo iko majini na inayotegemewa na samaki kwa kupumua na hivyo kuadhiri afya ya samaki, lakini kiwango cha madhara ambayo yanaweza kutokea hakijadhihirishwa na kwa hivyo, utafiti zaidi unahitajika. Usimamizi bora zaidi wa matumizi ya Ziwa Naivasha na huduma ambazo Ziwa hili linatekeleza ita sababisha uboreshaji wa afya na mazingira mazuri kwa ziwa na wakaazi wa eneo hili wanaotegemea ziwa hili kwa matumizi na maisha yao kwa jumla.

To Mom, Dad, Tommy and Matthew,

You may not have always understood the science,
but thank you for always supporting my passion.

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I was a lost soul during my undergraduate years at Western, but Dr. Trick showed me that it was possible to do good scientific research in a way that tells a story, has an impact, and can be used to enhance the well-being of human lives. It has been a tough road, but I am so grateful he stuck with me. I'd also like to thank my advisors Drs. Bend, Creed and Morbey for their valuable feedback and advice with my project. Thank you to those who assisted with sample collection throughout the study period: Ryan Sorichetti, Jace McLaughlin, Ruth Lewo, Rachel White, Chelsea Hicks, Vincent Kinyua and Sumedha Arya. Asante sana marafiki! The support of the Ministry of Fisheries in Naivasha for all our field work activities has been invaluable. To my boat driver, Stephen Muchai, who became more like my 'Kenyan Baba': You are an expert at steering clear of hippos, chasing poachers and maneuvering those rough waters of Lake Naivasha, and I am forever grateful for your support during these past two years. Mr. Eric Enanga has been the rock for this Lake Naivasha Sustainability Project. His dedication and ability to just get things done is awe inspiring and I thank him from the bottom of my heart for all his passion, support and advice. And finally, to all my wonderful colleagues and friends in the Trick and Creed Labs: You are all superstars. And remember, the 'toilet phone' may be gone, but will never be forgotten.

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"*I am I plus my surroundings and if I do not preserve the latter I do not preserve myself*."

Jose Ortega y Gasset, Meditations on Quixote, 1914

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Acronyms and Abbreviations

chl-*a*	chlorophyll-*a*
chl-*b*	chlorophyll-*b*
chl-*c*	chlorophyll-*c*
C.I.	Crescent Island
cyanoHAB	Cyanobacteria Harmful Algal Bloom
DO	Dissolved Oxygen
Ecohealth	Ecosystem Health
ELISA	Enzyme Linked ImmunoSorbent Assay
IDRC	International Development Research Council
IPAT	Impact = Population x Affluence x Technology
LNRA	Lake Naivasha Riparian Association
LANAWRUA	Lake Naivasha Water Resource Users Association
MAC	Maximum Acceptable Concentration
MC-LR	Microcystin-LR
M.L.	Main Lake
PC	Phycocyanin
PE	Phycoerythrin
PVC	Polyvinyl chloride
TDI	Tolerable Daily Intake
WHO	World Health Organization

1. INTRODUCTION

1.1. Cyanobacteria blooms

Cyanobacteria, previously known as blue-green algae, are microscopic photosynthetic prokaryotes known for their ability to survive in a wide range of terrestrial and aquatic habitats. Under ideal conditions and low grazing pressure, they can proliferate rapidly enough to accumulate and form visible scums known as cyanobacterial blooms in freshwater environments (Reynolds and Walsby, 1975). Compared to blooms of other eukaryotic algae (chlorophytes, diatoms, dinoflagellates), cyanobacteria blooms are generally the most common and problematic in freshwater systems (Paerl et al., 2001).

1.1.1. Characteristics of cyanobacteria

As prokaryotes, cyanobacteria are structurally similar to bacteria in that they lack a nucleus and organelles, yet they can also photosynthesize similar to eukaryotic algae (Paerl et al., 2001). They exist in two basic forms: 1) coccoid cells that may be individual and free floating or aggregated as colony (example: *Microcystis*, *Merismopedia*) or 2) filamentous forms that may (*Anabaena*, *Aphanizomenon*) or may not (*Spirulina, Planktothrix*) contain heterocysts, which are specialized cells that fix nitrogen.

Cyanobacteria produce chlorophyll-*a* (chl-*a*) as their major light harvesting pigment but also contain specific accessory pigments called phycobiliproteins that allow them to harvest light at lower wavelengths—which other algae are not capable of doing—giving them a high photosynthetic efficiency (Gantt, 1975). These pigments are known as phycocyanin (PC) and phycoerythrin (PE) and are responsible for giving cyanobacteria their blue-green or red colours, respectively. Measurements of these algal pigments can be used as indicators of overall and group specific biomass (Schagerl, 2007): chl-*a* represents overall biomass, PC and PE will infer cyanobacteria biomass and the accessory chlorophylls—chlorophyll-*b* (chl-*b*) and chlorophyll-*c* (chl-*c*)—represent the biomass of chlorophytes (green algae) and diatoms/dinoflagellates, respectively.

A special feature of cyanobacteria is the presence of gas vacuoles that allow them to regulate their buoyancy in the water column to optimize conditions of light and nutrients (Oliver and Ganf, 2000). This gives cyanobacteria a particular advantage when the environment is stable, as competing groups of algae (green algae and diatoms) are

unable to control their movements in the water column. Furthermore, turbulence or mixing can re-cycle nutrients and redistribute all algae giving cyanobacteria less of an advantage. Additionally, changes in pressure due to turbulence can disrupt gas vesicles causing them to collapse (Oliver and Ganf, 2000).

Some of the filamentous genera are able to fix nitrogen from the atmosphere to use when nitrogen is limited in the water (Paerl et al., 2001). In addition, cyanobacteria have efficient phosphorus uptake mechanisms that enable them to obtain and store phosphorus when it is available and then utilize it when the nutrient becomes limited in the environment (Oliver and Ganf, 2000). Iron requirements are higher in cyanobacteria compared to eukaryotic algae because it is needed in greater amounts for photosynthesis and it also plays a role in nitrogen fixation (Morton and Lee, 1974; Gress et al., 2004). In order to obtain iron for these needs cyanobacteria can produce iron chelators known as siderophores that can bind and transport iron to cyanobacteria cells.

1.1.2. Factors influencing cyanobacterial bloom occurrences

Specific factors that may play a role in cyanobacterial bloom occurrences include physical parameters (temperature, light, turbulence, mixing, and stability), biological interactions (grazing), and chemical characteristics (macronutrients (N and P), micronutrients (Iron)) (Paerl et al., 2001). In a natural environment it is rarely just one of these factors alone that will lead to bloom production but rather a combination of some or all of them.

The light harvesting features and buoyancy characteristics described above can give cyanobacteria an advantage to bloom over other algae in stable and turbid environments. Additionally, cyanobacteria can proliferate at higher temperatures than diatoms and green algae (Paerl and Paul, 2012), so cyanobacterial blooms tend to occur when water temperatures are greater. Cyanobacteria are also grazed less than eukaryotic algae for reasons relating to shape, indigestibility, and chemical production and therefore are less impacted by top-down controls (Paerl et al., 2001).

In general, cyanobacteria commonly proliferate in nutrient rich or eutrophic systems which are able to support excessive algal growth. Generally, when chl-*a* concentrations are consistently greater than 10 μg/L, a lake is considered eutrophic (OECD, 1982). Through anthropogenic or human-induced changes, more lake

ecosystems have become eutrophic and there has been an increase in cyanobacterial bloom occurrences in recent decades (Smith, 2003). Within these eutrophic systems however, there are a number of possible ways that nutrient regimes can lead to a bloom. Firstly, if a nutrient is limited (but most other conditions, such as sufficient light and temperature are met), cyanobacteria may be able to utilize one of the nutrient acquisition mechanisms it has to gain an advantage and bloom. However, different genera and/or species of cyanobacteria are capable of those adaptations at different magnitudes. For example, *Anabaena* has the ability to fix nitrogen, whereas *Microcystis* does not. However, *Microcystis* is better known for its phosphorus uptake mechanisms, as well as its buoyancy capabilities, so if nitrogen is not limiting it may be more likely to have the advantage and bloom.

It has also been suggested that low nitrogen to phosphorus ratios may promote cyanobacteria dominance and bloom formation over other eukaryotic algae (Paerl et al., 2001). That being said, for this to be a factor one of the nutrients must be limiting. In highly eutrophic systems there may just be more than enough of all the essential nutrients (saturation) and when this occurs, ratios and actual comparisons of nutrient concentrations must be interpreted properly. If all nutrients are present in high amounts, then the other influences such as grazing, turbidity, light and temperature can still play a key role in the proliferation of cyanobacteria.

1.1.3. Temperate versus tropical ecosystems

Tropical lakes only make up about 10% of lake ecosystems (Lewis, 1996) which is why most literature on cyanobacterial blooms tends to discuss attributes of their occurrences in temperate systems. However the dynamics are very different between these two types of ecosystems, and therefore generalizations and assumptions must be made cautiously when applying temperate climate studies to a tropical climate (Lewis, 2000). Temperate ecosystems experience seasonal changes in light and temperature, which tend to impact their mixing and limit the periods where phytoplankton and cyanobacteria can become dominant. In tropical ecosystems light regimes are more consistent year long, thereby allowing more energy to be focused on the other essential needs for algal production such as nutrients; this feature is why tropical lakes tend to be overall more productive than temperate lakes (Lewis, 1996). Seasonality in tropical

ecosystems is more defined by rainy and dry seasons (Melack, 1979a), which tend to also impact mixing and nutrient input. However, in general, community composition (at least to the genus level) has been found to be quite similar in temperate and tropical ecosystems as many genera have been found to inhabit a number of lakes in both the temperate and tropical zones (Lewis, 1996).

1.1.4. Shallow versus deep lakes

Nutrient availability, turbidity, phytoplankton communities and cyanobacterial bloom occurrences are also impacted by lake morphometry. There has been much discussion as to what defines a shallow lake, but it is more or less accepted that a shallow lake is approximately less than 5 m deep and lacks consistent stratification (Pasisak and Reynolds, 2003). A key characteristic of deep lakes is that they can stratify and often remain that way for long period of time depending on seasonality (Scheffer, 1998). This water column stability can be advantageous for cyanobacteria especially if other growth requirements are met. Shallow lakes tend to mix frequently due to wave action and this mixing creates substantial sediment-water interactions and continuously resuspends and redistributes nutrients, phytoplankton and particles throughout the water column (Scheffer, 1998). This physical disruption of the sediments by wave action can be further aided by benthic fish, or chemical reactions (such as dissolved oxygen depletion).

1.2. Adverse effects of cyanobacterial blooms

There are a number of adverse effects associated with cyanobacterial blooms including the production of nuisance taste and odour compounds, and the formation of a thick scum on the surface of the water that can prevent light penetration and therefore inhibit plant growth. When an algal bloom decomposes it can cause major changes in dissolved oxygen (DO). In particular, cyanobacterial blooms can produce harmful cyanotoxins in high amounts.

1.2.1. What makes a cyanobacterial bloom harmful?

The term cyanobacteria Harmful Algal Bloom (cyanoHAB) is used very loosely in literature. The ‘harmful” label often comes in reference to the adverse effects that blooms have on humans, animals, plants and the environment (Paerl et al., 2001; Backer, 2002). Based on that general definition, all blooms could then be considered harmful in

some way. However, sometimes the term cyanoHAB will only be used in reference to a cyanobacterial bloom that is associated with the production of cyanotoxins. For the purpose of this thesis, the same logic will be used: a cyanobacterial bloom is considered a cyanoHAB if cyanotoxins are also detected within the bloom.

1.2.2. Cyanotoxins

Cyanotoxins are naturally occurring low molecular weight compounds and in freshwater they fall into three main groups: 1) hepatotoxic cyclic peptides (microcystins and nodularins); 2) alkaloids, both neurotoxic (anatoxin-a, saxitoxin, β-Methylamino-L-alanine) and cytotoxic/hepatotoxic (cylindrospermopsin); and 3) irritant lipopolysaccharides (Sivonen and Jones 1999; Carmichael 2001).

The most widespread and intensely studied of these toxins is microcystin which is a toxin that targets the liver (Sivonen and Jones 1999; Carmichael 2001). Different cyanobacteria genera have been found to produce microcystin (*Microcystis, Anabaena, Oscillatoria, Nostoc*), but not all species or strains within these genera are toxic (Neilan et al., 1997; Codd et al., 1999**;** Davis et al., 2009; Rinta-Kanto et al., 2009).

More than 80 variants of microcystin have been discovered, each with a different protein L-amino acid combination (highlighted in blue; Figure 1.1). The most toxic microcystins are the ones with more hydrophobic L-amino acids, such as Microcystin-LR (Leucine and Arginine), and Microcystin-YR (Tyrosine and Arginine) (Falconer, 2005). Another feature of microcystin is what is known as the "ADDA" group (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid) (Figure 1.1), which also plays a key role in toxicity as it binds the protein phosphatases in the liver (Falconer, 2005). This ADDA group is also what is targeted for many of the methods to positively identify and quantify for the presence of microcystin.

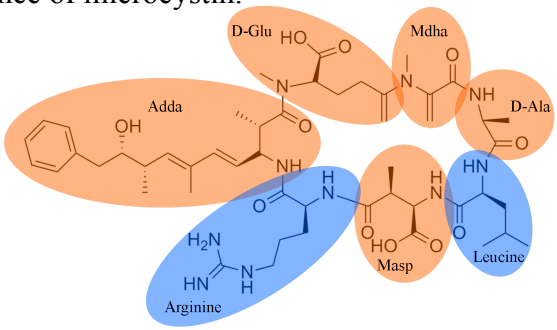

Figure 1.1. Chemical structure of microcystin-LR. The amino acids highlighted in blue are what differ amoung the different variants of microcystin.

The controls and regulations of microcystin production are still not completely understood. It is generally thought that for a single strain of toxin-producing cyanobacteria, microcystin content is directly related to growth (cell divisions) (Orr and Jones, 1998). Other studies have furthered this concept by showing that in uni-algal cultures, where macronutrients (nitrogen and phosphorus) are not limiting growth, high rates of microcystin production were observed (Sivonen et al., 1990; Vezie et al 2002). The regular production of microcystin under these stress-free conditions, in addition to the fact that it is produced from the start of log phase, suggests that microcystin is an essential intracellular compound (Orr and Jones, 1998; Kaplan et al., 2012); however the essential role that it plays is also not completely understood. Rohrlack and Hyenstrand (2007) found that microcystin was not exported from the cell nor broken down within the cell, suggesting that it is not produced for extracellular defense mechanism or metabolic cellular processes.

The key to identifying the essential role for microcystin may come from studying the variety of environmental factors that have been found to impact the regular production of microcystin. Commonly when a given factor acts to limit growth, a response in greater microcystin production is seen. These factors vary widely and include low iron concentrations, high iron concentrations, low nitrogen to phosphorus ratio, light, temperature, and pH (Kaplan et al., 2012). One difficulty when understanding the roles and regulations of microcystin is that relationships often seen in laboratory, strain specific experiments are hard to elucidate in natural blooms because toxic and non-toxic strains are both generally present and there are usually multiple changes occurring in the environment at a given time.

This research has been considerably advanced in the last decade due to determination of the *mcy* gene clusters responsible for microcystin biosynthesis which is common in the toxin-producing strains of a genus (Nishizawa et al., 1999, 2000; Tillett et al., 2000). With this knowledge, phylogenetic studies determined that these genes were ancient and common to the last ancestor of cyanobacteria (Rantala et al., 2004) and that gene deletions have led to the non-toxic strains of cyanobacteria (Schatz et al., 2005). These findings also indicated that microcystin's main role was not as a feeding deterrent to eukaryotic grazers because the production of microcystin in ancient cyanobacteria was

long before metazoan ancestry (Rantala et al., 2004). This was further shown when *Daphnia* could not differentiate or select between wild-type (for microcystin production) and mutant strains when feeding (Rohrlack et al., 1999).

Early studies identifying iron limitation as a condition for increased microcystins (Lukac and Aegerter, 1993) led to the suggestion that the toxin may act as an intracellular iron chelator (Utkilen and Gjolme, 1995; Humble et al., 1997), storing iron and allowing cells to live under iron-stressed conditions. Support of the iron chelation and storage hypothesis has been mixed (Saito et al., 2008; Fujii et al., 2011, Alexova et al., 2011) and there has yet to be identification of actual microcystin storage molecules in cells. Nevertheless, there seems to be more agreement that general iron limitation does increase toxin production (Sevilla et al., 2008; Alexova et al., 2011; Kaplan et al., 2012). The working hypothesis for this is that ferric uptake regulator (Fur) represses transcription of the *mcy* genes when iron is replete and allows transcription under iron-stressed conditions (Martin-Luna et al., 2006; Alexova et al., 2011; Kaplan et al., 2012). Furthermore, iron alone may not be the sole control, as nitrogen limitation (and global nitrogen transcription regulator NTcA) (Kaebernick et al., 2002; Ginn et al., 2010) and high light irradiance (Kabernick et al., 2000) might also impact microcystin production. In fact all three of these factors may play a cooperative role in allowing transcription of *mcy* genes due to redox states (Alexova et al., 2011; Kaplan et al., 2012). These types of controls could be factors in both initiation and maintenance of a cyanobacterial bloom.

Separate to the above hypothesis, there is also some evidence that microcystin may play a part in actual colony formation, and therefore potentially help to initiate a bloom event. This idea was supported by studies that observed mutant *Microcystis* strains deficient in microcystin that had changes in the extracellular proteins involved in cell-to-cell contact (Kehr et al., 2006; Zilliges et al., 2008). Finally, increased microcystin production may also occur as a response to extracellular microcystin presence (Schatz et al., 2007). Results from the study led to the suggestion that when cells that are lysed due to any type of stress, they would release microcystin (into the media) which might act as an info-chemical signaling increased cell death to the live cells and stimulating microcystin production in them (Schatz et al., 2007).

1.3. Ecosystem health and cyanoHABs

1.3.1. Principles of ecosystem health

Ecosystem Health (often shortened to ecohealth) is centered on the concept that one's interactions with their surrounding ecosystem can impact their overall health and well-being (Charron, 2012). We often regard the term 'environment' only in reference to the surroundings (of an individual or community). An 'ecosystem' on the other hand, considers the interactions and dynamics within the environment and therefore must incorporate the central role that humans play in those relationships. (Forget and Lebel, 2001; Charron, 2012). Our understanding of the term 'health' has also evolved; it is no longer just the absence of disease but rather is looked at as physical, mental and social well-being (WHO, 1948). Taking this into account within a given ecosystem there can be many categories of health determinants—physical, ecological, social, economic, and cultural, to name a few.

On a global scale key issues in ecohealth research include climate change, urbanization, food insecurity, and deforestation (Charron, 2012). In any local community an unhealthy ecosystem could be defined by many specific and different things such as poor sanitation, inadequate water access, and unemployment. What is common on both a global and local scale is that there is this dependence on the services (water, food, wood etc.) that the environment can provide to humans that in turn impact their quality of life. These are collectively referred to as ecosystem or ecological services (Forget and Lebel, 2001; WHO, 2005). These services can be further divided into 1) provisioning services (products obtained from ecosystems such as water, food, wood); 2) Regulating services (benefits obtained from regulation of ecosystem process, including water regulation, and pollination); 3) cultural services (nonmaterial benefits obtained from ecosystems such as aesthetics, recreation, ecotourism); and 4) supporting services (services needed for the production of all other ecosystem services including nutrient cycling and soil formation) (WHO, 2005).

Given all of these different determinants, services and ecosystem relations, it is obvious that any study with an ecohealth approach will not be simple. But when faced with a specific problem (whether it originates as a health concern or an environmental concern, or both) and a new community, you have to start somewhere to build your

understanding of the complex relationships. One tool that is often used as a first step to examining ecosystem impact is known casually as IPAT and is formally defined as: Environmental Impact= Population x Affluence x Technology (Chertow, 2001). The IPAT equation was first introduced by Elrich and Holdren in 1971 in order to demonstrate how much population was contributing to environmental degradation; but since then it has taken on many different variants and been used to address how technology can balance out the detrimental impacts of population and affluence (Chertow, 2001). On a simpler level, when faced with a specific ecosystem health issue, examining the three factors of Population, Affluence (the level of consumption of each person in the population) and Technology (how resource intensive the production of affluence is) can reveal some of the key issues that must be considered and lead you to the most relevant questions to investigate within the complex web of interactions. The majority of ecosystem issues will somehow be rooted in this IPAT model.

The framework of ecosystem health research used in this study and promoted by the International Development Research Centre (IDRC) (Charron, 2012) involved six key components that have been developed in the literature for a number of years (see Kay and Schneider, 1994; Rapport, 1998; Waltner-Toews et al., 2003; Lebel, 2003; Waltner Toews and Kay, 2005).

1) Systems thinking is the process of understanding all the different links and interactions that may exist when first beginning an ecohealth study, and then using that same approach for each specific question asked or issue studied. The IPAT model provides a good base for systems thinking.
2) In order to have effective systems thinking you need to approach the research in a transdisciplinary manner. Multidisciplinary approaches involve members from different disciplines working independently on different aspects of a problem/project with individual goals and separate methodologies (Choi and Pak, 2006). Interdisciplinary approaches are more interactive and have members from different disciplines working together towards a shared goal. The boundaries between disciplines are blurred, participants learn about and from each other and use common methodologies (Choi and Pak, 2006). Transdisciplinary approaches work across and beyond disciplines and in addition to scientists, involve stakeholders and non-

academic participants who work together using a shared conceptual framework. There is greater integration and collaboration in which the disciplinary boundaries are transcended (Choi and Pak, 2006).

3) One of the most important aspects of systems thinking and transdisciplinary research is the participation of the community and stakeholders. The community has to recognize that there is a problem and want to work together to address it, and the research is then based on community driven objectives. Understanding what the community identifies as important to them will then inform the different nodes of system thinking and define how the transdisciplinary team creates their conceptual framework.
4) Ecohealth research aims to achieve sustainability whether that is socially or ecologically. An ideal outcome will involve ethical, positive and lasting changes that are environmentally sound, socially sustainable and culturally appropriate. These changes must be owned and implemented by the community to ensure lasting impacts beyond the years of a research project/funding.
5) Throughout all aspects of an ecohealth project there must be gender and social equity. Different gender, age, economic and social groups will have different exposures to health risks and also various concerns about their well-being; these must be taken into account in order to fully research and represent a community.
6) Utilizing the knowledge gained from research to promote better health and well-being is essential in the ecohealth approach. This knowledge to action is an ongoing cycle in which changes made will then introduce new knowledge that then promotes new or improved changes.

1.3.2. Freshwater ecosystems, cyanoHABS and human health

Freshwater ecosystems are a major component to human health (Figure 1.2) and essential to consider within the ecosystem health approach. When all other factors and relationships are considered, it really comes down to a matter of water quantity and quality, as highlighted in Figure 1.2. Globally, the issue of water scarcity is an important one, and water quality issues such as cyanoHABs further limit the available water resources.

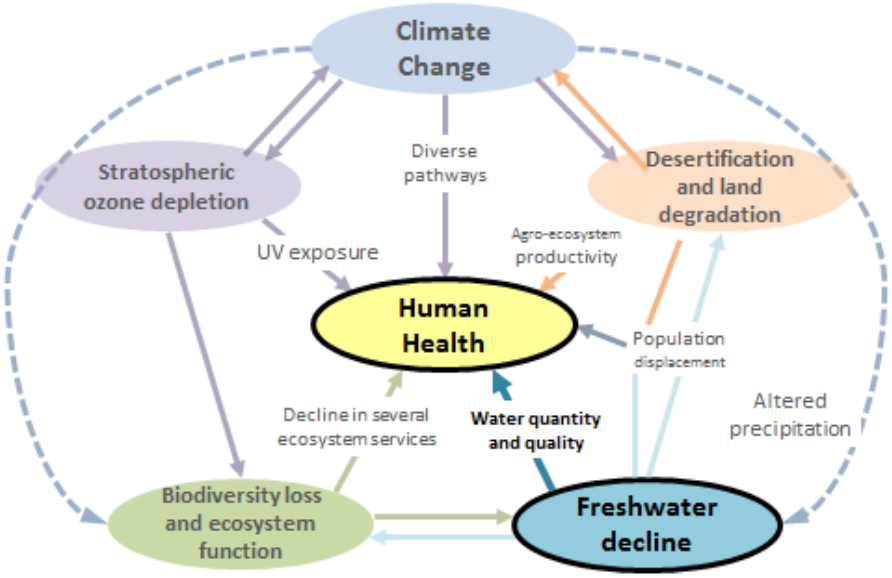

Figure 1.2. The links between environmental change and human health.
Adapted from: World Health Organization Climate Change and Human Health Program
http://www.who.int/globalchange/ecosystems/en/

The leading ecohealth concern with regards to cyanoHABs is their potential to produce harmful toxins, such as microcystin, which can be present in drinking water sources. Both the Environmental Protection Agency (EPA) and the World Health Organization (WHO) have recognized this and published thorough reviews on the topic (see Chorus and Bartram 1999; Hudnell 2008).

Microcystin is chemically stable and water soluble, which is why it poses such a large threat in freshwater ecosystems. As mentioned previously, microcystin targets the liver and specifically inhibits essential protein phosphatases (Falconer, 2005). Acute exposure to high concentrations of microcystin can therefore lead to gastrointestinal illness, liver hemorrhage, or possibly death (Carmichael, 2001; Backer, 2002). It has also been suggested that microcystin can result in long term health effects through chronic, low level exposure to the toxin, which may be of greater concern than acute exposure (Sivonen and Jones, 1999; Hitzfeld et al., 2000; Carmichael, 2001). Specifically, microcystin may act as a potential tumor promoter and while more laboratory, epidemiological and monitoring data are needed, microcystin has so far been linked to liver cancer (Ueno et al., 1996) and colorectal cancer (Zhou et al., 2002) in China.

Teratogenic impacts of microcystin have not yet been conclusively determined (Falconer, 2005).

During a cyanoHAB event, microcystin remains within cells (intracellular microcystin) and is only released when cells are lysed due to natural cell death and senescence or through chemical algaecide treatment. These free or dissolved microcystins may then be in very high concentrations if a large biomass of bloom was decomposed all at once compared to when cells were intact.

It is often hard to pinpoint a cyanoHAB and toxin exposure as a direct cause of illness because often the exposure and side effects are not seen until after a bloom has dissipated. Side effects of microcystin poisoning are similar to many other gastro-intestinal illnesses and, only once other potential origins (i.e. virus, pathogens, bacteria) are ruled out, does the focus change to whether there is evidence of a cyanoHAB as the cause of illness (Sivonen and Jones, 1999). Epidemiology is therefore very important in the study of cyanoHAB impacts, as physical and quantitative evidence is not always available (Baker, 2002). Currently there are quite a few incidents on record (with varying certainty) of human illness or death due to cyanoHABs and microcystin all over the globe (see Sivonen and Jones, 1999; Carmichael, 2001; Baker 2002). The most devastating occurred in 1996 and was due to contaminated water used intravenously on patients at a dialysis clinic in Brazil, resulting in 75 deaths (Carmichael, 2001).

Free microcystins can persist in water bodies anywhere from days to weeks after cell lysis because the toxins are very stable. They can be naturally deactivated and degraded by bacteria or by natural high UV exposure, which can also be aided by the phycobiliprotein pigments (Sivonen and Jones, 1999). Normal municipal water treatment processes that are shown to be effective in the removal of microcystins include filtration with granulated activated carbon and powdered activated carbon as well as specific chlorine and ozone oxidizing treatments (Hitzfield et al., 2000; Falconer, 2005). Coagulation or cell filtration can also remove intact toxic cells (Carmichael, 2001).

While the greatest potential for exposure to microcystin is from drinking water supplies, other contact to the toxin could still occur by inhalation of airborne particles through nasal tissues and mucous membranes (Codd et al., 1999; WHO, 2003; Cheng at

al., 2007), or with consumption of fish that have accumulated toxin through food web interactions (Sivonen and Jones, 1999).

The WHO has a provisional guideline value for microcystins in drinking water based on an oral 13-week study with mice and a 44-week study with pigs. The Tolerable Daily Intake (TDI) (which took into consideration the uncertainty and lack of data in some areas) is expressed as 0.04 μg/kg per day. The provisional guideline for maximum acceptable concentration (MAC) was then valued to be 1 μg/L of total microcystin-LR (intracellular plus dissolved) based on an allocation to water of 80%, and an adult of 60 kg consuming 2 L/d (WHO 2003). Other countries have established their own MACs including Canada (1.5 μg/L, based upon a 60 kg person consuming 1.5 L/day), Australia (1.3 μg/L) and New Zealand (1.0 μg/L and also 0.1 μg/L for tumor promotion factor) (Carmichael 2001). Obviously, the guidelines must be interpreted cautiously, as the TDI for a child would result in an MAC lower than 1.0 μg/L.

Another interesting aspect considering cyanobacterial blooms and cyanoHABs from an ecohealth perspective is that they are a global issue, occurring in every continent (Fristachi and Sinclair, 2008), in both developed and developing nations. The major difference, however, is how water is accessed and how cyanoHABs are addressed and monitored. In developed nations, piped water is generally transported to a home after a rigorous water treatment process. When a cyanoHAB occurs toxins are routinely monitored to assess the risk and there will be warning signs at public places if the risk is considered high. In less developed nations or communities where water treatment facilities are not present, people will often obtain water directly from a lake or reservoir and routine monitoring schemes are often not in place to assess the risk. This is the case in Lake Naivasha, Kenya, in which reports of cyanobacterial blooms became common in 2005 (Harper et al., 2011).

1.4. Lake Naivasha, Kenya

1.4.1. Rift Valley lakes of Kenya

The Great Rift Valley of Kenya is home to seven lakes including the largest alkaline lake in the world (Lake Turkana) and a lake with a 40 cm layer of trona which is the second largest supplier of sodium bicarbonate in the world (Lake Magadi). Of the 5

lakes in between that are a part of the Central Rift Valley of Kenya, three of them are saline lakes that are known for their flamingo populations who feed on the cyanobacteria *Spirulina* (Lakes Elmenteita, Nakuru and Bogoria). Lake Baringo, which is a freshwater lake (although not as fresh as Lake Naivasha), has been dominated by cyanobacteria blooms since at least 1929 (Rich, 1932) and while it still supports blooms of *Microcystis* the extreme turbidity makes the lake productivity much lower than expected (Odada et al., 2005). The land surrounding Lake Baringo is under government ownership allowing indigenous communities to live on and use the land (Harper et al., 2011). This is not the case in Lake Naivasha, which is the largest freshwater lake in the Kenyan Rift Valley, and second largest in Kenya after Lake Victoria. As such, it is an important freshwater resource in Kenya and many rely on its ecosystem services.

1.4.2. Lake Naivasha characteristics

Lake Naivasha is located about 80 km northwest of Nairobi and lies about 1890 metres above sea level making it the highest elevated of the Kenyan Rift Valley Lakes and because of this its climate is sometimes not regarded as truly tropical (Beadle, 1932). The surface area of Naivasha ranges from 100-150 km^2 and there is one major river, the Malewa River, which contributes the majority of inflow. There are two seasonal rivers—the Gilgil and Karati—that account for the other 10%. The total catchment area of Naivasha is approximately 3376 km^2 (Otiang'a-Owiti and Oswe, 2007). There is no surface outlet in Naivasha despite its freshwater status, so it has been hypothesized that a subterranean outlet keeps the lake fresh (Beadle, 1932).

Rainfall in the basin is typically on a bimodal cycle that roughly experiences short rains from October to December, a hot and dry period from December to February, long rains from March to May and finally a relatively cool and calm period from June to September (Beadle 1932). These patterns can be highly variable, especially in more recent years. Lake Naivasha has always experienced large lake level fluctuations due to these natural cycles of inflow and evaporation. For example from July 1929 to November 1930, the lake level increased about 1.5 m (Jenkin 1932; Worthington 1932).

There are 3 separate basins to Lake Naivasha: Lake Oloidien, Main Lake basin and Crescent Island basin. In the southwest corner is Lake Oloidien which is a volcanic crater once attached to the Main Lake basin but has been separated since 1982 (Harper et

al., 2011). Lake Oloidien's water chemistry and biology had always been different from the Main Lake basin even when they were attached (Beadle, 1932), but it now has changed to a more alkaline state and supports a *Spirulina* population and many lesser flamingos (*Phoenicopterus minor*). The Main Lake basin is circular and shallow (maximum depth ranges from 3 m to 6 m) and is known for its rough waters and strong winds. Finally in the northeast part of the lake is another volcanic crater known as the Crescent Island basin, named for the crescent-shaped land mass that used to be an actual island when water levels were higher. Although now connected to the mainland on one shore, the 'Crescent Island' forms the barrier around the basin and when water levels are extremely low it extends enough to completely cut this Crescent Island basin off from the Main Lake basin. The Crescent Island basin is deeper (approximately 12 m) compared to the Main Lake basin. Because it is more isolated and sheltered it does not experience the same intensity of wind and turbulence as the Main Lake. However, in both the Main Lake basin and Crescent Island basin evidence of daily mixing has been observed due to a consistent thermal profile and well oxygenated water at the surface and sediment (Melack 1979b). This is likely due to any wind action as well as substantial diel nocturnal cooling (Melack 1979b).

1.4.3. Human-induced changes in Lake Naivasha

Beginning in the late 1970's and early 1980's the farming industry, which previously was predominately agriculture, started to transition to horticulture due to the ideal weather and sunlight conditions, fertile, volcanic soil availability and affordable labour (Everard and Harper, 2002). It quickly thrived, and the flower farm industry has become a top exporter for Kenya. In Naivasha, this industry success led to rapid development in the catchment and a population of approximately 20,000 in the 1970's has grown to more than 400,000 today (Harper et al., 2011).

The increased industry and population have led to numerous land use changes. Many of the agriculture and floriculture farms are situated along the shoreline of the lake; therefore areas that used to consist of natural, undisturbed vegetation were destroyed. In particular, the fringe of *Cyperus papyrus* (commonly known as papyrus) was reduced by 70% from the late 1960's to 1995 (Boar and Harper, 1999), and now is only 10% of what it once was (Morrison and Harper, 2009). This decline came about because of the

physical destruction of the plant for more farms and built-up land, but also from the lake level declines that coincided with the start of horticulture. When the water levels decline they dry out the soil that the papyrus is rooted in and between that and wildlife disruption, it cannot regenerate (Morrison and Harper, 2009). The papyrus around Lake Naivasha acts as filter to any contaminants or nutrients entering the system (Gaudet, 1977; Boar and Harper, 2002). The noticeable reduction of papyrus in what is known as the 'North Swamp' of the lake is also important to note as that is the position where the Malewa inflows into the lake. In addition to the farming occurring directly around the Lake, it also increased the upper catchment specifically along the path of the Malewa and so everything entering the river from the upper catchment makes its way into the lake.

The population increase led to more urban development as formal and informal settlements. The informal settlements lack proper structure and sewage treatment, while the formal Naivasha Town, which supports the bulk of the population, generally has proper building infrastructure. However, the sewage treatment plant responsible for the waste from town has not been functioning for a number of years, and was only designed to support the former population of 20,000 that the town once was.

The pressures of the industry and population on the lake are far reaching, and the demand for ecosystem services in the basin is profound. While the natural fluctuations in Naivasha's lake levels were always recognized, since about the 1980's when the horticulture industry started expanding, it was found that the decline in water levels was no longer due to solely natural causes (Becht and Harper, 2002). All these various detriments led to Naivasha being named a RAMSAR site in 1995 by the Kenyan Government, which makes it a wetland of international importance (Harper et al., 2011).

1.4.4. Eutrophication of Lake Naivasha

According to chl-*a* concentrations, Lake Naivasha has been considered eutrophic since at least the 1970's, however since the 1980's eutrophication has increased as evidenced by the increase in peak chl-*a* since that time (Figure 1.3). The anthropogenic influences in the catchment have increased fertilizer and pesticide use, decreased the vegetation buffer, and therefore more nutrients were brought into the lake through surface run-off and the river inflows. The sediments of Lake Naivasha have always been rich in iron (Harper, 1993), and Kitaka et al. (2002) found that they formed a sink for

phosphorus as well. So soluble phosphorus and iron may not always be readily available, however physical or chemical sediment disruption could release them into the water column. Hubble and Harper (2002a) suggest that a major deoxygenation event could release those nutrients into the water and potentially double productivity.

1.4.5. Historical record of phytoplankton dynamics in Lake Naivasha

The first studies on the phytoplankton community in Lake Naivasha (and other East African Lakes) occurred with two expeditions undertaken in 1929 (Jenkin 1932; Rich 1932) and in 1930 (Beadle 1932; Worthington 1932). The physical characteristics of the lake were very different during this time: the Malewa river clearly fed into the North Swamp, Lake Oloidien was still attached to the Main Lake basin (although still had a very different water and plankton composition), and Crescent Island was an actual island was much more integrated with the Main Lake basin. During both these expeditions a mix of diverse phytoplankton was found in Lake Naivasha, but densities were not high, especially in comparison to the alkaline lakes and Lake Baringo, which were reported to have cyanobacteria 'water blooms' but overall not much species diversity. The community was generally the same between the Main Lake basin and the Crescent Island basin (Beadle 1932). Diatoms were the most dominant in Naivasha, and in particular the genus *Melosira* (which has since been re-classified as *Aulacoseira*) was abundant at times. Cyanobacteria were not dominant, and at certain areas and times not observed at all: but some of species that were identified were *Microcystis flos-aqua, Aphanocapsa* sp. and *Merismopedia* sp. (Beadle 1932; Rich 1932). A 1960 study of seven lakes in Central East Africa found very few algal species in Naivasha and only identified the diatoms *Melosira nyassensis* (*Aulacoseira*) and *Gomphonema lanceolatum* (Evans, 1962).

In more recent studies from the 1970's to the present, chl-*a* values were recorded in the lake along with some of the community composition. These historical chl-*a* values were compiled into Figure 1.3, and seem to show an increasing trend. What we need to keep in mind however, is that most of these studies just represent a snapshot, rather than continuous changes. These studies also have each used varied site locations and methodologies, and represent both grey and white literature sources. Some also included comprehensive species lists (Kalff and Watson, 1986; Hubble and Harper, 2002b; Ballot

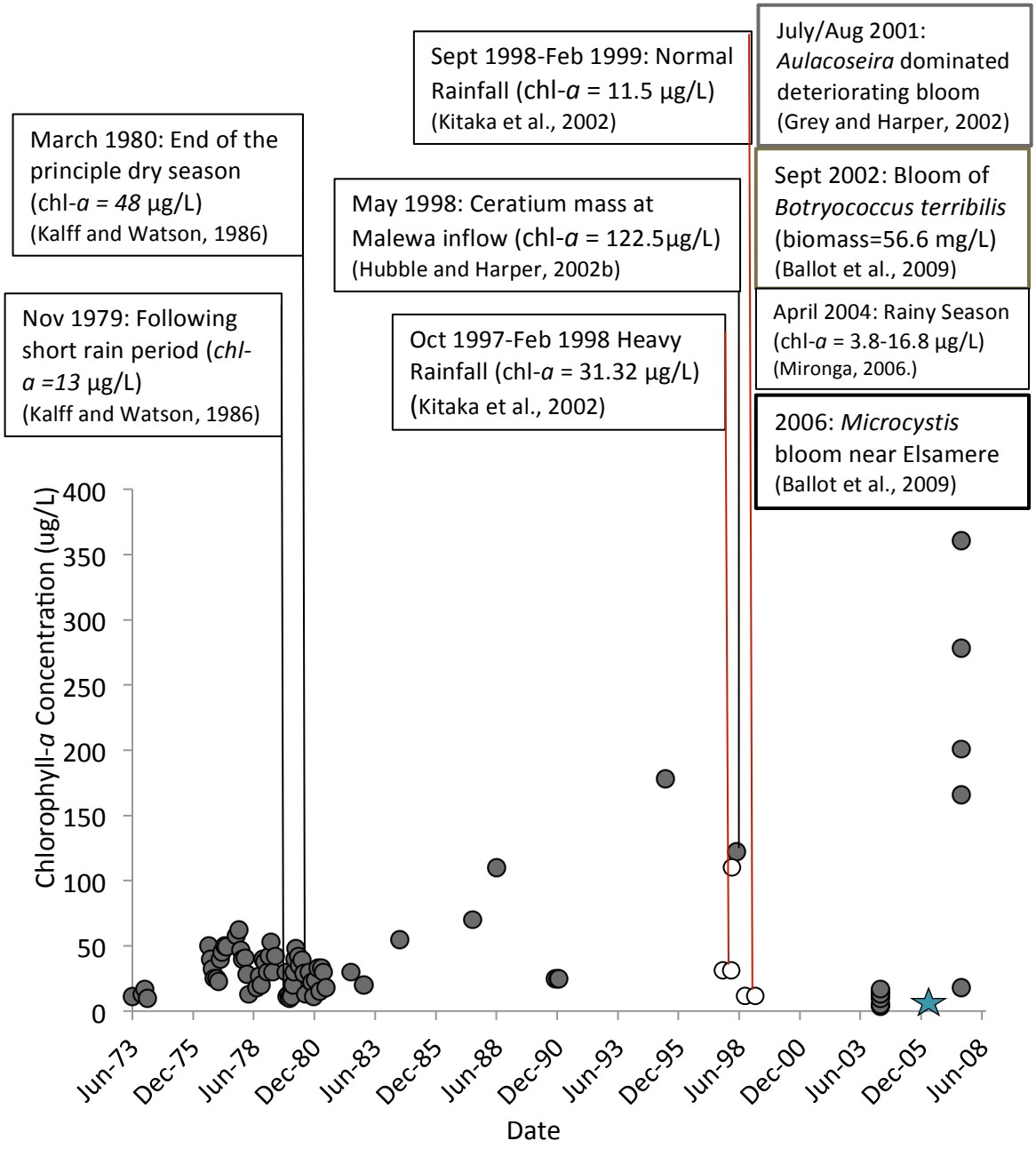

Figure 1.3. Historical chlorophyll-*a* levels in Lake Naivasha. Data are compiled from all literature sources and various studies of the lake. The star represents the approximate point in time when cyanoHABs started to become common in the lake.

Sources for data points: Melack, 1979a; Kalff and Watson, 1986 (also Kallquist, 1978, 1979 and Njuguna, 1983, as cited in Kalff and Watson, 1986); Harper, 1992; Uku and Mavuti, 1994; Kitaka et al., 2002; Hubble and Harper, 2002a; Mavuti and Harper, 2006; Mironga, 2006; Jimoh et al.. 2007.

et al., 2009). In addition to the chl-*a* levels, related notes of interest have been added to Figure 1.3, corresponding to the date of occurrence.

Prior to the human-induced changes in 1980, biomass was found to be lower, more seasonally variable, and never went much higher than 50 μg/L (Kalff and Watson, 1986). Melack (1979a) reported dominance by cyanobacteria from 1973 – 1974. From October 1979 to July 1980, Kalff and Watson (1986) found a shift between diatom dominance (low biomass) to green algae dominance (increasing biomass) to cyanobacteria dominance (peak biomass) (Figure 1.4a). The two studies from the 1990's, when eutrophication was said to have increased, were characterized by diatom dominance, although there was still a presence of cyanobacteria (Uku and Mavuti 1994; Hubble and Harper 2002b). In particular, the later study of community composition and succession (Hubble and Harper, 2002b) took place from December 1997 to October 1998, capturing the period of heavy El Niño rainfall. That study overlapped with the work of Kitaka et al. (2002) who measured chl-*a* and total phosphorus and found that the values suggested that the lake became hyper-eutrophic after the heavy rains. Higher chl-*a* and phosphorus loading were found during the heavy rain year, compared to the normal rain year, but interestingly there was also a localized area of the lake that had much higher biomass for about one month after the rains ended, showing how dynamic the biomass could be within a short period due to hydrological patterns and highlighting the potential spatial variation of phytoplankton (white dots, Figure 1.3; Kitaka et al., 2002). The community was consistently dominated by the diatom *Aulacoseira* spp., especially from January-February 1998, at the end of the heavy rainfall period, suggesting that the rains and corresponding nutrients contributed to the diatom dominance (Figure 1.4b). Kitaka et al. (2002) further determined that the phosphorus loading could be explained by the increased inflow from the Malewa at the time.

The last study of community composition in the literature took place intermittently from June 2001 to May 2004 giving a few time snapshots (Ballot et al., 2009). While continuous changes could not be assessed, the community was widely variable with cyanobacteria dominance (but low biomass) from June to November 2001, green algae dominance (moderate biomass) from February to May 2002 and desmid dominance (high biomass) in March-May 2004 (Figure 1.4c, Ballot et al., 2009). While

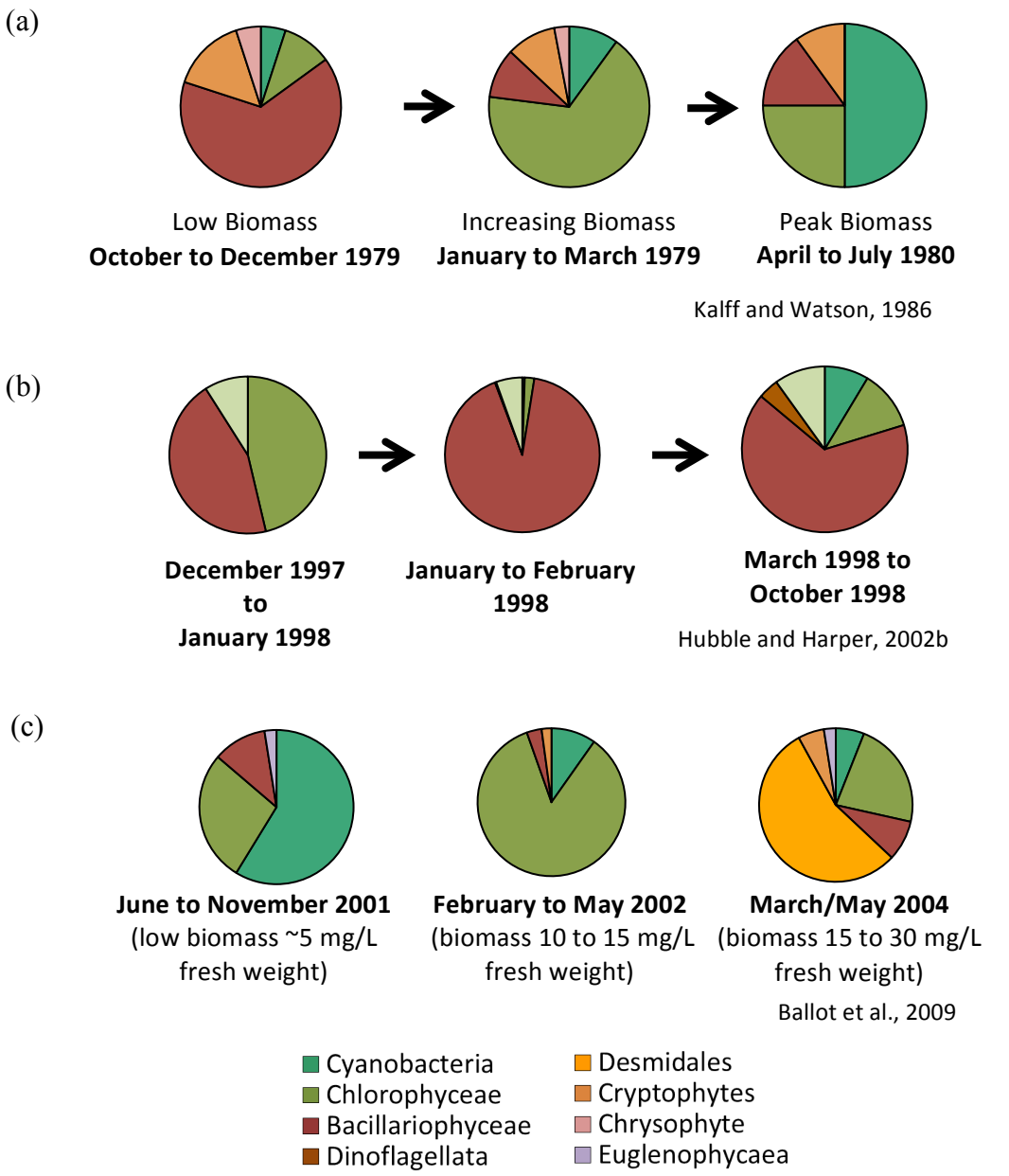

Figure 1.4. Historical phytoplankton community composition in Lake Naivasha. (a) Period of seasonal phytoplankton shifts b) Period of diatom (bacillariophyceae) dominance after eutrophication c) Snapshots indicating potential seasonality or transition state. Data from each literature source were compiled and graphed in a similar manner.

there are many gaps in this timeline of community composition, these historical studies do indicate that the lake has gone from a state of seasonal community shifts (Kalff and Watson, 1986) to diatom dominance due to eutrophication (Hubble and Harper, 2002b) to potentially another state of seasonality or transition (Ballot et al., 2009).

What is evident from the historical community composition is that from the 1970's to the present, regardless of what the chl-*a* levels were, there were periods where cyanobacteria were dominant. It was specifically expressed in Hubble and Harper (2001) that no cyanobacterial scums were occurring in 2000 and it has only recently been acknowledged in the literature that cyanobacterial blooms began occurring in 2005 in the lake (Harper et al., 2011). These blooms are therefore a relatively recent occurrence in the lake. The last study of the phytoplankton community described above (Ballot et al., 2009) also specifically stated that samples were found to contain low biomass of *Microcystis aeruginosa*, but the authors did report the presence of dense blooms of that same species in the southwestern part of the lake in 2006 (no details given on the exact date), and noted the potential health risk that could exist due to toxins (Ballot et al., 2009). In fact, a strain of *Microcystis aeruginosa* (AB2002/40), which was isolated from Lake Naivasha in 2002, was one of only 4 isolates from East Africa (24 isolates tested in total) to produce microcystins (Haande et al., 2007). The specific variant was found to be microcystin desmethyl-YR, and with this confirmation that there is a strain present from the lake that produces toxin in culture, it is very important to examine whether toxins are substantially produced in the natural population.

1.4.6. Community concern of cyanoHABs in Lake Naivasha

In a country where 80% of the land surface is arid or semi-arid (Otiang'a-Owiti and Oswe, 2007), ensuring that one of the few freshwater resources remains viable is of upmost importance at local and national levels. A small survey of 62 local community members indicated that the two top reasons that Lake Naivasha is so important from the local community's perspective is because of fishing and drinking water (Hickley et al., 2004). CyanoHABs can negatively impact both of these valuable ecosystem services. During a stakeholder's workshop for the Lake Naivasha Sustainability Project one of the top concerns expressed by the community was the occurrence of the potentially harmful cyanobacterial blooms, and whether they posed a health risk.

1.5. Questions, hypothesis, objectives and predictions

To assess the potential health risk of cyanoHABs, the first question to ask was what initiates the formation and maintenance of cyanobacterial blooms in Lake Naivasha? From this knowledge I was able to project if cyanobacterial bloom and/or cyanoHAB events are likely to occur in the future. Specifically, will they be common?; will they be predictable?; are there points of intervention that could help prevent or reduce cyanobacterial bloom formation? Furthermore, is microcystin, a potent liver toxin, associated with cyanobacterial bloom events, thereby officially making them cyanoHABs? How does the production of this compound relate to the initiation or maintenance of the bloom? Finally, are certain areas of the lake more likely to exhibit greater cyanobacterial bloom presence or higher microcystin levels?

Due to the presence of a toxic strain of *Microcystis aeruginosa* isolated from the lake (Haande et al., 2007), in addition to the reports of *Microcystis* blooms in recent literature (Ballot et al., 2009; Harper et al., 2011) it is hypothesized that the production of microcystin, corresponding to cyanobacteria blooms, will pose a risk to human health in the Lake Naivasha region. To test this hypothesis, two objectives were chosen. The first was to conduct a survey of the natural levels of cyanobacterial and algal biomass, the physical environment (temperature, rainfall, evaporation, and lake level) and chemical characteristics (salinity, pH, dissolved oxygen) to determine the occurrence of bloom events and the factors influencing their formation. This will help define the current and future situations that might lead to cyanoHABs and ways to mitigate or prevent their occurrence. It is predicted that hydrological changes will play a key role in cyanobacteria bloom formation due to their ability to cause large nutrient influxes.

The second objective was to conduct a survey of the natural levels of microcystin in correspondence to the survey of cyanobacterial biomass. Toxin levels were compared to established guidelines to assess the present risk. It was predicted that the levels of particulate microcystins would be higher with bloom presence versus absence and that the larger the biomass of cyanobacteria, the greater the concentration of particulate microcystin. Additionally particulate toxin level dynamics were examined on a per cell basis to identify controls on the production of microcystin, which is useful to know for future risk assessment. It was predicted that increased particulate microcystin per cell

would be observed in the later stages of the bloom when growth stressors would be greater.

The data from the two objectives will also allow for a comparison between the Main Lake basin and the Crescent Island basin of the lake. It is predicted that the Crescent Island basin will be less likely to support a cyanobacterial bloom compared to the Main Lake basin due to its more sheltered location, considered to be ‘pristine’, that experiences less wind-induced mixing and nutrient influxes from runoff and the Malewa inflow.

2. MATERIALS AND METHODS

2.1. Field methodology

Two field campaigns were completed from August 2010 to March 2011 (Field Season 1) and May to August 2011 (Field Season 2). During Field Season 1, I organized and established the sampling routine during August and September. Standardized operating protocol was then continued by Canadian and Kenyan colleagues until March 2011. During Field Season 2, some changes were made to the sampling sites and field methodology, and are noted where applicable. During both field seasons samples and measurements were typically taken on a weekly basis.

2.1.1. Sampling Sites

Sampling sites were selected to cover a range of areas around the lake, including well-known sites traditionally sampled by other researchers and the Lake Naivasha Riparian Association (LNRA), and other visited sites of interest (Figure 2.1, Table 2.1). Criteria for site selection also included locations where the Naivasha community or likely to influence the lake water quality or likely to access the water. During Field Season 1, more open water locations were visited to represent average, well-mixed lake conditions. During Field Season 2, there was a greater focus on the changing water characteristics from shoreline to open water.

2.1.2. Qualitative and quantitative in situ *water observations and measurements*

Both *in situ* qualitative observations and quantitative measurements were recorded at each site visited. Qualitative observations included weather notes, the presence or absence of floating vegetation and visible water characteristics such as colour, turbidity and algal bloom presence.

Other quantitative *in situ* water parameters were measured using a multi-parameter water sonde (YSI 6600V2, Yellow Springs Instruments, Yellow Springs, Ohio) (Table 2.2). At each sampling site throughout Field Season 1, the sonde was lowered to approximately 1 m below the surface. It was tethered to a wooden pole for stabilization, and left to collect measurements for approximately two minutes. During the surface *in situ* characteristics were measured on a discrete water sample. This integrated surface water sample was collected with a 1 m polyvinyl chloride (PVC) tube and put

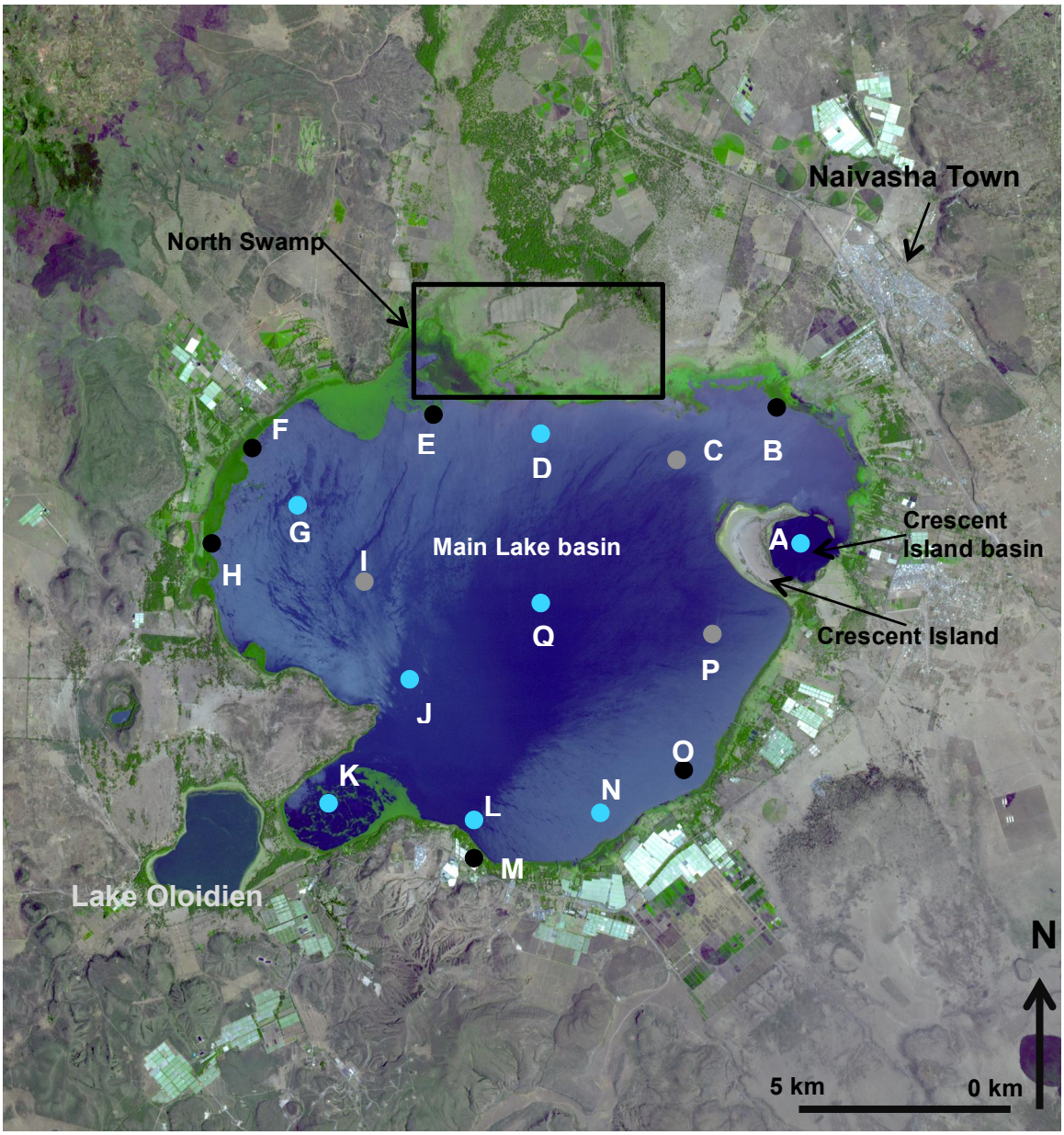

Figure 2.1. Lake Naivasha sampling locations. Blue symbols represent sites visited during Field Season 1 and 2, grey symbols were only visited during Field Season 1 and black symbols were only sampled in Field Season 2.

Satellite Image credit: ASA/GSFC/METI/Japan Space Systems, and U.S./Japan ASTER Science Team (http://asterweb.jpl.nasa.gov/gallery-detail.asp?name=naivasha) Image captured February 2, 2008.

Table 2.1. Description and GPS coordinates of sampling locations.

Site ID	Name/Description	GPS coordinates (WGS 84 37M UTM)	
		Easting	**Northing**
A	Middle of Crescent Island basin*	0211814	9915042
B	Sewage Treatment Plant *	0211779	9918769
C	Main Lake Entrance	0209319	9917431
D	Near Malewa River Inflow *	0205876	9919011
E	Near Gil Gil River Inflow	0202903	9919804
F	Bilishaka/Shalimar farms	0200167	9919465
G	Northwest 1	0200257	9918399
H	Korongo Farm	0198097	9917534
I	Northwest 2	0201369	9917005
J	Hippo Point	0201354	9912591
K	Oserian Bay *	0199816	9910628
L	Kamere Public Beach	0202251	9909986
M	Kamere public water collection area	0202553	9909785
N	Offshore from Greenhouses	0205638	9909093
O	Sher Red House *	0206713	9909112
P	Main Lake East	0208082	9916472
Q	Middle of Main Lake basin *	0204977	9914267

* Sites routinely sampled by the Lake Naivasha Riparian Association in the past.

Table 2.2. Water sonde *in situ* water parameter measurements.

***In situ* Measurement**	**Units**
Depth	Metres (m)
Temperature	Degrees Celsius (°C)
Specific Conductivity	Microsiemens per centimeter (µS/cm)
Conductivity	Millisiemens per centimeter (mS/cm)
Resistivity	Ohm/Metre (Ω/m)
pH	n/a
Salinity	Parts per thousand (ppt)
Total Dissolved Solids (TDS)	Grams per litre (g/L)
Dissolved Oxygen Saturation	Percent (%)
Dissolved Oxygen Concentration	Milligrams per litre (mg/L)
Chlorophyll Concentration	Micrograms per litre (µg/L)
Chlorophyll Fluorescence	Raw Florescence Units (RFU)

into a cup designed for the sonde instrument and the measurements for each parameter recorded. This water was then used as a sample as described below in Section 2.1.3. The surface measurements from Field Season 1 were stored as data files in the YSI sonde. These files were uploaded to a computer weekly using EcoWatch® for Windows® data analysis software and exported to Microsoft Excel® to be used for further data analysis.

2.1.3. Water sample collection

During Field Season 1, water samples for both microscopic identification (Section 2.1.5) and Laboratory Analysis (Section 2.2) were collected with a 64 μm mesh plankton tow net that was lowered 1 m into the water column and immediately drawn back up. During Field Season 2 the plankton net was used to collect the microscope sample (which was preserved with a few drops of Lugol's Solution), and the sample for laboratory analysis was taken from the integrated surface water sample that was collected with the PVC tube for the *in situ* parameter measurements. All samples were collected in plastic bottles and remained in the dark and on ice until processing at the end of the day.

2.1.4. Water sample filtration

A known volume of each water sample was filtered onto a glass fiber filter (Whatman, GF/C) using a plastic filter apparatus and manual hand pump. This collected all cells within the water onto the GF/C filter, which is a common and standard filter type when collecting field samples of phytoplankton for later laboratory analysis (Holm-Hansen, 1978; Lind, 1985). The filter was folded in half, covered in aluminum foil or placed in a cryovial and frozen at -20 °C. The frozen filters were brought back to London, ON, Canada for laboratory analysis.

2.1.5. Microscopy and taxonomic identification

Taxonomic identification was carried out by examination using a compound microscope. A web-based taxonomic key created by The Freshwater Ecology Laboratory at Connecticut College was used to assist in identification of the algae to a genus level (Shayler and Siver, 2006). Specifically, the three dominant genera were identified along with other algae that were present in the sample but not dominant using a similar approach to Uku and Mavuti (1994). The dominant rankings were converted to proportions (most dominant = 0.5, second most dominant = 0.3, third most dominant = 0.2) and averaged between all the sites to obtain an estimate of overall community

composition for the lake on a given week. Digital pictures were taken of the cyanobacteria and eukaryotic algae that were observed.

2.2. Laboratory methodology

2.2.1. Filter preparation

A single filter representing one water sample was used for three different types of analyses, and to do so the filter was divided and cut up. The filtration process distributes cell material from the water sample uniformly over the filter and since the filters were stored while folded in half, they were each divided as depicted in Figure 2.2. Each frozen filter was given a lab ID number (from 001 to 428) and one half of the filter was used for toxin analysis (Section 2.23), while one quarter of the filter was used for chlorophyll quantification and the final quarter of the filter used for phycobiliprotein quantification (Section 2.22). Each portion of the filter was weighed on a balance (Sartorius TE1502S) so that the appropriate volume of water represented by the algal biomass on the specific piece of filter could be determined and used for final calculations. Each filter portion was stored in a labeled 1.8 mL cryovial (Nalgene), at -20°C in the dark until analysis.

2.2.2. Extracted pigment (chlorophyll and phycobiliprotein) analysis

Measurement of the chlorophylls—chlorophyll *a* (chl-*a*), chlorophyll *b* (chl-*b*) and chlorophyll *c* (chl-*c*)—and phycobiliproteins—phycocyanin (PC) and phycoerythrin (PE)—was performed as outlined in Figure 2.3. For each class of pigment, analysis was kept as consistent and similar as possible, but the different extraction solvents acetone (Strickland and Parsons, 1972; Bowles et al., 1985) and phosphate buffer (Bennett and Bogorad, 1973; Otsuki et al., 1994; Lee et al., 1994; Lee et al., 1995; Silveira, 2007; Lawrenz et al., 2011) were used to optimize extraction of the chlorophylls and phycobiliproteins, respectively. The organic solvent acetone (90%:10% acetone to water) (Strickland and Parsons, 1972; Jeffrey and Humphrey, 1975; Bowles et al., 1985; Arar and Collins, 1997) was used to extract the non-water soluble chlorophylls, which are present within the thylakoid membrane of the chloroplasts. In contrast, the water soluble phycobiliproteins are attached on the surface of the thylakoid membrane (Gantt, 1975; Tandeau de Marsac, 2003), and were extracted with the phosphate buffer (0.1M, pH of 6.0) (Lawrenz et al., 2011).

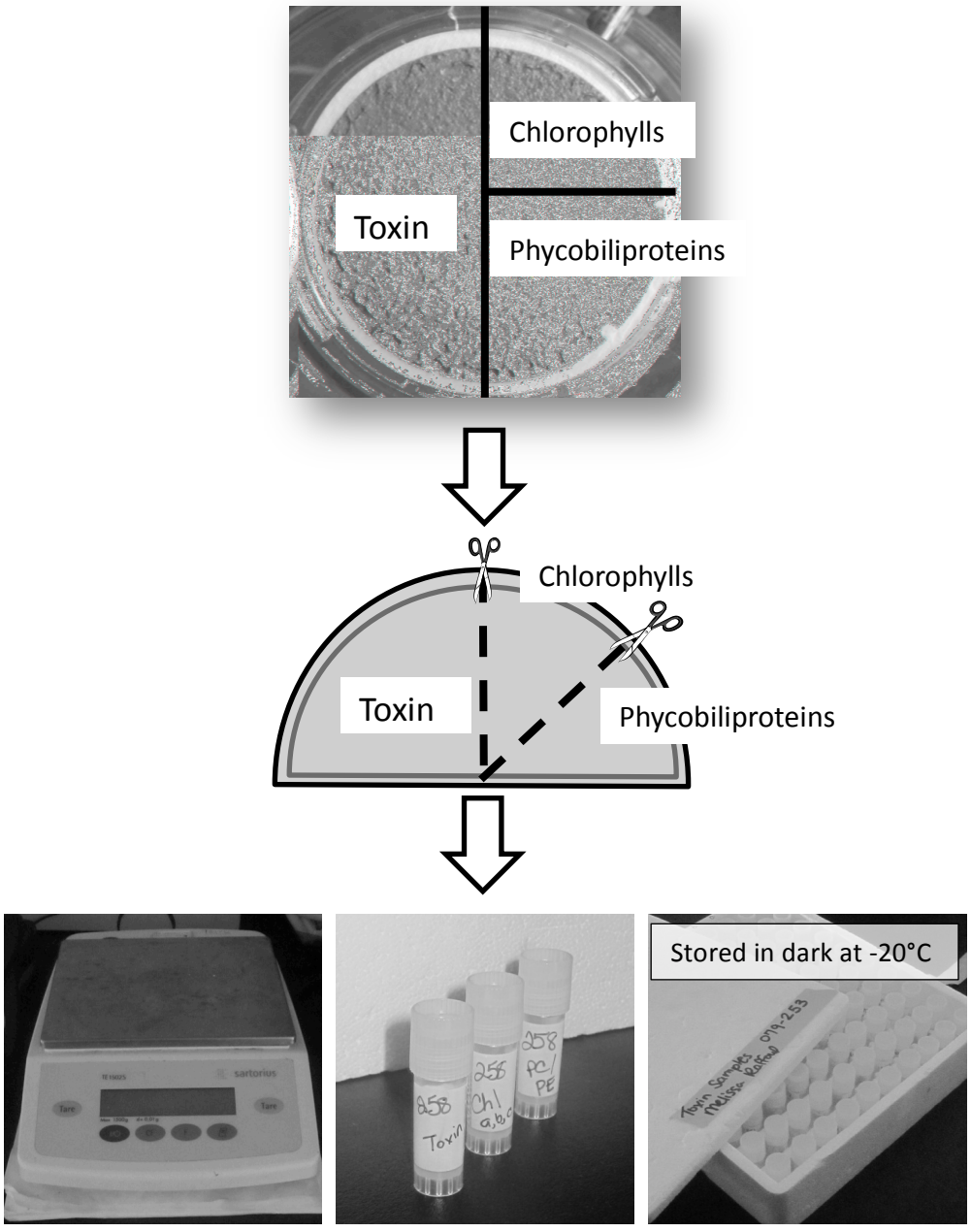

Figure 2.2. Preparation of filter for laboratory analysis.

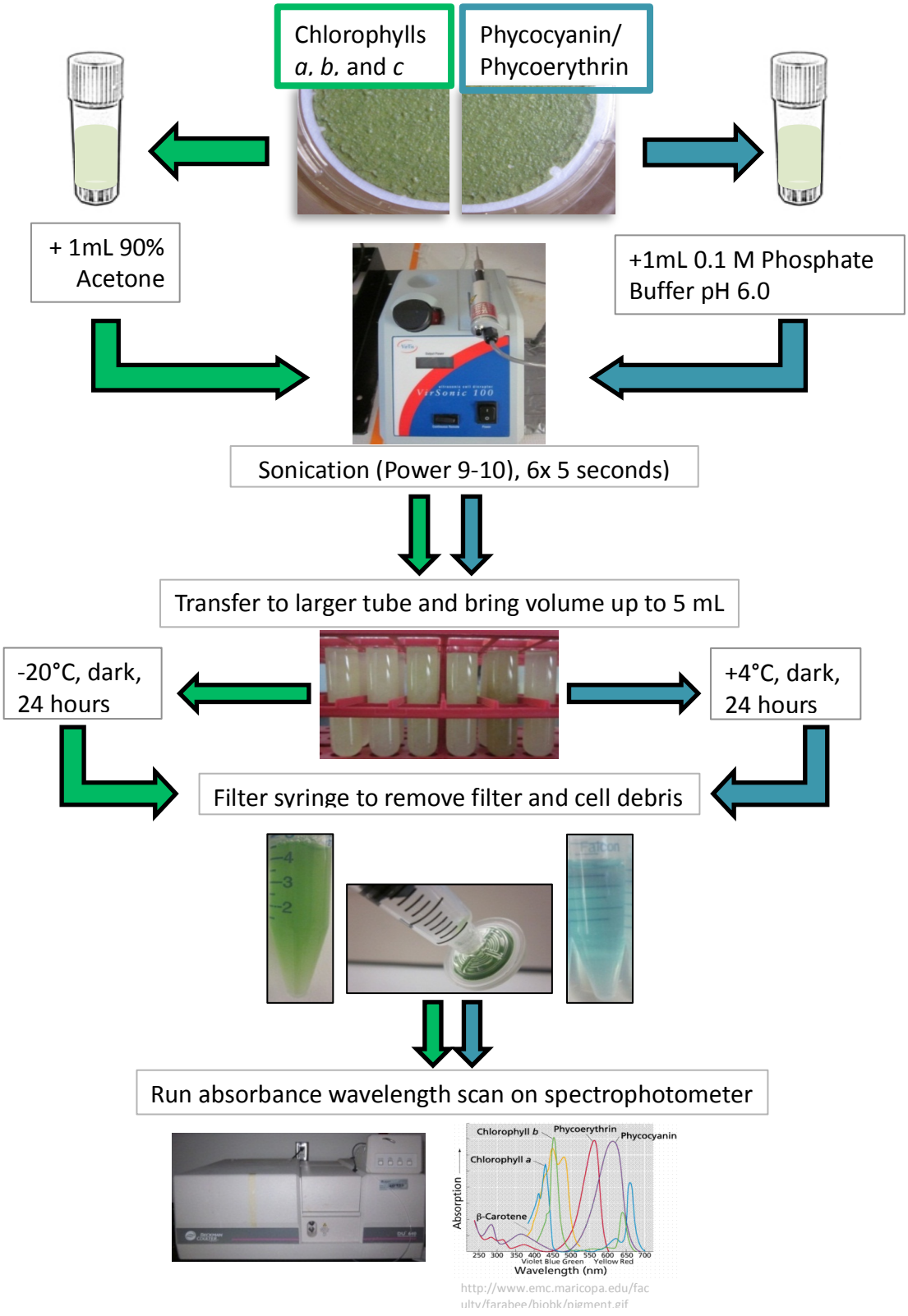

Figure 2.3. Steps for extracted pigment analysis.

There have been a number of studies done to determine the best solvent (acetone, methanol, or ethanol) and methods for chlorophyll extraction, all with varying results (Holm-Hansen, 1978; Riemann and Ernst, 1982; Sartory and Grobbelaar, 1984; Bowles et al., 1985; Simon and Helliwell, 1998; Wasmund et al., 2006; Schagerl and Kunzl, 2007). However, spectrophotometric equations for 90% acetone extractions have been comprehensively derived (Strickland and Parsons, 1972; Jeffrey and Humphrey, 1975) and this solvent is supported and used by the United States Environmental Protection Agency (Arar and Collins, 1997). While properties of acetone allow it to easily penetrate membranes, burst cells and release chlorophyll—sometimes deemed sufficient for extraction (Strickland and Parsons. 1972)—it is more often recommended that some form of mechanical cell disruption take place (Holm-Hansen, 1978; Riemann and Ernst, 1982; Lind, 1985; Bowles et al., 1985; Wright et al., 1997; Wasmund et al., 2006; Schagerl and Kunzl, 2007), especially when dealing with natural samples of mixed phytoplankton, and cyanobacteria, which are known for their strong cell walls. Phosphate buffer does not easily rupture cells, so mechanical disruption is highly recommended for phycobiliprotein analysis (Siegelman and Kycia, 1978; Lee et al., 1994; Lee et al., 1995; Furuki at al., 2003; Silveira, 2007; Lawrenz et al., 2011; Moraes et al., 2011).

One mL of extract solvent was added to each cryovial (containing the prepared filter as described in Section 2.2.1) and sonicated (VirTis Virsonic 100 Ultrasonic Cell Disruptor™) on ice water for 30 seconds (six 5 second pulses). The resulting slurries were transferred to larger tubes and the appropriate extract solvent was added to bring the volume to 5 mL. The chlorophyll extractions used borosilicate glass tubes and were stored at -20°C and the phycobiliprotein extractions were stored in plastic Falcon™ tubes at 4°C. All extractions incubated for 24 hours. After this period, the extract was clarified to remove filter pieces and cell debris using a filter syringe. Chlorophyll extracts were passed through a 25 mm glass fiber filter (Whatman GF/F grade) and the phycobiliprotein extracts were passed through a 25 mm 0.45 µm Acrodisc Supor Membrane™ filter (Pall Life Sciences) (Figure 2.3).

The absorbance of the resulting solutions was measured by performing a wavelength scan (400-800 nm) on a spectrophotometer (Beckman-Coulter DU60) in a 4 mL glass cuvette with a 1 cm path length. The spectrophotometer was blanked with either

90% acetone or 10 mM phosphate buffer, depending on which samples were being scanned. In order to calculate pigment concentrations from the wavelength scans the absorbance at specific wavelengths was used (Appendix 1.1) in the trichromatic equations of chl-*a*, chl-*b*, chl-c (Jeffrey and Humphrey, 1975) and the equations for PC and PE concentration (Lawrenz et al., 2011).

The equations used to calculate chlorophyll concentrations were as follows (Jeffrey and Humphrey, 1975):

$$[\text{Chl-a}]\ \mu g/L = [11.85(A_{664}) - 1.54(A_{647}) - 0.08(A_{630})] \times \frac{V_{acetone}}{V_{sample}\ (d)}$$

$$[\text{Chl-b}]\ \mu g/L = -5.43(A_{664}) + 21.03(A_{647}) - 2.66(A_{630}) \times \frac{V_{acetone}}{V_{sample}\ (d)}$$

$$[\text{Chl-c}]\ \mu g/L = -1.67(A_{664}) + 7.6(A_{647}) + 24.52\ (A_{630}) \times \frac{V_{acetone}}{V_{sample}\ (d)}$$

Where,

A### = Absorbance at a given wavelength

V = Volume in mL

d = cuvette path length in cm

The equations used to calculate phycobiliprotein concentrations were (Lawrenz et al., 2011):

$$[\text{PC}]\ \mu g/L = \frac{A_{620}\ (MW_{PC})}{\varepsilon_{PC}\ d} \times \frac{V_{phosphate\ buffer}}{V_{sample}} \times 10^6$$

$$[\text{PE}]\ \mu g/L = \frac{A_{545}\ (MW_{PE})}{\varepsilon_{PE}\ d} \times \frac{V_{phosphate\ buffer}}{V_{sample}} \times 10^6$$

Where,

MW = Molecular weight (PC = 264,000 g/mol, PE = 240,000 g/mol)

ε = Molar extinction coefficient (PC=1.9 x 10^6 L mol^{-1} cm^{-1}, PE=2.41 x 10^6 L mol^{-1} cm^{-1})

d = cuvette path length in cm (for this study d = 1 cm)

V = Volume in mL

2.2.3. Toxin analysis: sample preparation

One half of each filter was used to measure the concentration of total intracellular microcystin through an Enzyme Linked Immunosorbent Assay (ELISA). Most studies using an ELISA to measure environmental samples perform the assay with natural water samples or freeze-dried cells and few describe the ELISA procedure for intracellular microcystin with cells on a glass fiber filter. However, extraction protocols used on freeze-dried cells were noted to be applicable to cells on a filter (Barco et al., 2005) and a number of studies have used HPLC and similar analysis techniques when measuring intracellular microcystin from filters (Lawton et al., 1994; Coyle and Lawton, 1996; Spoof et al., 2003).

An additional concern when extracting microcystins for ELISA use, is that some of the material may adsorb to plastic polymers (such as microcentrifuge tubes and pipette tips), which was shown in Metcalfe et al. (2000). This adsorption was evident when the solvent used was less than 25-30% methanol, and each pipette tip action represented an approximate 4% decrease in microcystin concentration (Metcalfe et al., 2000; Hyenstrand et al., 2001). However, Hyenstrand et al., (2001) also found no differences in microcystin concentrations when methanol ranged from 25-96%, and suggested that under the proposed optimal extraction of 75% methanol—recommended by Fastner et al. (1998) and used in this study—hydrophobic interactions with plastics should not cause a significant loss of microcystins, but the use of plastic equipment should be minimized when possible.

Initial analysis of a few samples indicated that intracellular microcystin concentrations were low. In order to gather data effectively for a large amount of samples, a protocol was developed to extract and pellet the microcystins in a consistent manner. The samples were prepared according to the scheme in Figure 2.4, which drew from and adapted a number of protocols from previous studies (Lawton et al., 1994; Coyle and Lawton, 1996; Gjolme and Utkilen, 1996; Fastner et al,. 1998; Harada et al., 1999; Meriluoto et al., 2000; Spoof et al., 2003; Barco et al., 2005). Where appropriate,

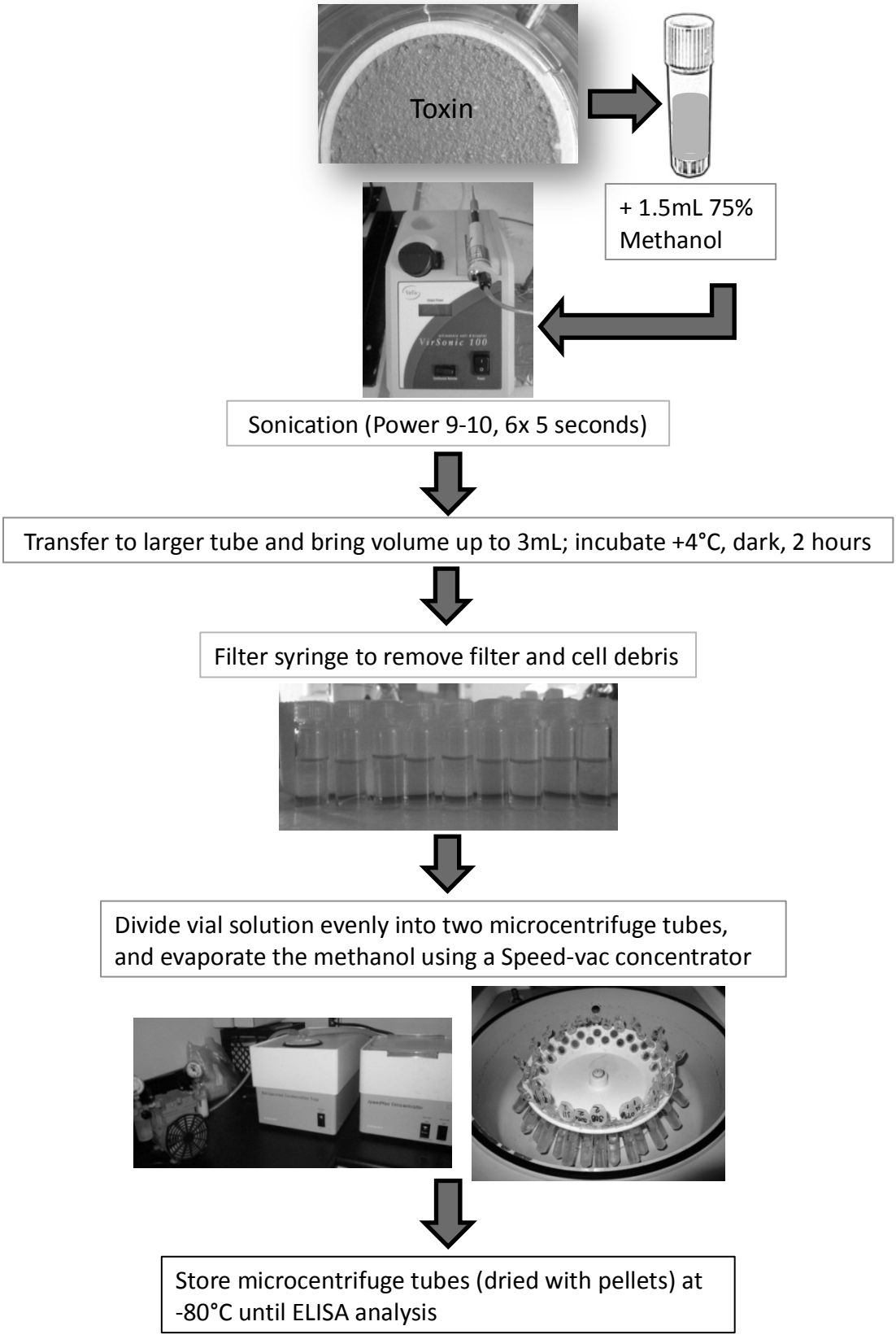

Figure 2.4. Microcystin extraction and sample preparation for ELISA kit.

this protocol also mirrored the methodology used for the pigment extractions.

First, 1.5 mL of 75% methanol (75% methanol: 25% water) (Fastner et al., 1998) was added to the cryovial containing the filter. The cryovial containing the filter and 75% methanol were then sonicated (VirTis Virsonic 100 Ultrasonic Cell Disruptor™) at a power of 9-10, for six 5 second intervals while in an ice bath. The resulting slurry was then transferred to a larger borosilicate glass tube and the volume brought up to 3 mL. The tubes were incubated at 4°C in the dark for 2 hours to allow extraction. After incubation, the extract was filter-syringed using a Whatman GF/F grade glass fiber filter to remove the sample filter and cell debris. The remaining solution was divided equally into two 2 mL microcentrifuge tubes and the 75% methanol solution was evaporated from these tubes, using a Savant Speed-Vac™ concentrator. The microcentrifuge tubes containing dried pellets of extracted microcystin were stored at -80 °C until analysis with the ELISA.

2.2.4. Microcystins (Adda specific) ELISA

The Microcystins (Adda specific) ELISA kit (Enzo Life Sciences) allows for the congener-independent quantification of microcystins and nodularins as it shows very good cross-reactivity with other microcystin variants (Fischer et al., 2001). This test kit does not differentiate between the many variants of microcystin, but uses Microcystin-LR for calibration and standards; therefore the final concentrations are officially reported as Microcystin-LR equivalents. It is one of the most highly regarded methods for rapid and sensitive microcystin screening when compared to other procedures available because of its low cost, simplicity of use and reliability (Carmichael and An, 1999; Hawkins et al., 2005)

The dried samples (Section 2.2.2), were reconstituted with 125-200 µL of 5% methanol, which allowed for the measured concentrations to fit within the sensitivity range (0.1-5.0 µg/L) of the assay. A 5% methanol solution was used to minimize the interference of methanol with the assay as methanol concentrations greater than 20% were found to produce false positive in other brands of the ELISA kit (Metcalf et al., 2000).

Fifty µL of each standard, sample and control were pipetted in duplicate onto the 96-well plate which was set up according to the general scheme in Figure 2.5, and the

protocol carried out according to manufacturer directions. This ELISA kit utilized the principles of indirect antibody competition, in which a non-specific antigen (microcystin-LR-Bovine Serum Albumin) came pre-coated on a 96-well plate. A monoclonal antibody competed with the microcystins present in the sample for the antigen binding sites, and then a second antibody was added to bring on a colour change.

The results of the ELISA were qualitatively observed by colour intensity—which is inversely proportional to the concentration of microcystins—and were quantified by reading the absorbance at 450 nm using a plate reader (Molecular Devices Versamax™ microplate reader). The standards run on the plate were used to create a standard curve (Figure 2.6), and the equation of the standard curve was used to infer the concentration of microcystin-LR equivalents in the samples. Since the measured samples were concentrated from a water sample of known volume, and only reconstituted in 125-200 µL of solution, the concentrations measured by the ELISA were further adjusted as follows:

$$\text{Total intracellular microcystins } (\mu g/L \text{ of microcystin-LR equivalents}) = C_{\text{std curve}} \times \frac{V_{5\%\text{methanol}}}{V_{\text{water sample}}}$$

Where,

C = sample concentration obtained from ELISA standard curve

V = Volume in mL for the given sample

2.3. Statistical Analysis

Statistical analysis was performed using SigmaPlot (Systat Software Inc., San Jose, CA). If data met the assumptions for regressions, linear or non-linear regression analysis was performed. Spearman-Rank correlations were performed when analyzing the relationship between the pigments as the data were not normally distributed. Tests were performed with an alpha of 0.05 and relationships were deemed significant when $p < 0.05$.

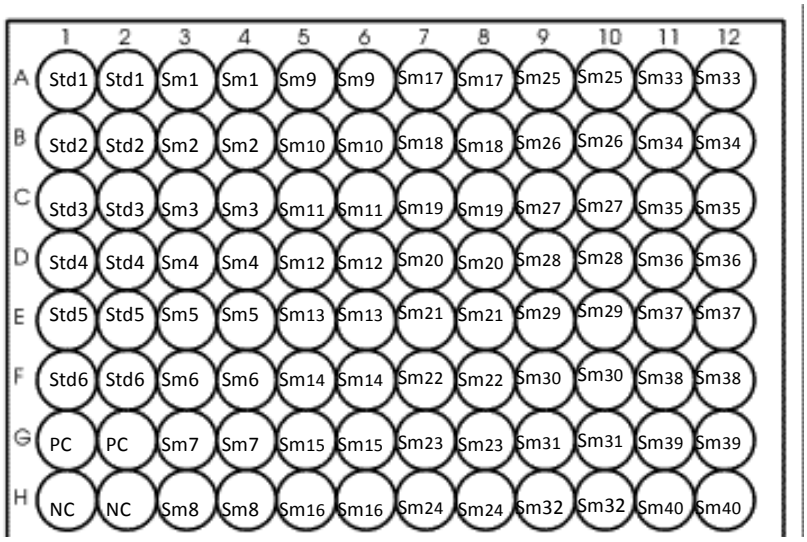

Figure 2.5. ELISA 96-well plate set-up. Std = Standard (0, 0.15, 0.40, 1.0, 2.0, 5.0 ppb); PC = positive control (0.75 ppb); NC = Negative control (5% methanol); Sm = Sample

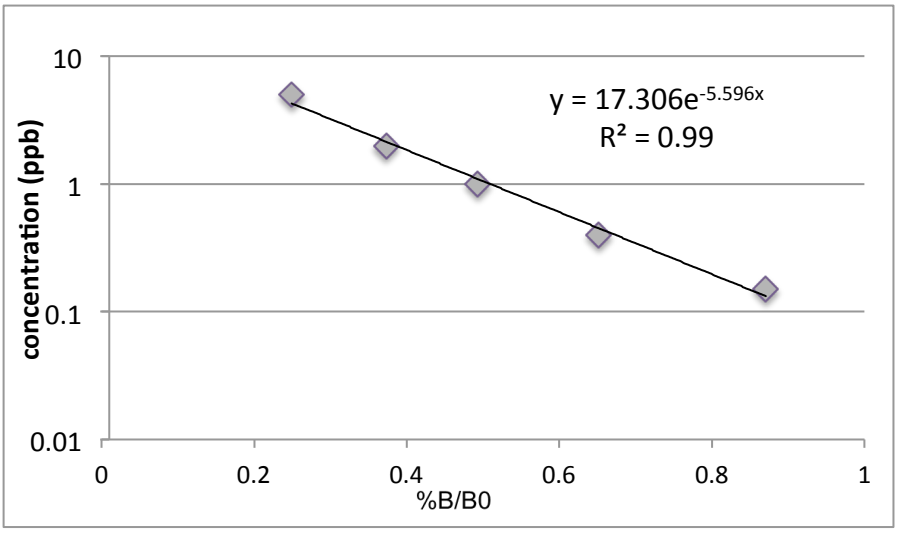

Figure 2.6. A standard curve example generated from an ELISA plate.

3. RESULTS

3.1. Weather data for the Lake Naivasha region

The Lake Naivasha Water Resource Users Association (LANAWRUA) provided raw data for weather related parameters from 1991 to 2012. Data are shown from January 2009 to December 2011, which encompasses more than a year and a half prior to sampling, the sampling period from September 2010 to August 2011 and the four months following the study (Figure 3.1 and 3.2).

The average daily temperature in Naivasha remained fairly consistent over the study period. The mean daily maximum temperature was always above 20 °C and was highest from January to March and lowest from June to August (Figure 3.1). The average radiation had some variation that showed some consistency with maximum temperature and evaporation, but overall the levels were high and within a relatively small range (Figure 3.1). Although there was no consistent annual pattern in total evaporation over these three years, it was almost always greater than rainfall and constantly fell in the approximate range of 120-180 mm. In 2009 rainfall was generally low except for April and May, and correspondingly, the lake level declined steadily throughout the year. A large increase in rainfall and minor decrease in evaporation led to a rapid rise in lake level during the first half of 2010. This was followed by a few months of low rainfall and a stable lake level from June to August 2010 (Figure 3.2).

When Field Season 1 began in September 2010, there was characterized by a three month period of rain and lake level rise, followed by another three months of low rainfall, high evaporation and lake level decline. The remaining six months of the study period saw moderate rainfall each month and moderate to high evaporation which led to small but steady lake level declines each month (Figure 3.2). During Field Season 2, rainfall was moderate and fairly consistent and lake level continued to decline at small and steady increments (Figure 3.2). After Field Season 2, rainfall increased, evaporation decreased and lake levels began to rise sharply.

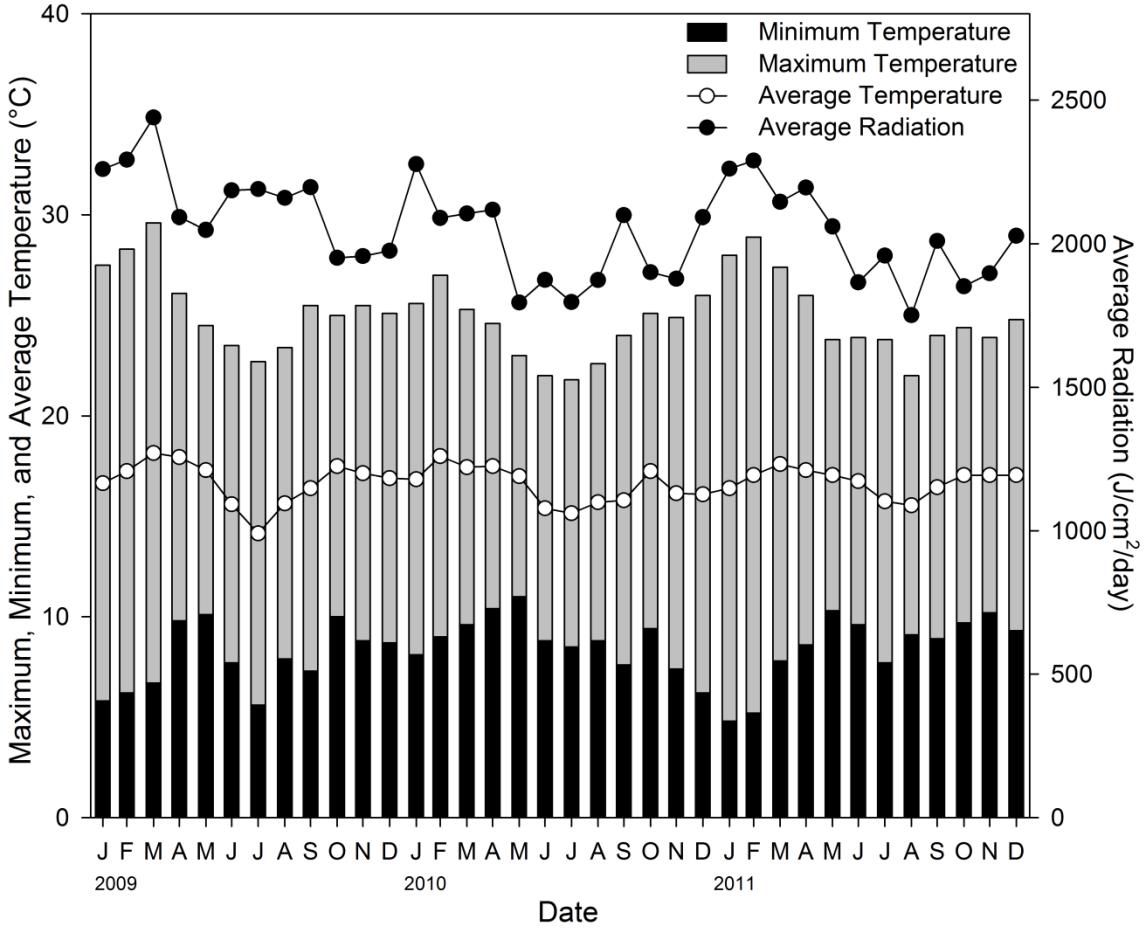

Figure 3.1. Mean daily maximum and minimum temperatures, average daily temperature and average radiation in Lake Naivasha (2009-2011). Raw data provided by the Lake Naivasha Water Resource Users Association (LANAWRUA).

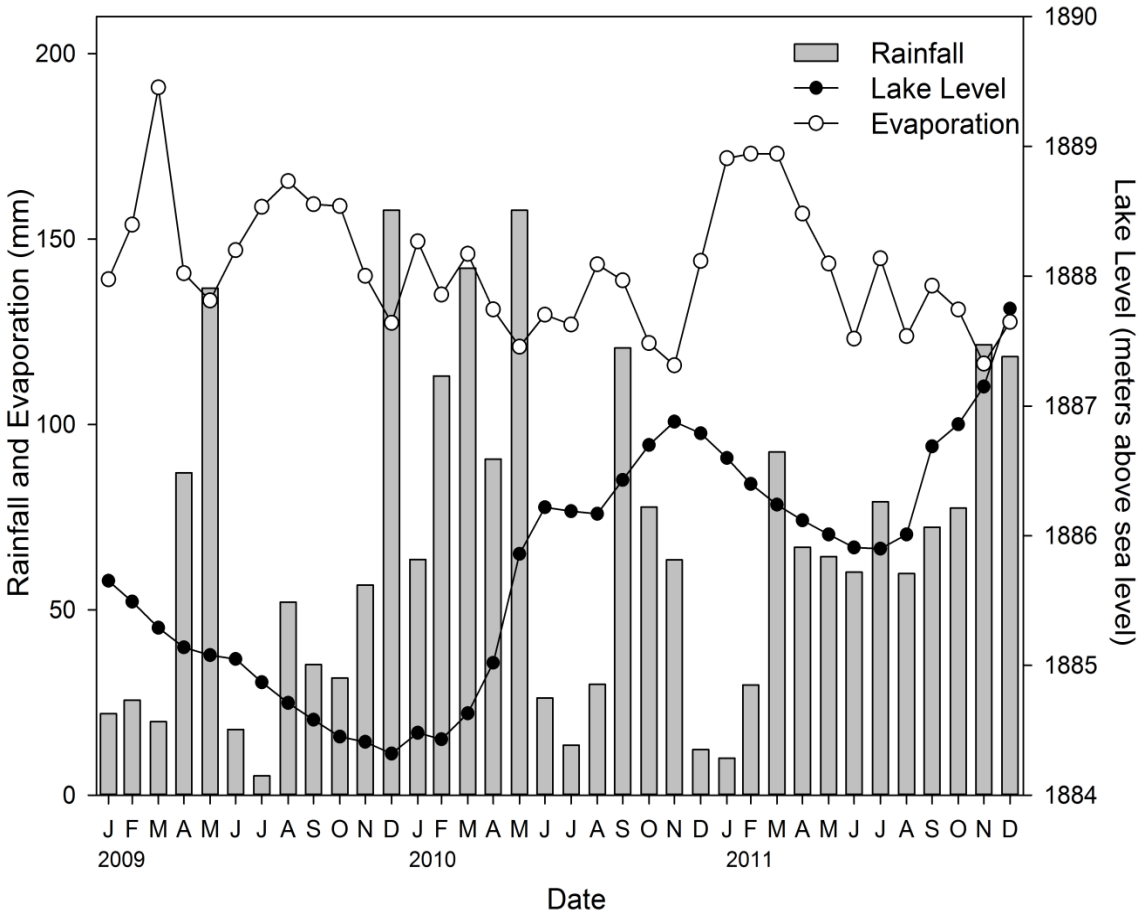

Figure 3.2. Total monthly rainfall, evaporation, and lake level in Lake Naivasha (2009-2011). Raw data provided by the LANAWRUA.

3.2. Physical and chemical surface water characteristics in Lake Naivasha

The general surface water measurements for the middle of the Main Lake and Crescent Island basins of Lake Naivasha are summarized for the entire study period in Table 3.1. For many of the parameters (conductivity, salinity, resistivity, total dissolved solids, and Secchi depth) overall differences are observed between the Main Lake and Crescent Island basins. Some magnitude of variation exists for all parameters, but of particular importance is the water temperature, which is quite consistent and high, and the pH, dissolved oxygen and Secchi depth.

Table 3.1. Summary of general surface water parameters and coefficient of variations (COV) from September 2010 to August 2011 for the Middle of the Main Lake basin and the Middle of the Crescent Island basin of Lake Naivasha.

	Middle of the Main Lake range (average)	**COV (%) M.L.***	**Middle of Crescent Island** range (average)	**COV (%) C.I.****
Water Temperature (°C)	20.03-23.83 (21.13)	4.1	20.24-22.95 (21.19)	2.9
Specific Conductivity (mS/cm)	0.211-0.301 (0.249)	9.1	0.328-0.464 (0.404)	8.2
Salinity (ppt)	0.10-0.14 (0.12)	9.6	0.16-0.22 (0.19)	7.7
Resistivity (Ω/m)	3598.02-5109.10 (4395.41)	9.4	2339.67-3173.78 (2697.96)	8.0
Total Dissolved Solids (g/L)	0.14-0.20 (0.16)	9.8	0.21-0.30 (0.26)	8.1
pH	7.72-9.26 (8.54)	5.0	7.53-8.75 (8.19)	4.4
Dissolved Oxygen (mg/L)	5.71-11.22 (7.59)	15.9	2.15-9.08 (6.27)	24.7
Secchi depth (m)	0.29-0.55 (0.43)	18.8	0.55-1.05 (0.74)	16.8

* Main Lake (M.L.)
**Crescent Island (C.I.)

3.3. Cyanobacterial bloom presence in Lake Naivasha

Field Season 1 was characterized by a lake-wide cyanobacterial bloom which was consistently present in the Main Lake basin. The bloom was not evenly distributed from a visual perspective across the surface of the lake. Sometimes it would look more uniform and homogenous while other times it would be patchy and clumped (Figure 3.3a). In the Crescent Island basin a bloom was not visible on the surface until December 2010. No lake wide bloom was observed during Field Season 2, however clumps of algae were occasionally observed at a few locations along the shoreline of the Main Lake basin. During the morning hours of May 19th, 2011, a shoreline bloom in the Crescent Island basin was observed and sampled (Figure 3.3b). The qualitative water observations for the Main Lake basin and Crescent Island basin are summarized for the entire study period in Tables 3.2 and 3.3, respectively.

3.4. Algal community composition

The algal community in the lake was generally dominated by cyanobacteria and diatoms (Figure 3.4) but there was also a presence of green algae and dinoflagellates (Tables 3.4 and 3.5). In the Main Lake basin the proportion of dominant genera remained fairly consistent until the beginning of December and was comprised of colonies of the cyanobacteria *Microcystis*—which was what specifically formed the green algal clumps visible in the water, the diatom *Synedra*, and similar amounts of the cyanobacteria *Anabaena* and the diatom *Aulacoseira* (Figure 3.4a). A large number of other genera were observed to be present, but not dominant (Table 3.4). From December to March *Microcystis* still remained the most dominant genus, but was closely followed by an increasing *Aulacoseira*. The dominance of *Anabaena* considerably decreased, and there was also less of *Synedra* and a greater dominance of some green algae genera Figure 3.4a). Additionally, the diatom genus *Achnanthes* started to be observed in November (Table 3.4), and was occasionally noted as a dominant genus until the end of the first field season. When sampling was resumed in May 2011, *Achnanthes* completely dominated the algal community (Figure 3.4a), while other cyanobacteria, diatoms and green algae were also noted (Table 3.4). Over the next couple of months *Microcystis* became more dominant, but was still not as abundant as *Achnanthes* (Figure 3.4a).

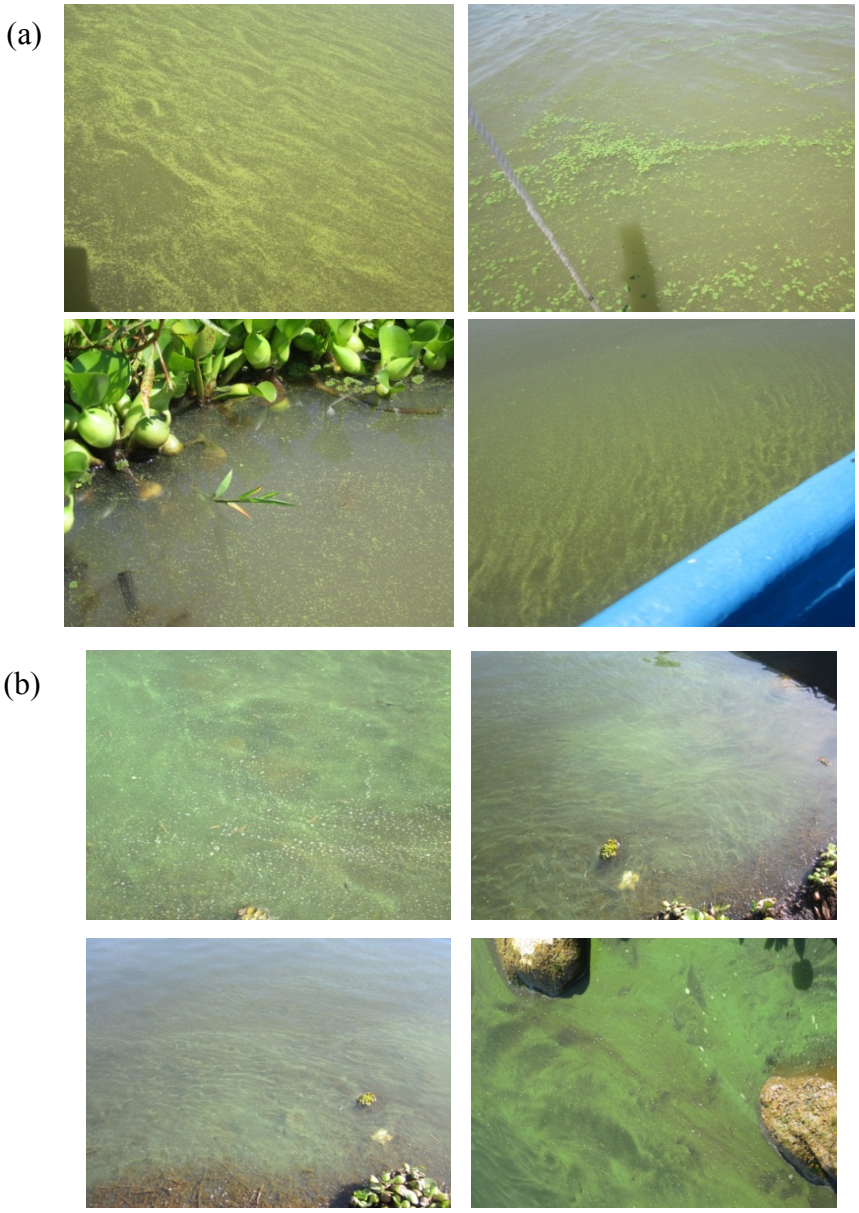

Figure 3.3. Cyanobacterial blooms in Lake Naivasha. (a) Lake-wide bloom present in the Main Lake basin from August 2010 to March 2011. (b) Shoreline bloom present in the Crescent Island basin in the morning hours of May 19, 2011.

Table 3.2. Qualitative observations for the Main Lake basin of Lake Naivasha. Qualitative observations represent a summary of the characteristics at the majority of sites around the lake for the time period.

Time Period	Water colouration	Algal presence or absence	Other observations and notes
1-Sept-2010 to 15-Nov-2010	Murky green water	Visible surface algae or bloom at most sites - Dense, soupy, foamy, foul odours at some sites beginning late-October	Occasional dense water hyacinth (*Eichhornia crassipes)*
22-Nov-2010 to 20-Dec-2010	Murky green water	Visible surface algae or bloom at most sites - Dense and foamy at some sites	Occasional dense water hyacinth cover
29-Dec-2010 to 15-Mar-2011	Murky green or brown water	Visible surface algae or bloom - Dense or foamy at many sites	Regular scattered or dense water hyacinth cover
15-May-2011 to 8-Jun-2012	Murky green-brown water Turbid	No visible surface algae	Regular scattered hyacinth/ salvinia cover in open water, occasionally dense and patchy - Access to North Shore limited because vegetation all concentrated in the area.

Table 3.3. Qualitative observations for the Crescent Island basin of Lake Naivasha. Qualitative observations represent a summary of the characteristics at the site for the time period.

Time Period	Water colouration	Algal presence or absence	Other observations and notes
1-Sept-201 to 4-Oct-2010	murky green	No visible surface algae	
11-Oct-201 to 22-Nov-2010	murky dark green	No visible surface algae	
29-Nov-2010 to 13-Dec-2010	murky green to dark green	No visible surface algae	Dense water hyacinth cover on 6-Dec-2010
20-Dec-2010 to 15-Mar-2011	murky green	Visible surface algae - Foamy- 25-Jan-11 - Dense- 31-Jan-11, 1-Mar-11 to 15-Mar-11	
18-May-2011 to 8-Aug-2011	murky dark green	No visible surface algae*	Water is definitely greener and less turbid than the Main Lake basin

*on May 19, 2011 on a shoreline area (not the regular sampling site) of Crescent Island, a bloom was briefly present for a few hours in the morning. The water was fairly clear, but there were dense surface algal clumps that were a deep green colour.

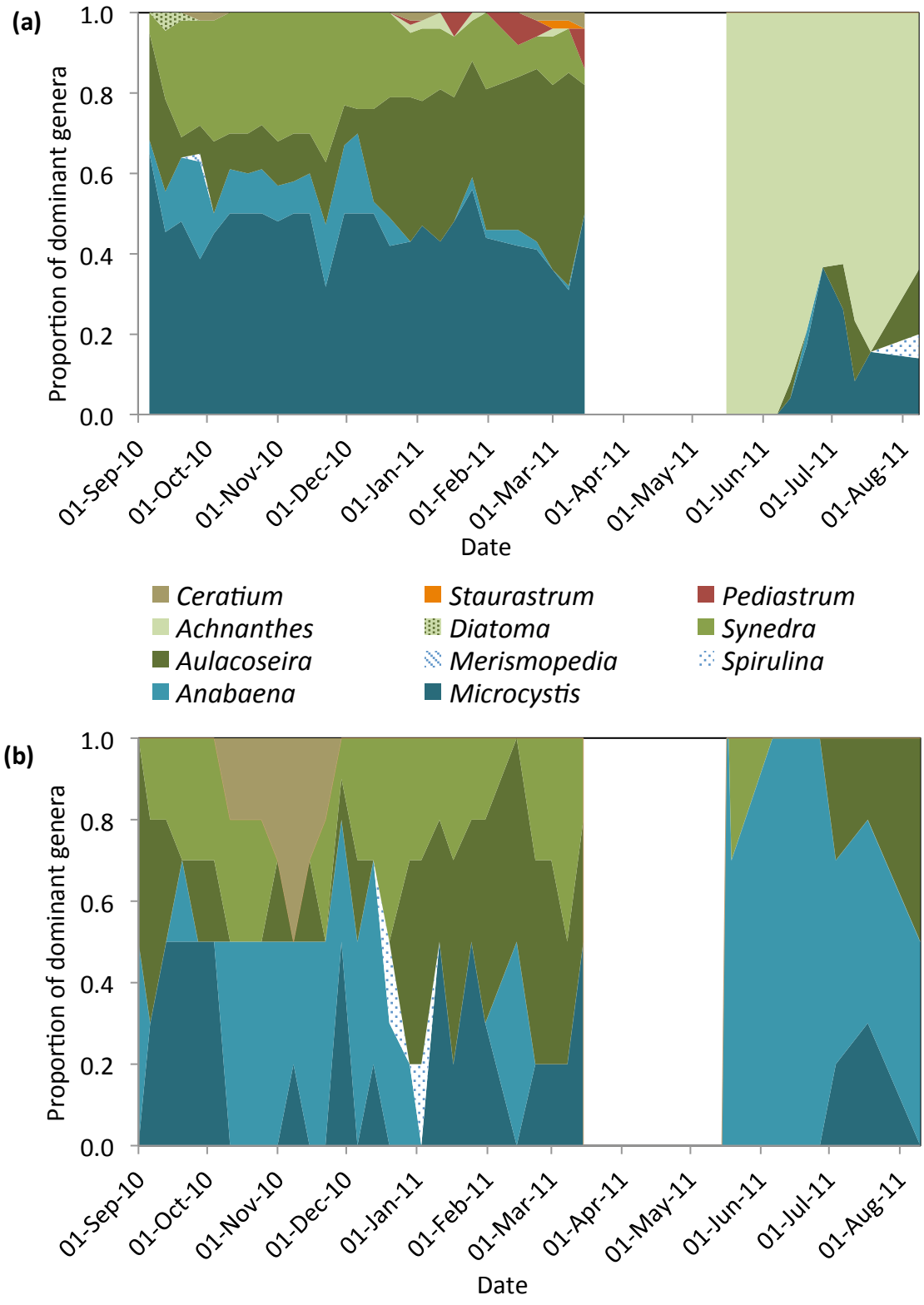

Figure 3.4. Community compositions of the dominant genera present over the study period. (a) Main Lake basin of Lake Naivasha and (b) Crescent Island basin of Lake Naivasha. The gap of white represents a period where no sampling was taking place.

Table 3.4. Other non-dominant algal genera present for the Main Lake basin of Lake Naivasha.

Time Period	Other Cyanobacteria Genera	Other Bacillariophyceae (Diatom) Genera	Other Chlorophyceae (Green Algae) Genera	Dinoflagellate Genera
1-Sept-2010 to 15-Nov-2010	*Anabaena* *Merismopedia* *Anacystis*	*Aulacoseira* *Synedra* *Diatoma* *Achnanthes Nitzshia*	*Pediastrum* *Staurastrum* *Scendesmus* *Coelastrum* *Closteriopsis* *Eremophaera* *Chlorococcum* *Actinastrum* *Planktosphaeria*	*Ceratium*
22-Nov-2010 to 20-Dec-2010	*Microcystis* *Anabaena* *Meismopedia* *Anacystis*	*Aulacoseira* *Diatoma* *Achnanthes Nitzshia*	*Pediastrum* *Staurastrum* *Euglena*	*Ceratium*
29-Dec-2010 to 15-Mar-2011	*Anabaena*	*Synedra* *Diatoma* *Achnanthes*	*Pediustrum* *Scenedesmus* *Straurastrum*	*Ceratium*
15-May-2011 to 8-Aug-2011	*Microcystis* *Aphanocapsa* *Anabaena* *Merismopedia* *Anacystis*	*Aulacoseira* *Synedra*	*Pediastrum* *Scenedesmus*	

Table 3.5. Other non-dominant algal genera present for the Crescent Island basin of Lake Naivasha.

Time Period	Other Cyanobacteria Genera	Other Bacillariophyceae (Diatom) Genera	Other Chlorophyceae (Green Algae) Genera	Dinoflagellate Genera
1-Sept-2010 to 4-Oct-2010	*Anabaena* *Merismopedia* *Spirulina*	*Diatoma* *Aulacoseira*	*Coleastrum* *Pediastrum* *Staurastram*	
11-Oct-2010 to 22-Nov-2010	*Microcystis*	*Aulacoseira* *Synedra* *Nitzshia*	*Pediastrum* *Actinastrum* *Planktosphaeria* *Chlorococcum*	
29-Nov-2010 to 13-Dec-2010	*Spirulina*			*Ceratium*
20-Dec-2010 to 15-Mar-2011	*Anabaena* *Spirulina*		*Pediastrum* *Spirogyra*	*Ceratium*
18-May-2011 to 8-Aug-2011	*Microcystis*	*Aulacoseira* *Synedra*	*Pediastrum*	*Ceratium*

The community composition in the Crescent Island basin of the lake was more dynamic than the Main Lake basin. While some genera common in the Main Lake sampling sites were dominant (*Microcystis, Anabaena, Aulacoseira, Synedra*), the proportions were different (Figure 3.4b) and variances could also be seen within the non-dominant genera recorded (Table 3.5). In October and November, there was dominance by *Ceratium* and *Anabaena,* with the later maintaining a strong presence until January (Figure 3.4b). *Microcystis* had intermittent spikes of dominance up until January (most notably on November 29, 2010), and became more consistently dominant after January when the presence of *Aulacoseira* climbed (Figure 3.4b). *Anabaena* once again dominated starting in May 2011 and while *Microcystis* was present but not dominant for most of Field Season 2 (Figure 3.4b; Table 3.5), the shoreline bloom that occurred on May 19, 2011 was equally dominated by both *Anabaena* and *Microcystis*. *Achnanthes* was also not observed in any capacity in the Crescent Island basin

Although algae could only be determined to the genus level, microscope images suggested that there were different species and/or isolates present of *Microcystis* (Figure 3.5) and *Anabaena* (Figure 3.6) and potentially *Aulacoseira* (Figure 3.8). Images of *Synedra* (Figure 3.9) and *Achnanthes* (Figure 3.7) were not as visually diverse. Images of some of the other common algae present in Lake Naivasha are displayed in Figure 3.10.

3.5. Levels of algal pigments

3.5.1. Main Lake basin

The chlorophyll concentrations at various sites in the main lake basin are presented in Figure 3.11. Chlorophyll-*a* concentrations varied over the study period and during Field Season 1, when there was a lake-wide cyanobacterial bloom, concentrations widely ranged from 40-1400 µg/L. There were a few instances where extremely high chl-*a* values (>1000 µg/L) were captured in September and in October 2010; however overall, the highest concentrations at the majority of sites occurred throughout October and November 2010, and generally fell between 300-700 µg/L. An overall shift in chl-*a* concentrations took place around approximately mid-November and on November 22nd, there was a noticeable decrease in chl-*a* concentration at nearly all sites compared to previous weeks (Figure 3.11a). From this point up until the end of Field Season 1in

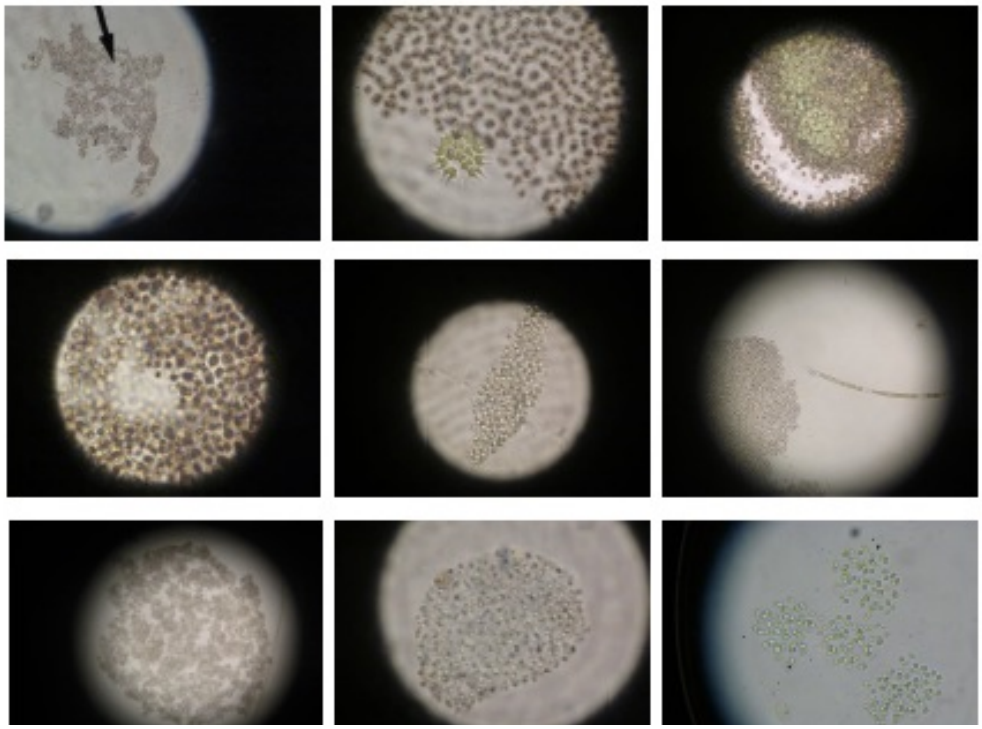

Figure 3.5. Microscope images of *Microcystis* spp. in Lake Naivasha. *Pediastrum* sp. (top middle) and *Aulacoseira* sp. (middle right) are also pictured.

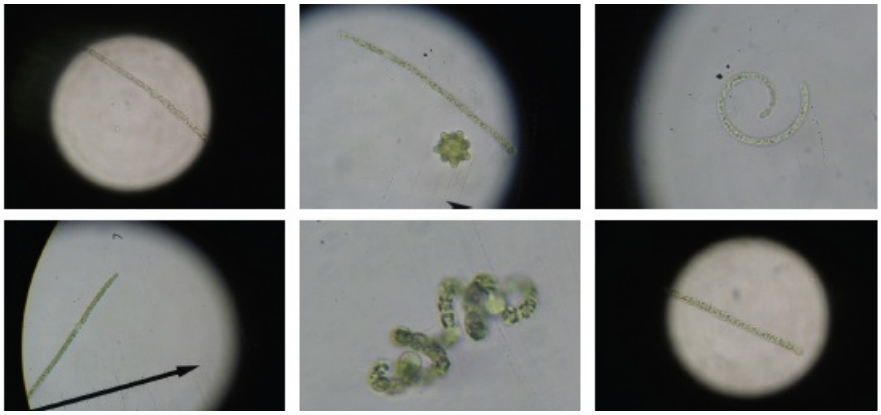

Figure 3.6. Microscope images of *Anabaena* spp. in Lake Naivasha. Also pictured is *Coelastrum* sp. (top middle).

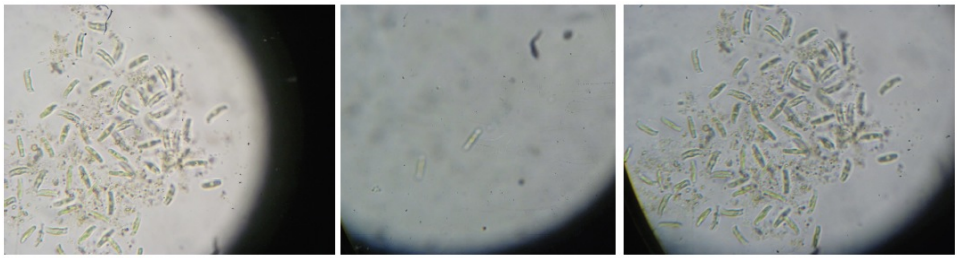

Figure 3.7. Microscope images of *Achnanthe*s sp.

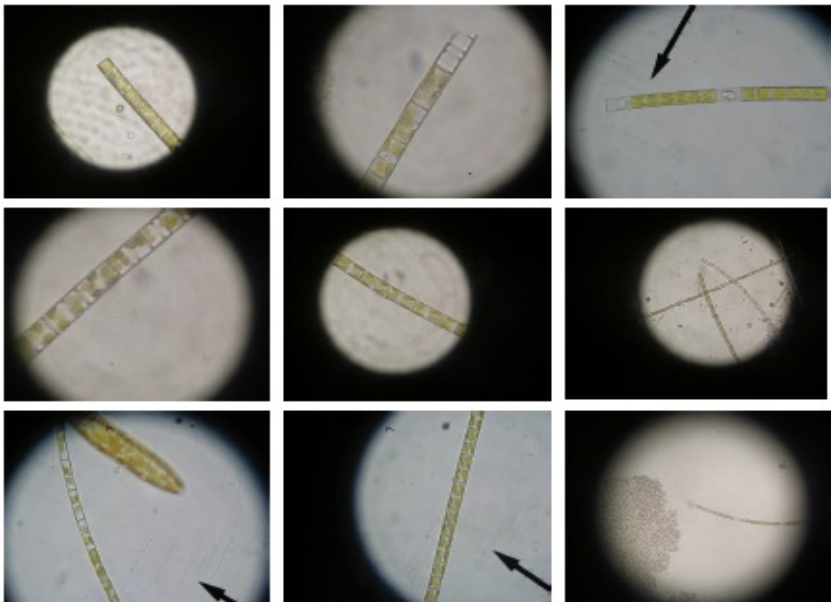

Figure 3.8. Microscope images of *Aulacoseira* spp. Also pictured are *Diatoma* sp. (bottom left) and *Microcystis* sp. (bottom right).

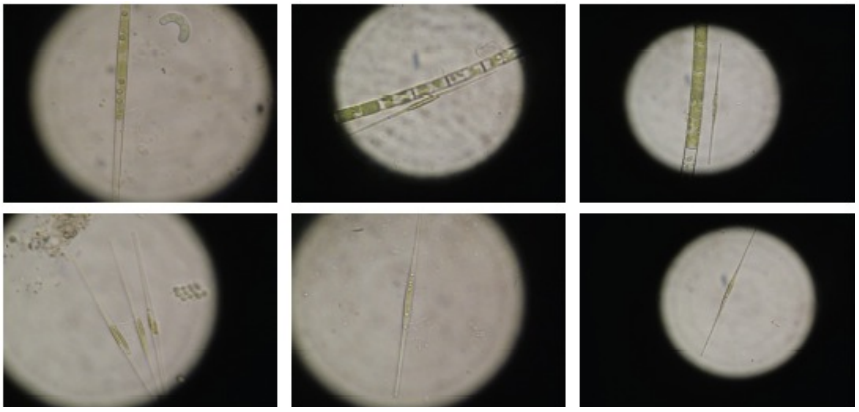

Figure 3.9. Microscope images of *Synedra*. Also pictured are *Spirulina* (top left) and *Aulacoseira* (top middle and right).

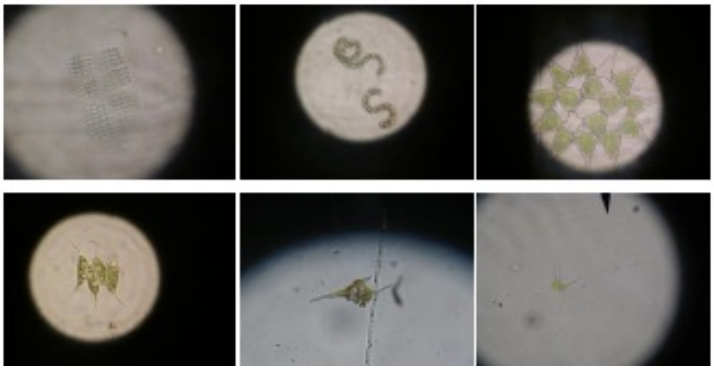

Figure 3.10. Microscope images of other Lake Naivasha algae. Top row (L-R): *Merismopedia* sp., *Spirulina* sp., *Pediastrum* sp. Bottom Row (L-R): *Scenedesmus* sp., *Ceratium* sp., *Staurastrum* sp

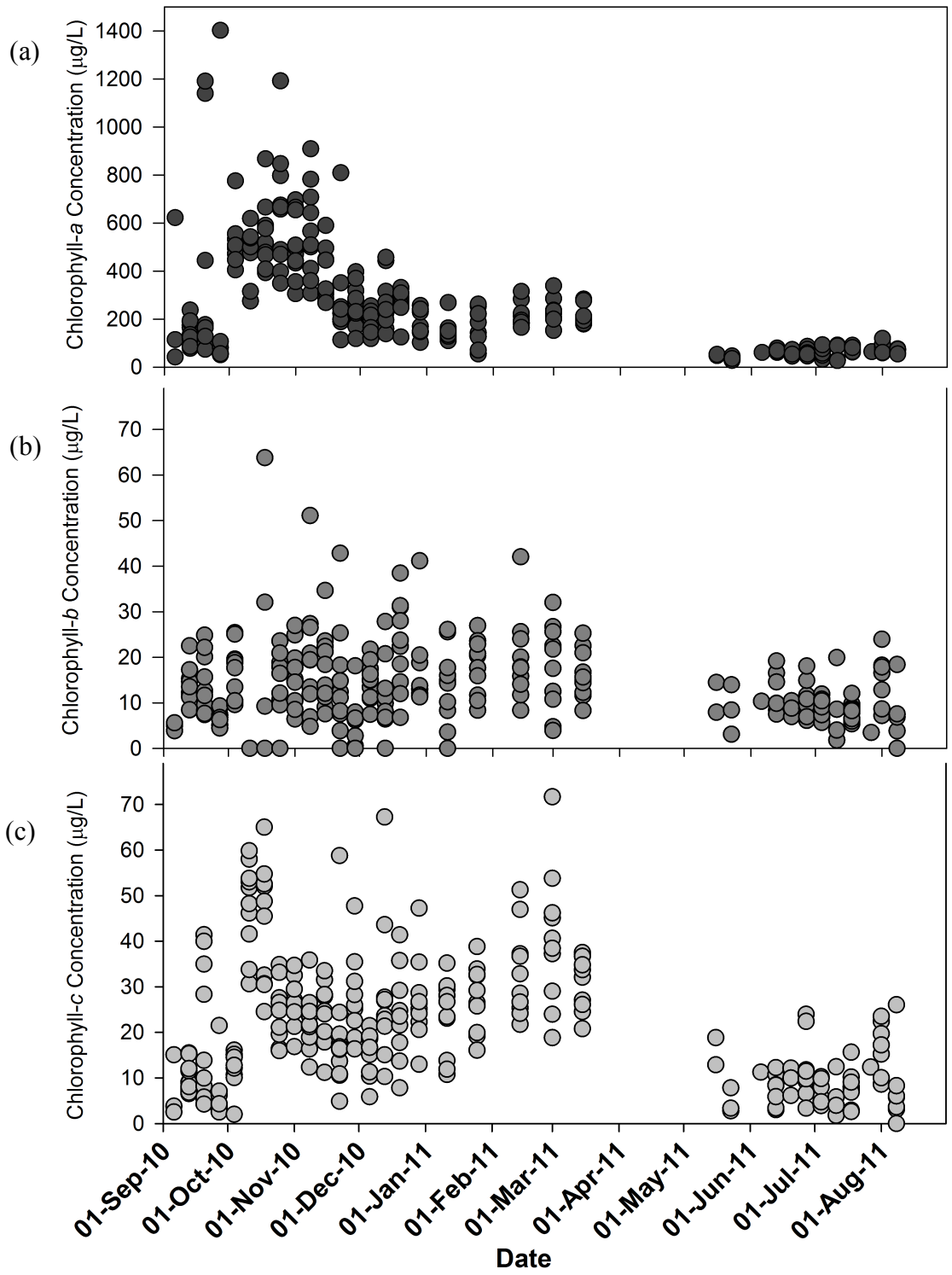

Figure 3.11. Extracted pigment levels in the Main basin of Lake Naivasha. Each point represents a different sampling site in the lake on that given day (ranging from N=3 to N=12). (a) Chlorophyll-*a* concentrations, (b) Chlorophyll-*b* concentrations, and (c) Chlorophyll-c concentrations. Note the different y-axis scales. Total points on each graph = 279)

March 2011, the chl-*a* levels remained similar from week-to-week with slight increases in the last month of sampling. During May to August 2012, when there was no lake-wide cyanobacterial bloom, the chl-*a* concentrations were overall relatively lower (27-120 μg/L) but the maximum concentration generally increased over the time period (Figure 3.12a, inset).

In general there was no noticeable trend in chlorophyll-*b* over both field seasons (Figure 3.11b). Most concentrations on a given day generally fell within the same range of 5-30 μg/L, with concentrations during Field Season 2 being slightly lower overall. During October when chl-*a* was high, chl-*b* concentrations were very low, and often 0 at many sites (Figure 3.11b).

Chlorophyll-*c* was elevated for two weeks in October and remained fairly steady with a slight decrease for the remainder of October and November (Figure 3.11c). Chl-*c* concentrations seemed to gradually increase from week to week starting in December, which was particularly noticeable trend with the minimum values from each week. On December 6th 2010 the minimum chl-*b* concentration was 5.87 μg/L, and that minimum increased almost every week and was 21 μg/L on March 15, 2011 (Figure 3.11c). There was no evident trend in chl-*c* from May to August 2011, although values were more elevated during the second last week of sampling and concentrations were lower overall compared to Field Season 1 (Figure 3.11c).

The primary accessory pigment associated with changes in chl-*a* concentrations was phycocyanin. Overall, phycocyanin was elevated during the October and November 2010, and while there were some instances of extremely high values (> 1500 μg/L), most concentrations ranged from approximately 200-1200 μg/L during this period (Figure 3.12b). The highest peak in phycocyanin (2642.72 μg/L) did not correspond with the maximum chl-*a* concentration measured (Figure 3.12a,b). Similar to chl-*a,* on November 22nd there was also a major decline in phycocyanin concentrations at nearly all sites. Maximum values gradually declined and were usually less than 350 μg/L for the remainder of the calendar year. From January to March, phycocyanin concentrations remained fairly stable and generally less than 150 μg/L (Figure 3.12b). From May to August 2011, overall phycocyanin concentrations were lower than the previous field season, but there did seem to be a slight trend of the values increasing over the time

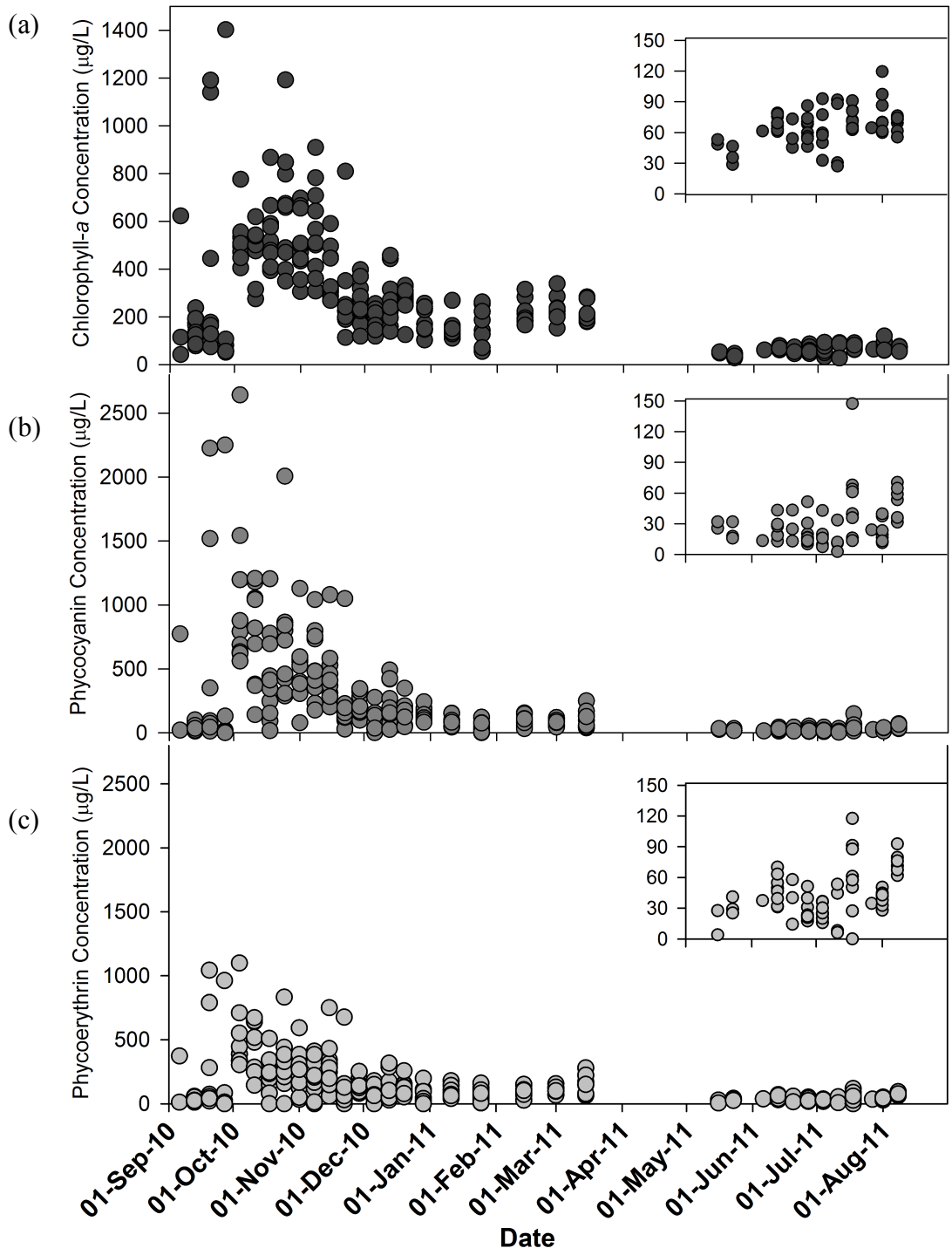

Figure 3.12. Extracted pigment levels in the Main basin of Lake Naivasha. Each point represents a different sampling site in the lake on that given day (ranging from N=3 to N=12). (a) Chlorophyll-*a* concentrations (total points on graph = 279), (b) Phycocyanin concentrations (total points on graph = 276), and (c) Phycoerythrin concentrations (total points on graph = 276). Inset on each graph are the data from May to August 2011, displayed on a more defined axis.

period (Figure 3.12b, inset).

The overall trend of phycoerythrin was the same as phycocyanin although up until January 2011, phycocyanin was generally greater than phycoerythrin (Figure 3.12c). Starting in January 2011, the levels of both these pigments were quite similar, and from May to August 2011 phycoerythrin seemed to be slightly greater overall in addition to also having that small increasing trend also observed with phycocyanin (Figure 3.12c, inset).

3.5.2. Crescent Island basin

In Crescent Island basin the chlorophyll-*a* concentrations reached a maximum (2760– 2830 µg/L) from October 11, 2010 to October 25, 2010 (Figure 3.13a). There was a sharp decline in chl-*a* levels at the start of November, although the overall values were still considerably high. After a slight increase to 785 µg/L a couple of weeks later, chl-*a* concentrations continued to decrease into January to 126 µg/L and only small variations were measured until mid-March which was the end of the first field season (Figure 3.13a). From May to August 2011, chl-*a* was lower and less variable with concentrations ranging from 50-65 µg/L (Figure 3.13a).

Chl-*b* was generally stable between 10-25 µg/L during Field Season 1, except for the period of October 4, 2010 to November 22, 2010, where it and peaked and dipped over the weeks (Figure 13.13b). This variable period coincided with the general period when chl-*a* was greatest, indicating a change in algal speciation.

Chl-*c* concentrations were generally greater than Chl-*b* during Field Season 1, and similar to chl-*a* peaked from October 11 to 25 with extreme relative highs from 400-490 µg/L (Figure 3.13c). The period of this chl-*c* increase also coincides with the dominance of the dinoflagellate *Ceratium* in the basin (Figure 3.4b). During Field Season 2, the concentrations of chl-*b* (3-9 µg/L) were higher than chl-*c* (0.00-5 µg/L) on weekly basis, although both pigments were considerably lower and less variable than during Field Season 1 (Figure 3.13 b and c).

Similar to chl-*c* and chl-*a*, phycocyanin was at its highest from October 11 to 25, however during these three weeks these high concentrations were more variable, reaching an extreme high of 7230 µg/L and a low of ~1900 µg/L (Figure 3.14b). This period of

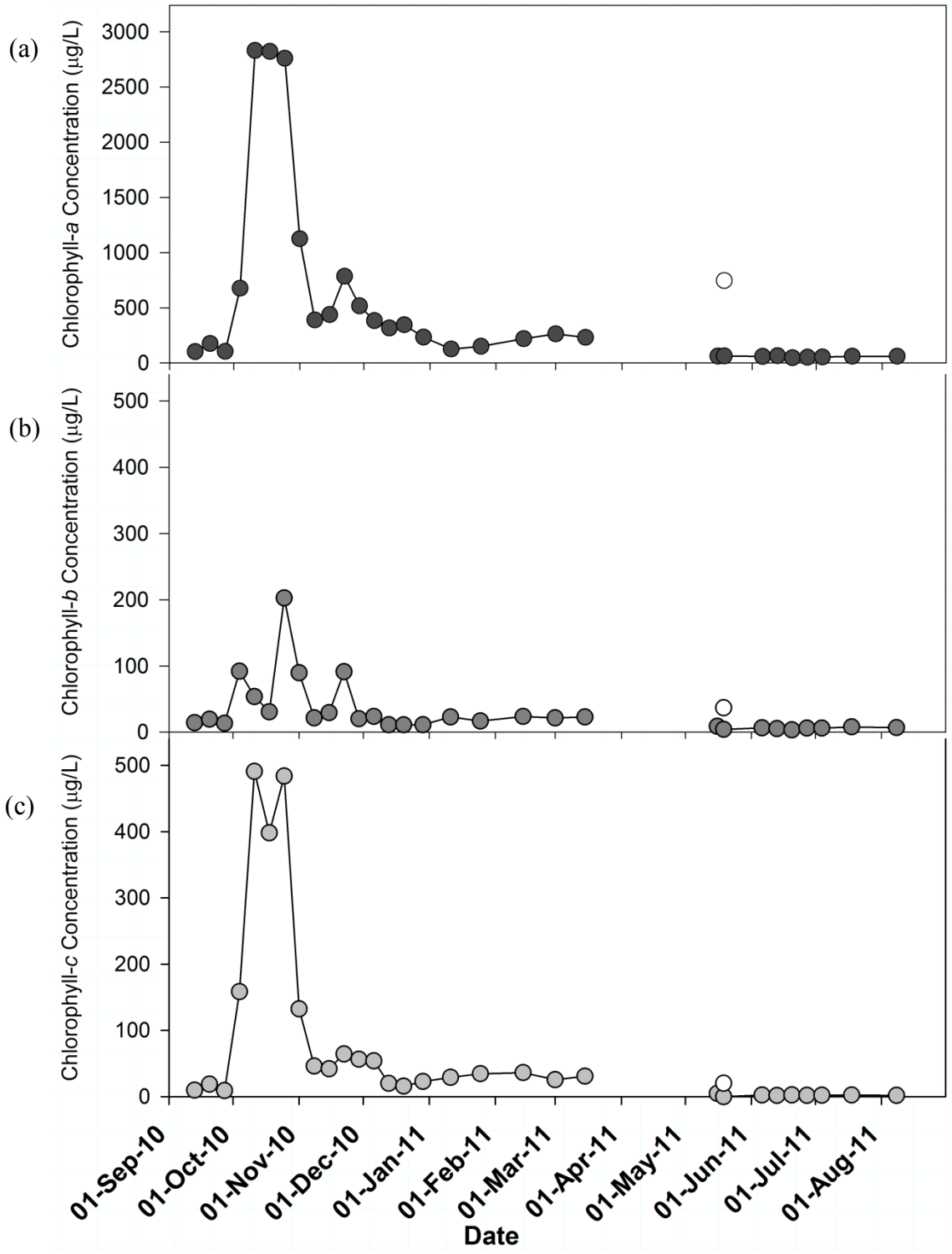

Figure 3.13. Extracted pigment levels in the Crescent Island (C.I.) basin of Lake Naivasha. Only one site was sampled in C.I. on a given day, with the exception being on May 19, 2011 (represented by the -o- symbol) when a sample from a shoreline cyanobacterial bloom was taken. (a) Chlorophyll-*a* concentrations, (b) Chlorophyll-*b* concentrations, and (c) Chlorophyll-*c* concentrations. Note the different y-axis scales for (b) and (c) which are 6 times smaller than (a). Total points on each graph = 31.

elevated phycocyanin occurred at the same time of *Anabaena* dominance in Crescent Island (Figure 3.14b). After the peak phycocyanin period ended on November 1, concentrations fluctuated but generally ranged from approximately 100-180 μg/L (Figure 3.14b). Phycocyanin concentrations remained fairly stable from May to August 2011 ranging from approximately 40-55 μg/L for all but one week (Figure 3.14b). The trends in phycoerythrin were similar to phycocyanin, with the exception that the phycoerythrin concentrations were generally lower, most notably during the peak period (Figure 3.14c).

The shoreline bloom that occurred in Crescent Island on May 19, 2011 had an elevated chl-*a* concentration (745 μg/L) compared to the sample from the regular sampling location (Figure 3.13a). This increase in biomass, visually evident due to the bloom, was microscopically determined to be due to fairly equal proportions of *Microcystis* colonies and *Anabaena* filaments. A small amount of the *Synedra* filament was also seen. While all pigments were understandably greater in the bloom compared to the regular sampling site (Figure 3.13 and 3.14), the largest relative increase was in phycocyanin concentration, which increased by a factor of about 21.

Compared to the Main Lake basin, overall trends in chl-*a* were similar in Crescent Island from September 2010 to March 2011 (Figure 3.11a and 3.13a). However, during May to August 2011, chl-*a* remained steady in Crescent Island (Figure 3.13a), but was more variable and showed a slight increase over time in the Main Lake (Figure 3.11a). The trends and peaks in phycocyanin were also similar between the Main Lake and Crescent Island except that Crescent Island had a maximum phycocyanin concentration that was far greater than the maximum in the Main Lake. Similar to chl-*a,* from May to August 2011, the levels of phycobiliproteins were generally more stable over time compared to the slight increases observed in the Main Lake basin (Figures 3.12, 3.14).

3.6. Pigment correlations

The relationship between chl-*a* and each of the pigments can demonstrate relative dominance of each pigment (and therefore group of algae) as well as show which group may be controlling the overall biomass. In the Main Lake basin during Field Season 1, when a cyanobacterial bloom was present, chl-*a* was most strongly correlated to

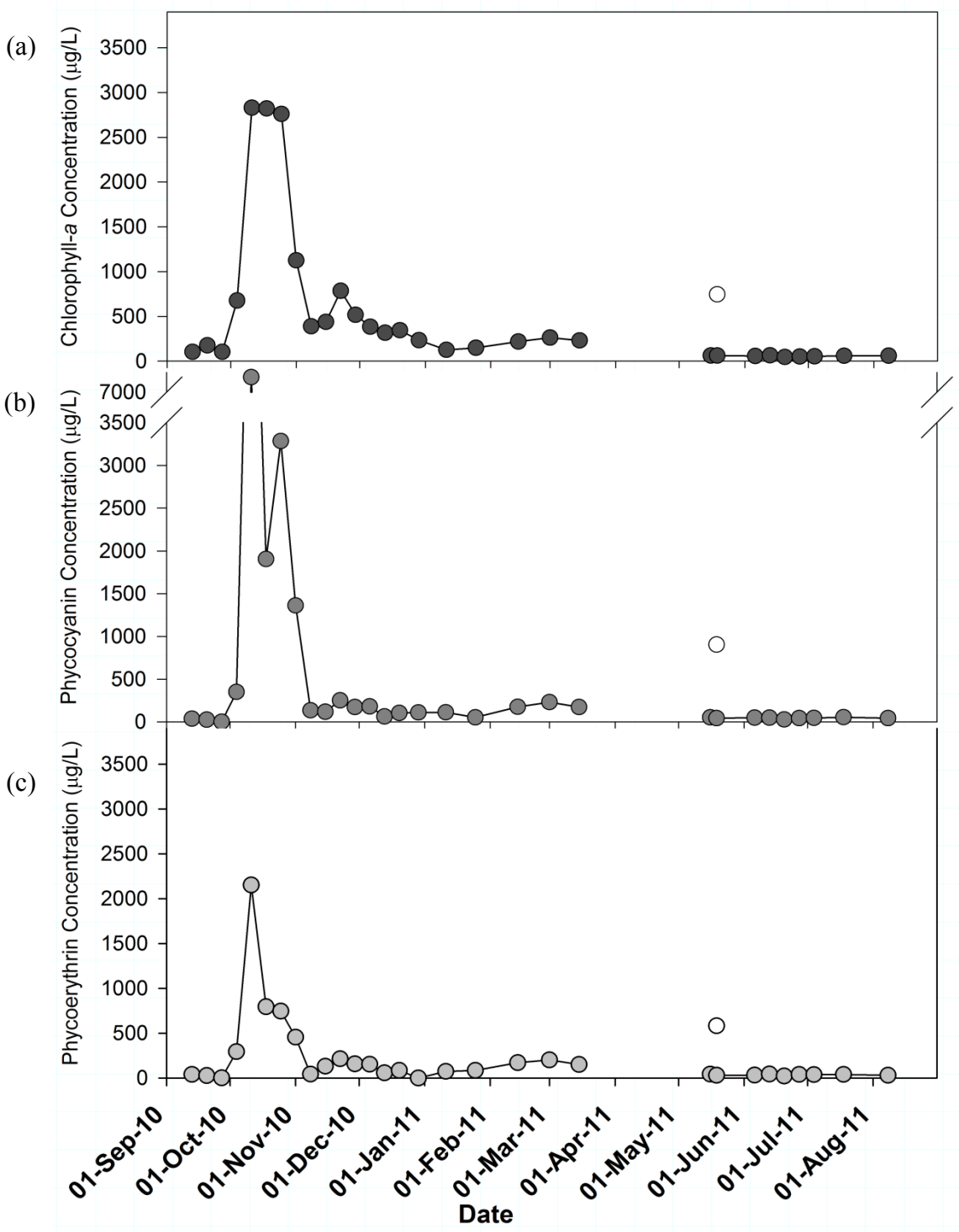

Figure 3.14. Extracted pigment levels in the Crescent Island (C.I.) basin of Lake Naivasha. Only one site was sampled in C.I. on a given day, with the exception being on May 19, 2011 (represented by the -o- symbol) when a sample from a shoreline cyanobacterial bloom was taken (a) Chlorophyll-*a* concentrations, (b) Phycocyanin concentrations (note the break in the y-axis), and (c) Phycoerythrin concentrations. Total points on each graph = 31.

phycocyanin (Correlation Coefficient (R) = 0.86, $p < 0.001$, N = 214). Chl-*c* was weakly correlated to chl-*a* (R = 0.39, $P < 0.001$, N = 216) and there was no significant relationship between chl-b and chl-*a*. During Field Season 2, when no cyanobacterial bloom was present, there was no significant correlation between chl-*a* and phycocyanin and chl-*a* and chl-*c*, and a weak correlations between chl-*a* and chl-*b* (R = 0.39, $p < 0.05$, N = 63).

In Crescent Island, both phycocyanin (R = 0.83, $p < 0.001$, N = 21) and chl-*c* (R = 0.87, $P < 0.001$, N = 21) were strongly correlated with chl-*a* in Field Season 1, and chl-*b* was moderately correlated (R = 0.68, $p < 0.001$, N = 21). No significant relationships were seen between the pigments in Field Season 2.

3.7. Microcystin levels

3.7.1. Total particulate microcystin-LR equivalents

Microcystin levels were measureable in the lake indicating that the cyanobacterial bloom event was in fact a cyanoHAB. The concentrations of total particulate microcystin-LR (MC-LR) equivalents for both the Main Lake and Crescent Island basins are presented in Figure 3.15. Of the samples analyzed, the total concentration of particulate microcystin-LR equivalents generally decreased over the study period. The maximum concentration of intracellular microcystin-LR equivalents measured in the Main Lake basin was 0.11 μg/L (October 25, 2010) (Figure 3.15a). During the period up to and including November 15th 2010, (also the period of highest phycocyanin concentrations) all MC-LR equivalent concentrations were greater than 0.05 μg/L. A noticeable decline does occur on November 22, 2010, (range of MC-LR equivalents measured = 0.041 – 0.072 μg/L) but the relative difference in concentration ranges from previous weeks is not that substantial. The following weeks are characterized by similar or slight increases in MC-LR equivalent concentrations in the Main Lake basin, and eventually a gradual decline. From January to March 2011, the maximum MC-LR equivalent concentration was approximately 0.05 μg/L in the Main Lake basin. From May to August 2011, when no lake-wide bloom was present, all MC-LR equivalent concentrations measured were equal to or less than 0.01 μg/L (Figure 3.15a).

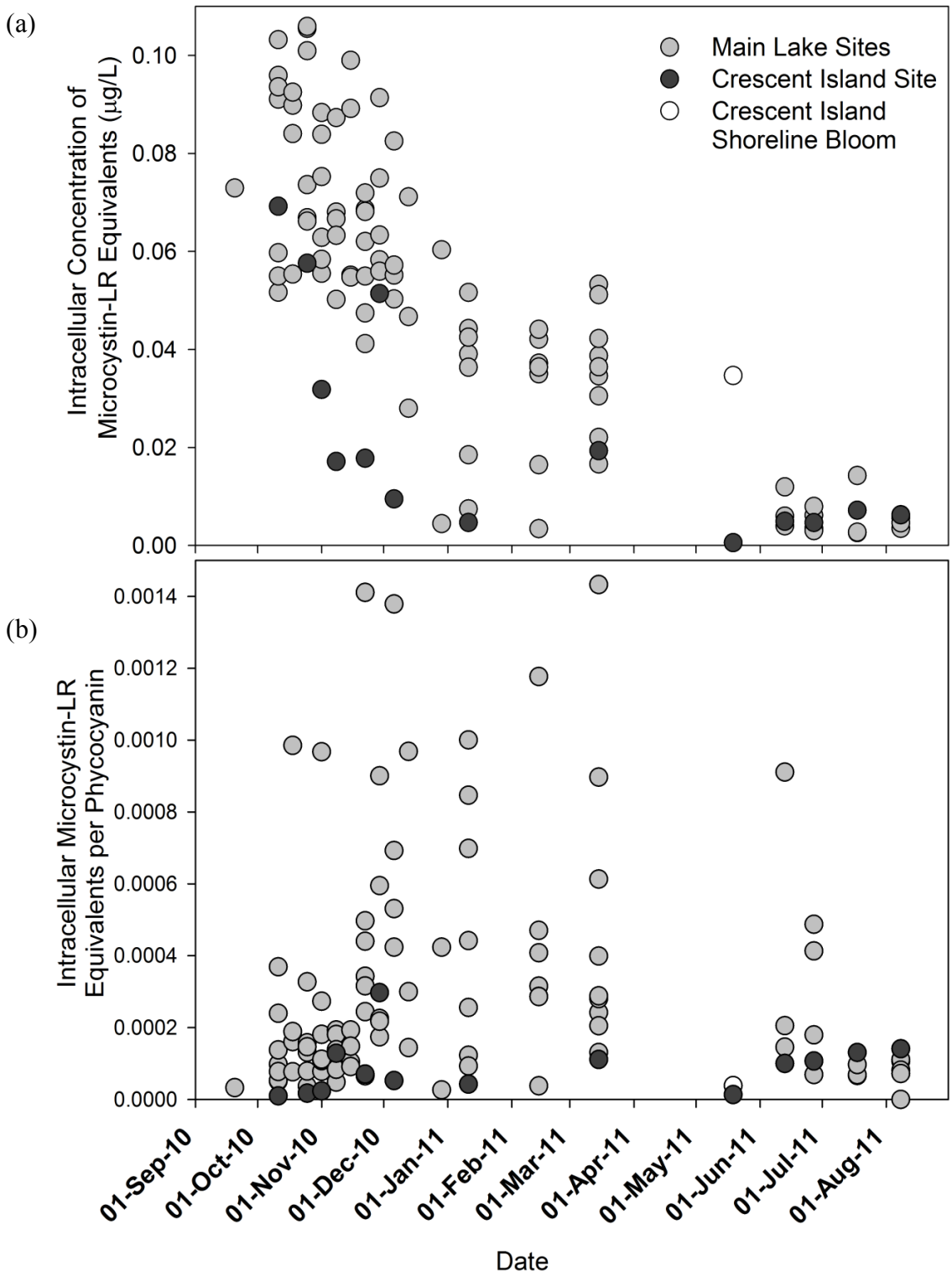

Figure 3.15. (a) Intracellular concentrations of microcystin-LR equivalents, and (b) Intracellular microcystin-LR equivalents per phycocyanin, in the Main Lake and Crescent Island basin of Lake Naivasha. Total points on graph for Main Lake Sites = 91, Crescent Island Site = 14.

During Field Season 1, the Crescent Island basin concentrations MC-LR equivalent were nearly always lower than the minimum measurement for the Main Lake basin (Figure 3.15a). The highest concentration was measured on October 11, 2010, and although this was the where the phycocyanin concentration was the highest recorded in this study (and nearly 3 times larger than the highest concentration recorded in the main lake), the concentration of MC-LR equivalents was 0.07 µg/L (Figure 3.15a). Concentrations proceeded to decline from this date, but did spike up for the week of November 29, 2010, when there was also an increase in *Microcystis* dominance (Figures 3.15a and 3.4b). From May to August 2011 concentrations were low and similar to the Main Lake basin. The shoreline bloom sample taken from the Crescent Island basin on May 19, 2011 had a MC-LR equivalent concentration of 0.03 µg/L, which was less than measurements taken during the previous lake-wide bloom but relatively high compared to other samples taken during Field Season 2 (Figure 3.15a).

3.7.2. Particulate Microcystin-LR equivalents per phycocyanin

Standardizing the microcystin concentrations to the phycocyanin concentrations for each sample emphasizes some other trends on a cellular basis (Figure 3.15b). In the Main Lake basin, the amount of particulate MC-LR equivalents per phycocyanin mostly remained within a tight range of values (0-0.0002) up until November 15, 2010 (Figure 3.15b). Starting November 22, 2010, this ratio becomes much more variable between the different sites on the Main Lake reaching as high as 0.0014 particulate MC-LR equivalents per phycocyanin (Figure 3.15b). The period from November 15 to 22 may therefore be characterized by a physiological change. Keeping in mind that there is only one site sampled, Crescent Island does not necessarily show the same pattern as the Main Lake basin. Each week, the ratio is relatively low, with the only major difference occurring on November 29, 2010 (Figure 3.15b).

3.7.3. Relationship between particulate microcystin-LR equivalents and phycocyanin

Figure 3.15b indicated that the relationship between the intracellular concentrations of MC-LR equivalents was not necessarily a simple or consistent one. The relationship between particulate microcystin and phycocyanin was not linear, and was best fitted with a hyperbolic curve ($R = 0.72$, $r^2 = 0.5$, $p < 0.001$, $N = 91$; Figure 3.16). At low levels of phycocyanin, particulate microcystin is very variable and increases quickly,

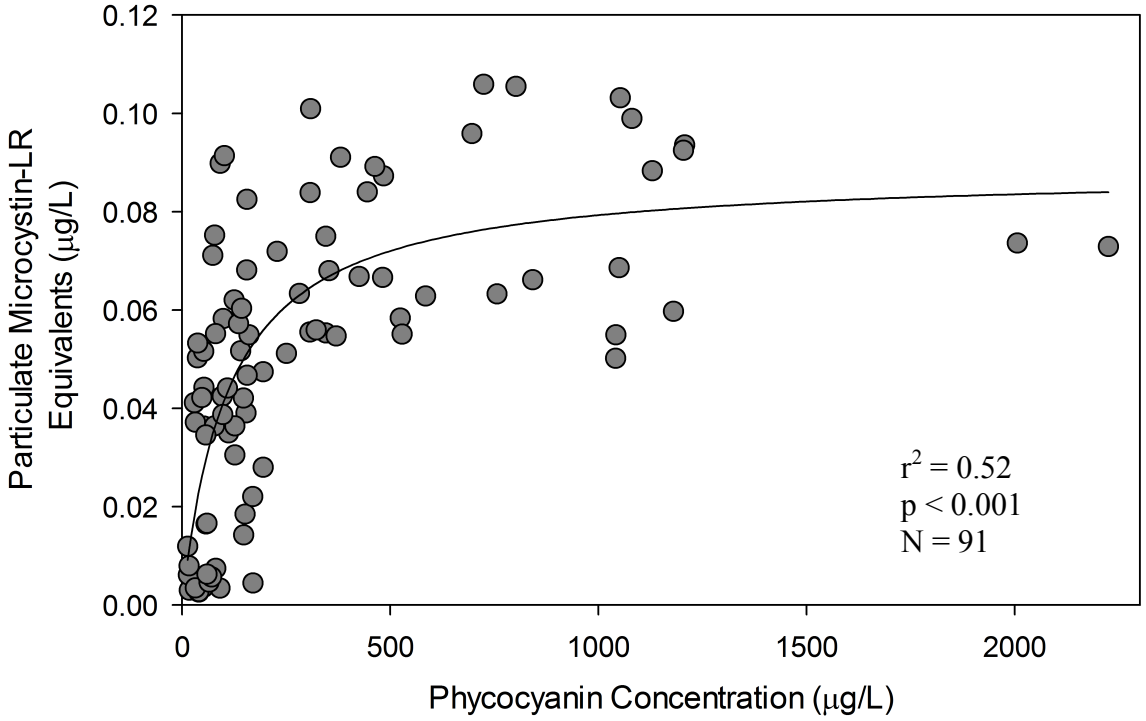

Figure 3.16. Particulate microcystin-LR equivalents vs. phycocyanin in the Main Lake basin of Lake Naivasha. Data are fitted to a hyperbolic curve, and show with a fairly strong and significant regression.

but after about 500 μg/L, the particulate microcystins seem to plateau, and specifically two there are two branches that plateau (Figure 3.16). Those two branches are primarily compromised of data points from the peak bloom period in October and November 2010 but there is not a clear difference between dates in the upper branch and lower branch (Figure 3.17a). Data from Field Season 2, when no bloom was present, can be seen in the cluster at the bottom left of the graph; although no bloom was present there was still production of microcystins that was much lower than the previous year (Figure 3.17a).

Specific sites are highlighted in Figure 3.17b. There was no major difference in the data distribution between the open water sites (Hippo Point, Middle of Main Lake basin) and the more isolated site (Oserian Bay) where cyanobacterial blooms or toxins could potentially accumulate. While phycocyanin was always relatively lower at the site near the Malewa inflow, particulate microcystins were widely variable (Figure 3.17b).

The relationship between particulate Microcystin-LR equivalents and phycocyanin in the Crescent Island basin of Lake Naivasha seems to show a similar trend inclining and plateau trend as the Main Lake basin (Figure 3.18). The data did not meet the assumptions of normality for regression analysis; therefore this trend can just be visually described at this point, and the curve is only present to help visualize the potential plateau.

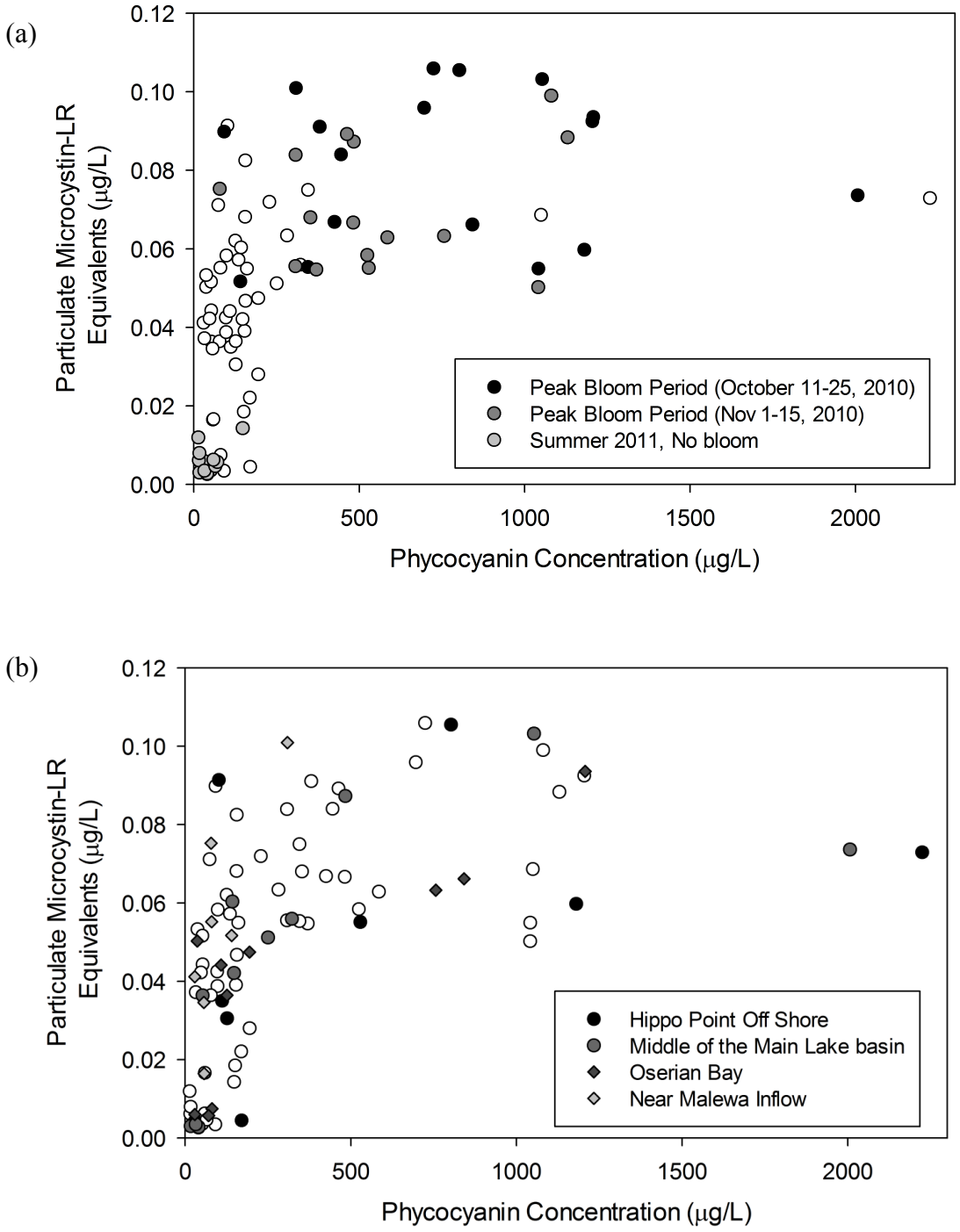

Figure 3.17. (a) Temporal patterns and (b) spatial patterns of particulate microcystin-LR equivalents vs. phycocyanin in the Main Lake basin of Lake Naivasha. Specific dates or sites are highlighted in the legend, and the white circular symbols represent all other data points.

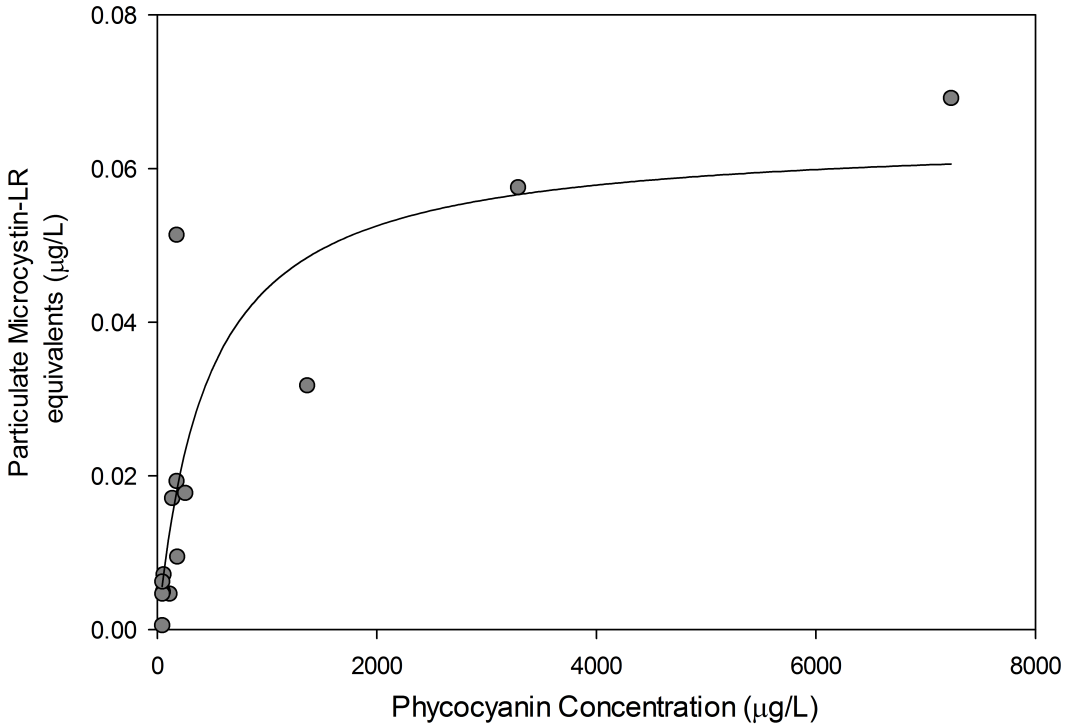

Figure 3.18. Particulate microcystin-LR equivalents vs. phycocyanin in the Crescent Island basin of Lake Naivasha. The curved line does not hold any statistical significant as data were not normally distributed and sample size was low (N = 14), but is there to show a potentially similar trend to the Main Lake basin (Figure 3.17).

4. DISCUSSION

Relative to the decades of anthropogenic change that have occurred in Lake Naivasha, cyanobacterial blooms are a recent occurrence, and this is the first study to focus on the initiators, characteristics and impacts of cyanobacterial blooms from an ecohealth perspective. The lake-wide cyanobacterial bloom studied from 2010 to 2011 was one of the most extensive to date and due to the detection of microcystin; it will now be referred to as a cyanoHAB event.

4.1. Factors influencing the cyanoHAB formation

Unlike the other lakes in the Rift Valley of Kenya that have supported blooms of cyanobacteria since at least 1929 (Beadle, 1932b; Jenkin, 1932; Rich, 1932), Lake Naivasha, which was sometimes dominated by cyanobacteria, only began to experience actual blooms starting in 2005 (Harper et al., 2011). The industry, population and development boom that occurred in the 1980's was the first large-scale initiator and led to increased eutrophication (Harper et al., 2011). Yet, given the timing of the blooms, eutrophication alone was obviously not enough to trigger the formation of cyanobacterial blooms. What actually led to the start of cyanobacteria blooms in approximately 2005 is out of the scope of this thesis, as there were many long-term ecological changes experienced in the lake over the past decades that could be responsible; however since that point what we do know is that cyanobacterial blooms have not occurred continuously.

Therefore, there must be very specific factors leading to each bloom event , and in the case of the lake-wide *Microcystis* cyanoHAB observed from 2010-2011 in this study, those drivers were a combination of primarily weather influences which began in 2009 with an extended drought in Naivasha (Figure 4.1). Although measurements of nutrient levels were not available, after the drought ended at the start of 2010, heavy rains came and the lake started to fill back up (Figure 3.2). The runoff from land, increased inflow from the Malewa River, and re-suspension of nutrients due to sediment disruption likely led to an increasing nutrient availability. After the El Niño rains of 1998 it was found that phosphorus loading transformed the lake to hyper-eutrophic for a period of time (Kitaka et al., 2002). While the rains in 2010 were not due to an El Niño period, the intensity of them could have led to a similar occurrence as in 1998.

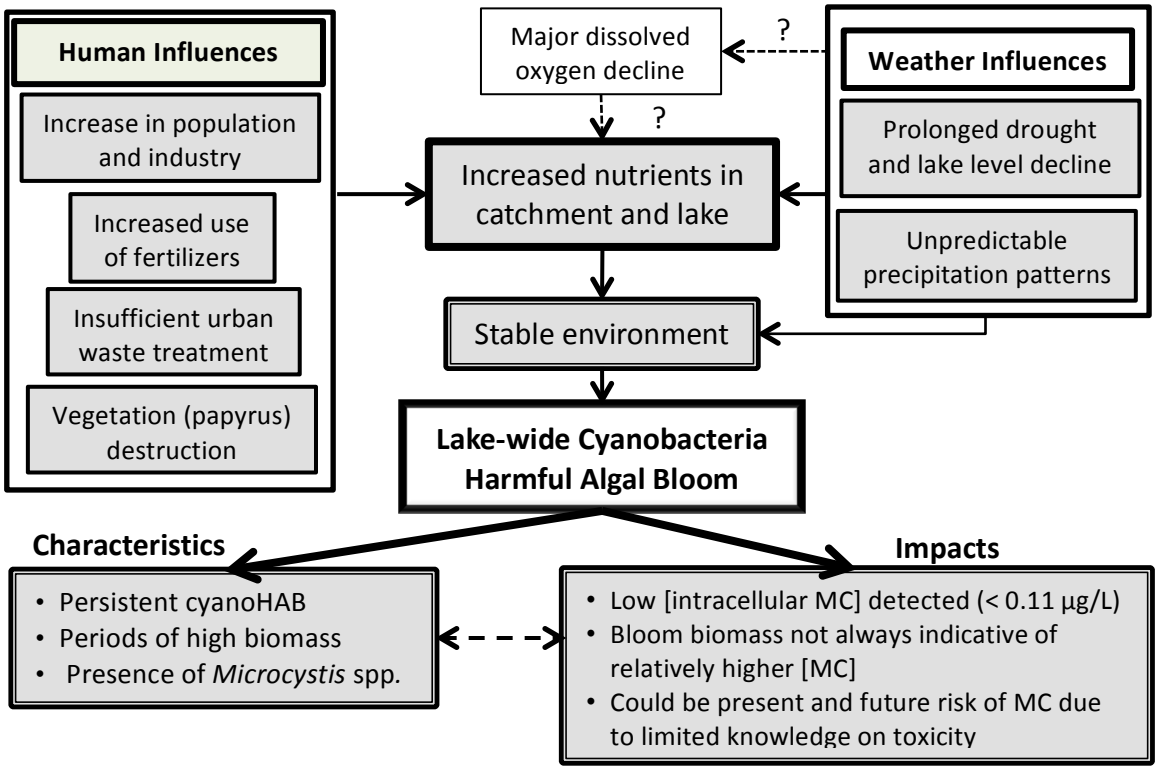

Figure 4.1. Influences, characteristics and impacts of cyanoHAB in Lake Naivasha.

Additionally, in February 2010 after the rains began and just prior to the major lake level increase there was a substantial decline in lake DO (also causing the major fish kill). Whether this de-oxygenation led to a large release of phosphorus and iron bound in the sediment (as predicted by Hubble and Harper (2002b)) cannot be determined without more information on productivity and biological oxygen demand; however, considering the importance of both iron and phosphorus to cyanobacteria growth, in addition to the historical decline of DO minima over time (Creed, unpublished data) this cannot be ruled out as an influencing factor to nutrient availability and warrants further study.

Despite all these potential routes for nutrient elevation, no cyanobacterial bloom was observed in May 2010 (Trick pers. comm. 2010); however in August 2010 the bloom was present. The key period and final factor occurred throughout June, July and August of 2010. After those many months of continuous rainfall and lake level elevation (Figure 3.2) that brought in essential nutrients to the water column, there was then a three-month period of stability and decreased rainfall (Figure 3.2). This is where cyanobacteria would have had a competitive advantage due to their buoyancy and advantage in stable environments (Paerl et al., 2001). In summary, while increased rainfall and lake level rise impacted nutrient availability, cyanoHAB formation was more dependent on the changes in those factors which in this instance, led to a stable environment. Unfortunately, in Naivasha these weather influences are very unpredictable, and therefore the occurrence of large-scale bloom events may be hard to predict.

4.2. Characteristics of cyanoHAB and other algae in Lake Naivasha

The clumps of algae from the cyanoHAB in the Main Lake basin that could be observed with the naked eye were made up of the genus *Microcystis* (Figure 3.5). The strong correlation between chl-*a* and phycocyanin indicate that the cyanobacteria played the largest role in the biomass changes. Despite the presence of the cyanoHAB, there was still substantial dominance of the diatom genera *Aulacoseira* and *Synedra* (Figures 3.7 and 3.8), although they had less of a relationship with chl-*a* changes. Cyanobacteria are known for their efficient nutrient uptake mechanisms that can also give them advantages in nutrient limited conditions, but diatoms do not have such abilities (Paerl et al., 2001). The dominance of both these groups of algae supports the suggestion that all nutrients

were elevated in the water column. Furthermore, *Microcystis* is generally considered to grow poorly in low nutrient conditions compared to other cyanobacteria genera; therefore the saturation of nutrients would be favourable for *Microcystis* proliferation over other genera (Finni et al., 2001).

A remarkable feature of the algae during the study period and particularly while the cyanoHAB was at its peak, was the levels of pigments measured that were used as indicators of biomass (Figures 3.11-3.14). These elevated levels, which are not generally seen in temperate lakes, are likely due to the consistently high temperature and radiation of the tropics (Figure 3.1). These levels are similar, however, to some tropical lakes that do consistency experience cyanobacterial blooms, such as Lake George, Uganda (Ganf, 1974). In comparison, the chl-*a* concentrations in the pristine lakes of the Muskoka region of Ontario rarely reach over 10 μg/L (Hutchinson 2012). Given the baseline chl-*a* concentrations seen in Naivasha in the past, the present levels of pigments measured both during the cyanoHAB (September 2010 to March 2011) and when there was no cyanobacterial bloom (May–August 2011) are consistent with a tropical eutrophic system. Chlorophyll values in 2011 as communicated by N. Pacini to Harper et al. (2011) were consistent with those measured in this study.

Once the cyanoHAB established itself in the lake, it persisted for an extended period of time. Biomass was highest from September to November, but after that period, on a weekly basis there were signs of bloom decomposition that were marked by more dense and foamy algae at the surface. The foam is representative of a large amount of proteins and organic matter being released and mixed in the water from lysed cells (BC Ministry of Environment, 2010), and it is common for more cells to migrate closer to the surface during the final stages of a bloom due to increased gas vacuoles and/or the decline in light from self-shading (Reynolds and Walsby, 1975). Nevertheless, although these signs were seen by the end of November, the bloom was still present up until March 15 and this persistence can be attributed to 1) the tropical climate and lack of major seasonality and 2) the elevated or constant flux of nutrients to support long term algal growth and 3) the ability of cyanobacteria to use mechanisms of nutrient uptake and storage, which may have been utilized in the final stages of the cyanoHAB, allowing it to persist for even longer.

Sometime during the period where sampling was not taking place (March 16-May 13, 2011) the bloom completely dissipated, and come May, the community composition was drastically different. We found that it was dominated by the diatom *Achnanthes (*Figure 3.4) which was also identified to the species level as *Achnanthes minutissima* (personally communicated by N. Pacini to Harper et al., 2011). While *Achnanthes* was occasionally present in the water during the bloom period (Table 3.4), it was observed more frequently as a non-dominant genus from the beginning of 2011. *Achnanthes minutissima* is a widespread diatom and is generally associated with low or moderate nutrients and organic pollution in lakes (Kelly et al., 2005), therefore its dominance indicates a change in the state of the lake compared to the previous year.

4.3. Impacts of the cyanoHAB and microcystin levels

4.3.1. Microcystin exposure from drinking water

All concentrations of particulate MC-LR equivalents measured in this study were at least 10 times lower than the WHO provisional guideline for maximum concentration of total microcystins in water (1.0 μg/L) (Figure 3.19a). However, these particulate concentrations represent under-estimates, as dissolved microcystin concentrations were not measured in this study due to the logistical issues of transporting water samples back to Canada. During a natural bloom the majority of microcystins will be found within intact cells as the bloom develops, but when a bloom begins to senesce there may be a greater amount of free microcystins present due to them being released from lysed cells (Sivonen and Jones, 1999). That being said even though Park et al. (1998) observed a relatively greater amount of free microcystins at the end of a bloom (>20%), the levels were still low compared to intracellular microcystin. So, when large declines in biomass are observed, there is a greater concern for high levels of dissolved microcystins being released from cells all at once, however free microcystins are also subject to a rapid factor of dilution in water especially in well mixed areas that are not enclosed or isolated. In this study, the months of September to November 2010, represent a period of high net growth in the Main Lake basin and so the intracellular concentrations measured were probably quite close to what total concentrations would be. But the biomass decline from November 15th to 22nd indicates that there may have been a relatively greater amount of

dissolved microcystins that had been released from cells and so this may be a point where particulate concentrations were likely underestimates of total microcystins.

Overall, even if the free microcystins could have been factored in, it is unlikely that total microcystin levels would have been much higher than that 1.0 μg/L guideline. This is a positive outcome as it indicates that the cyanoHAB occurrence did not pose any short or moderate term impacts on health through consumption of drinking water.

Knowledge on the long-term effects of microcystin is limited at this point. In particular, the literature is expanding on the carcinogenic impacts of chronic low-level microcystin exposure and studies continue to assess whether microcystin may impact more vulnerable populations (such as expectant mothers, children, and those with pre-existing conditions) in different ways (Kuiper-Goodman at al., 1999). Of certain importance is also that some of the symptoms of microcystin poisoning—stomach pain, nausea, vomiting, fever and muscle weakness—may present similar to malaria and typhoid, which are common ailments in Sub-Saharan Africa and so exposure to the toxin may not be fully realized. Due to the fact that cyanobacteria blooms are not continually occurring in the lake, impacts of chronic low-level exposure may also be minimal, but will be dependent on the occurrence of future potential bloom events.

4.3.2. Other impacts and routes of exposure to microcystin

Microcystin can have effects on various aquatic organisms, further disrupting the trophic web interactions and potentially fish health (Paerl et al., 2001; Ibellings et al., 2005) Cyanotoxins have been shown to cause rapid mortality and impact growth and reproduction in zooplankton, and this sensitivity increases with increases in water temperature (Paerl et al., 2001). Fish do not generally graze cyanobacteria directly, so the risk of intracellular (and therefore high) toxin exposure is minimal, although there is evidence that some fish kill events may have been due to microcystin (Penaloza et al., 1990; Tencalla et al., 1994). More common is the possibility for detrimental effects involving oxidative stress, growth and vital organ damage in juvenile fish due to microcystins and growth, deformations, development and oxidative stress impacts on developing embryos and larvae (Malbrouck and Kestemont, 2009). These effects have also been specifically observed in common carp (Zambrano and Canelo, 1996; Li et al., 2004; Palikova et al., 2004).

There is also the concern of microcystin exposure through consumption of fish. Numerous studies have observed bioaccumulation of microcystin and while accumulation most often takes place in the liver, intestine or gut (parts of the fish generally not consumed), a number of studies have observed microcystin concentrations in muscle tissue above (Freitas de Magalhaes et al., 2001; Xie et al., 2005; Malbrouck, 2009) and below (Adamovsky et al., 2007) the WHO limits for human consumption (0.04 μg/kg of body weight per day).

The low concentrations of microcystin measured in this study may indicate that the risk of bioaccumulation in fish is low. Additionally, the benthic and omnivore diet of common carp also limits the access of the fish to microcystin, although common carp have been shown to accumulate low microcystin (Adamovsky et al., 2007).

4.3.3. Pattern of microcystin levels

Since the levels of particulate microcystin were low, indicating no current risk to health, focus could shift to understand the pattern of microcystin levels over the course of the bloom, to try to predict what the toxin levels might be like in the future. In particular does microcystin help to initiate the bloom or maintain the bloom?

As predicted, the total particulate microcystin was higher during the presence of bloom versus absence (Figure 3.16a). Some phycocyanin levels from Field Season 2 (when no bloom was present), were similar to phycocyanin measurements from Field Season 1 when the cyanoHAB was occurring (see PC < 125 μg/L; Figure 3.17). But while those cyanobacteria biomass levels may have been similar, the particulate microcystin levels were not and there is a gap in the data points from about 0.20-0.35 μg/L of particulate microcystin. This could potentially represent the role of microcystin in helping to physically form large colonies and/or visible clumps of algae, whether that is through manipulating extracellular proteins as mentioned in the introduction (Kehr et al., 2006; Zilliges et al., 2008), or by some other means. This has implications for bloom initiation as there may always need to be a particular baseline of microcystins to allow for physical bloom formation.

Since phycocyanin is fairly constant between cells, standardizing the particulate microcystins to the phycocyanin levels allows examination of the changes in toxins on a per cell basis (Figure 3.16b). Particulate microcystin per unit biomass was relatively

lower for the period when the bloom was on the incline (up to November 15), but starting November 22, the toxin per cell seems to go up on decline of the bloom.

Although it was predicted that there would be increased microcystin production per cell during the later stages of the bloom (decline) due to growth stressors, the data also indicate that there could be some process limiting microcystin per cell during the incline period, therefore neither of these possibilities cannot be ruled out. This figure indicated that the amount of toxin per cell was not constant or linear over the study period, which was against what was predicted and further explored in Figure 3.17.

This non-linear relationship between microcystin and phycocyanin is characterized by a plateau at the high phycocyanin concentrations (representing the incline of the bloom) and also the high concentrations of microcystin are represented by a wide range of phycocyanin. Likewise within a tight range of phycocyanin values there is a wide range of particulate microcystin levels. These all make it very difficult to be able to predict the levels of microcystin based on solely cyanobacteria biomass. Distinguishing various dates during peak bloom biomass and certain geographical sites, also did not show a clear cluster or pattern (Figure3.18a,b) indicating that temporal or spatial characteristics are not the sole influence on the data.

We can speculate on some of the processes that could be causing these patterns. This includes both ecological and physiological processes that could be acting alone or these different influences could be acting in combination to give the response the data show. In the natural environment blooms are often made up of toxic and non-toxic strains (Sioven and Jones, 1999; Ranita-Kanto and Wilhem, 2006; Hotto and Joung, 2008). Given the low concentrations of toxin measured in this study, and the magnitude of the bloom, it could be that a non-toxic strain contributed more to the high biomass, and the changes in toxin per cell are more due to a shift in the community and proportions of non-toxic to toxic strains.

From a physiological standpoint, the plateau seen at the high concentrations of phycocyanin may be caused by the fact that during that peak biomass phase, cells were dividing so quickly that the rate of microcystin production could not keep up with the rate of cell division, and therefore microcystins were divided among many more cells and look as though they’ve reached a maximum. The relationship between microcystin cell

quota and growth rate was modeled and showed a similar plateau when there was no corresponding microcystin depletion rate (Jahnichen et al., 2008). It has been found that toxic cyanobacterial cells will always contain a minimum quota of microcystin per cell (why we measured low toxin production in cyanobacteria even without an actual bloom), but also have a maximum quota under nutrient saturation for a given light and temperature condition, which could correspond to the peak biomass plateau observed in this study (Long et al., 2001; Wiedner et al., 2003).

Another reason for the observed plateau that is not often considered in the literature is that microcystin could be actively exported from cells when a certain threshold is reached. This is not something widely supported as most scientific studies have not found any evidence for active release of the toxin: it is generally found that microcystins remain within cells until cell lysis. However, a few studies have indicated that microcystin can be present in the cell wall of intact cells (Shi et al., 1995), and that it may be exported at higher light threshold intensities (Kaebernick et al., 2000). Pearson et al., 2004, suggested that an ABC membrane transporter that is encoded by one of the *mcy* genes may in fact actively transport microcystin from cells, and further study is taking place.

The increased microcystin per cell evident at the decline of the bloom could be indicative of increased production for maintenance of the bloom due to nutrient and growth stress. In Lake Naivasha after the period of stability where the bloom established itself, it continued to grow in the following months as lake level continued to rise, which continually replenishing nutrients to the lake. When this continual lake level rise and increased rainfall ended in November, nutrient stores and availability in the lake may not have been replenished leading to some limitations, perhaps in iron. Iron limitation stimulating microcystin production is widely supported in the literature (Lukac and Aegerter, 1993; Utkilen and Gjolme, 1995; Sevilla et al., 2008; Alexova et al., 2011; Fujii et al., 2011; Kaplan et al., 2012), and is a hypothesis that should be explored further in Naivasha which naturally has iron-rich sediments due to the lateritic volcanic soils (Tarras-Wahlberg et al., 2002). Because of this, the nutrient influxes that helped establish the bloom could have been characterized by high soluble iron availability, and if over time iron became (relatively) less available due to decreased influxes or formation of

insoluble hydroxides, this could have led to the increased microcystin production per cell. It may be that the natural conditions of the Lake Naivasha region, which allow for relatively higher iron availability compared to other lakes, could be what keep microcystin concentrations at levels low enough not to pose a health risk—this should be a key area for future research on cyanoHABs in Naivasha.

4.4. Site specific considerations

4.4.1. Main Lake basin versus Crescent Island basin

Chl-*a* levels in the Crescent Island basin were high, but almost always lower than in the Main Lake basin. The major exception was during an approximate five week period from October to November where levels were considerably higher than the Main Lake. A similar pattern was observed with phycocyanin levels and chl-*c* and the sharp increases in those pigments were due to dominance of the cyanobacterium *Anabaena* and the dinoflagellate *Ceratium*, respectively. Despite the large increase in biomass, qualitative observations of the surface water indicated no visible algal bloom. The *in situ* chlorophyll fluorescence vertical profile (Appendix B) suggested that the large biomass was sitting approximately 1 m below the surface of the water, a depth that could not be seen from the surface of the water. Therefore, although it was predicted that the Crescent Island basin would be less likely to support a cyanobacterial bloom compared to the Main Lake basin, which is not entirely true. While the decreased wind-induced mixing would prevent distribution of nutrients, the potential for more stability and vertical stratification give some algae—such as the buoyant *Anabaena* and flagellated *Ceratium*—a chance to optimize their position in the water column and bloom below the surface. This is important because the Crescent Island basin is often referred to as an undisturbed and pristine area of the lake, but in fact certain water quality issues may be present similar to the Main Lake, but just not always directly observable.

Although not measured in this study, *Anabaena* is able to produce the neurotoxins anatoxin-a (Sivonen and Jones 1999; Carmichael, 2001) and saxitoxin—well known as a paralytic shellfish poison (Humpage at al., 1984; Al-Tebrineh at al., 2010)—which can also pose health risks. Another feature of this *Anabaena* is its ability to fix atmospheric

nitrogen into usable forms. The dominance of *Anabaena* at certain times in Crescent Island may indicate periods of more limited nitrogen availability (Paerl et al., 2001).

Since inflow from the Malewa largely only affects the Main Lake basin, and the majority of the Crescent Island basin border is relatively undeveloped, the main source of nutrients into the basin may be different from the Main Lake basin. For example there may be a greater influence due to re-suspension of nutrients from the swamp area leading to the Crescent Island basin that easily dries out. The outflow and waste from the defunct sewage treatment in the Northwest corner of the lake, enters into that shallow swampy area and may influence nutrients in the Crescent Island basin more than the Main Lake basin.

Particulate microcystin levels in the Crescent Island basin were almost always lower than all of the Main Lake sites on a given day (Figure 3.16a), indicating that the health risk of drinking water in the Crescent Island basin is even lower than the Main Lake basin. However, the potential for increased toxin production due to a *Microcystis* bloom was observed in the shoreline bloom that occurred in the basin on the morning of May 19, 2011. Compared to the regular sample from the middle of the Crescent Island basin the same day, particulate microcystins levels were 60 times greater in that shoreline bloom, although the level was still very low at 0.03 μg/L (Figure 3.16a).

4.4.2. Main Lake basin sites

A challenge was identified when comparing the various sites of the Main Lake basin due to the heterogeneity of the bloom. Although the lake is well mixed, the distribution of the bloom was not equal at all the sites, and from week to week there was also variability between where the highest biomass was found. This was due to the fact that algae move around the lake with wave action, wind and turbulence and it was impossible to separate those influences from specific site characteristics that might have actually been influencing biomass and toxins in this study. Unfortunately, although more detailed and intense sampling sites were added during Field Season 2, the lack of cyanobacteria and/or high biomass made it difficult to observe any relationships. However, there are a few points to make about two of the sites:

Near Malewa River Inflow (Site D)

This was the only site in the Main Lake basin that was consistently different from the others and this difference can be attributed to the constant inflow from the Malewa River. The water was generally more brown and turbid due to the loading of dissolved and particulate matter, and algal and cyanobacterial biomasses were overall lower. Despite the consistently low phycocyanin, particulate microcystin levels were widely variable at this site, ranging from 0.02-0.08 μg/L. Therefore the inflow seems to impact how well cyanobacteria can establish at this site, but other factors may be impacting the microcystin production.

Kamere public water collection area (Site M)

This is an area right on the southern shore where community members are often found filling up large jugs of water, whether it be for personal use or to sell. This site was only directly sampled during Field Season 2, although it is in close proximity to Site L (Kamere Beach) that was sampled during both field seasons. Although no cyanobacterial bloom was present during Field Season 2 biomass levels and community composition at this site were similar to the other sites in the Main Lake and it is recommended that this site continue to be monitored.

4.5. Do cyanoHABs pose a present or future health risk to the community?

Although there are some key areas where further research will enhance our understanding of the impacts of these cyanobacterial blooms and cyanoHABs in Naivasha, the data suggest that these cyanoHABs do not pose a health risk to the community with regards to drinking water toxins. Measured particulate microcystins were low in the 2010-2011 cyanoHAB, and while the impacts of chronic low-level exposure to microcystin are not well understood, cyanobacterial bloom events are not a regular occurrence in the lake. Besides the lake-wide cyanoHAB in 2010-2011 and the brief shoreline cyanoHAB in May 2011, our research team has not recorded any other cyanobacterial bloom event in the lake.

This study confirmed the production of microcystin in natural lake samples and observed that the controls to its production were not simple or straightforward. The dynamic nature of Lake Naivasha means that the potential for more cyanoHAB occurrences and increased toxin production might be possible albeit unpredictable. The

climate change forecast for the Naivasha region includes more extreme and unpredictable weather events such as drought conditions, followed by intense rainfall, followed by drought conditions: these would present the ideal conditions of nutrient influxes and stability for further cyanobacterial bloom events in the lake.

For decades, changes have been proposed for the better management of Lake Naivasha, and a number of suggestions in the next section echo similar words to those (Everard and Harper, 2002; Becht et al., 2005; Abiya, 2006; Harper et al., 2011)—only now the occurrence of cyanobacterial blooms can be added to the long list of the signs of Lake Naivasha's degradation. This continued repetition in the scientific literature just shows that the key to fulfilling these actions is within the community and not within the pages of limited access journal articles.

4.6. Knowledge-to-action

4.6.1. Continued monitoring

Going forward, further research on cyanobacterial blooms and cyanoHABs in Naivasha is necessary. In trying to fill knowledge gaps, it will be essential to first consider the past and present work being done by the numerous research groups studying Lake Naivasha. It may be that data on nutrient levels were collected during 2010 by another research group and so collaborations and sharing of collective knowledge must be a priority for all those interested in the well-being of the lake. By doing this, we will be better informed to effectively carry out any further research necessary.

Future cyanoHAB events must be monitored. The dynamic nature of the lake was very evident throughout this study, and the drivers and impacts of future bloom events may lead to different conclusions. The lake level is currently the highest it has been in decades and the lake may have shifted to a different ecological state. This leads to a potential roadblock in that although we can continuously monitor lake characteristics, we may never find any consistency or predictability with respect to the influences and impacts of cyanobacterial blooms and cyanoHABs. In this study, for example, it would have been near impossible at the time to have predicted that those precise weather patterns would have occurred and caused a bloom. The next bloom event may be driven by similar or different factors. While this unpredictability may seem like a drawback, it should be used as motivation to implement long-term sustainable solutions to completely

eliminate the potential for cyanoHABs. If we were able to predict each bloom event, it may lead to a cycle of just utilizing short-vision solutions (artificial mixing techniques, use of algaecides) to deal with each occurrence as it came while the overarching causes remained unaddressed.

4.6.2. Prevention of cyanoHABs, implementing change, and sustainability

Sustainability is one of the main principles of ecohealth and in the case of Lake Naivasha, a sustainable lake will be one that can provide the community the necessary ecosystem services for many generations into the future. When examining the influences to cyanobacterial bloom production in Lake Naivasha (Figure 4.1), it is clear that there are some things that cannot necessarily be reversed, such as the pressures from the population increase or industry presence. Additionally, the unpredictable weather factors that led to the cyanoHAB in this study cannot be controlled at the community level. The key areas of intervention impacting eutrophication and cyanoHABs are actually areas that impact other ecological issues such as organic loading and persistent organic pollutants and metal contamination.

I. *Restoration of papyrus fringe.* The contribution of the loss of papyrus to eutrophication has been recognized in the literature (Harper and Mavuti, 2004). Various interventions to reestablish the papyrus swamp in the north part of the lake have been proposed and are in stages of implementation (Morrison and Harper 2009). It is thought that the major trophic changes in Naivasha are due to the influence of the wider catchment along the inflowing rivers (and not just the immediate lake surroundings) (Kiataka et al. 2002) and so restoring the papyrus in that North swamp would moderate the nutrient transfer into the lake that allows for the higher biomass, particularly in the Main Lake basin.

II. *Regulation of catchment-wide farming practices*. The increased use of fertilizers and pesticides in the entire catchment needs to be regulated to ensure that runoff from farms contains minimal contaminants. More advanced and sustainable irrigation and pest control practices should be implemented to ensure that nutrients cannot enter the lake through runoff channels.

III. *Functioning urban sewage treatment*. The extent of the impact of human waste, from the growing Naivasha town , that enters the lake through the non-functional

sewage treatment plant has not been quantified, but it may substantially impact nutrient influxes to the Crescent Island basin and Main Lake basin. The status of the sewage treatment plant upgrade and repairs is still unclear, and therefore it could continue to play a role in future bloom or cyanoHAB events.

4.6.3. Education, engagement, and empowerment

A challenge faced in understanding cyanoHABs in the lake is that we are limited to what we have been able to observe. We were able to learn a lot about cyanoHAB dynamics through this study of one major event in 2010-2011, but we cannot say for sure whether these drivers and impacts will be the same every time. The Ontario Ministry of the Environment has a program in which citizens can report the occurrence of algal blooms and the Environmental Monitoring and Reporting Branch will collect and process samples and the information is compiled into a database representing all lakes and reports (Winter et al. 2011). The collection of all this information has led to the understanding of important trends in bloom timing and distribution in Ontario. Implementing a similar system in Naivasha may prove to be worthwhile, as it could bring about more information on cyanobacterial blooms in the lake, and potentially help in our understanding of the patterns and extent of their occurrences. The fishermen who work out on Lake Naivasha 8 months out of every year could be valuable contributors to a program like this.

Key groups to target in knowledge translation are children and youth. They will be the next generation of people capable of implementing the necessary changes in Naivasha, but unfortunately they also do not know what their water resource was like before development and increased eutrophication. This concept of environmental amnesia (in this case across generations) comes from the simple fact that the baseline that the younger generations have to compare Naivasha's health to is not the pristine lake that it once was, but rather a lake that, to them, began as a polluted and degraded ecosystem.

Overall, the major steps to promoting change is making sure that the community members are aware of the different environmental concerns around the lake and are properly informed of anything that might impact them, including cyanoHAB occurrences. Social media can play a huge role in this because although internet access may not be something regularly available for everyone, one thing that has massively expanded in

East Africa is cell phone usage, and mass communications (often through free or affordable text messaging services) are widely used and available. Using social media to promote greater awareness and understanding about water quality will foster greater empowerment, and by engaging the community in this way, it may provide the motivation needed for positive change to occur in Naivasha.

5. CONCLUSIONS

- **A lake wide cyanoHAB bloom occurred in 2010.** The bloom was made up of the cyanobacterium *Microcystis* but there was still a large presence of diatoms (*Aulacoseira* and *Synedra*) in the lake indicating that this was an instance where all nutrients were relatively high to support communities of both algae.
- **Factors that influenced bloom initiation** included the 1) overall eutrophication of Lake Naivasha; 2) prolonged drought (2009) followed by increased rains and lake level, and physical sediment disruption (Jan to May 2010) and; 3) stability from June-Aug 2010, providing conditions ideal for cyanobacterial dominance.
- **Particulate microcystins were detected** in the lake, and concentrations were lower than the World Health Organization Provisional Guideline of 1μg/L indicating no immediate health threat from drinking water.
- **Factors controlling microcystin** production are not straightforward, but indicate that physiological processes related to **nutrients and stressors to growth** may need to be considered more fully.
- The substantial presence of *Anabaena* is something that should be monitored as this genus is known for its **neurotoxin production** including anatoxin-a and saxitoxin.
- With erratic and unpredictable weather and climate events predicted, there is **potential for increased occurrences of major cyanoHABs in the future**.
- Since certain factors are unpredictable (weather conditions) or difficult to reverse (population increase, industry presence), interventions need to focus on the causes of eutrophication that can be controlled at the community/government level which include **restoring the papyrus fringe, ensuring proper sewage and waste treatment, and decreasing pesticide and fertilizer use in the entire basin.**

- **Knowledge translation, community education, engagement and empowerment** will be necessary in implementing these better management practices of Lake Naivasha's ecosystem services, and will ultimately contribute to enhanced health and well-being of the lake and community.

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7. APPENDICES

7.1. Supplements to pigment determination methodology

Phycocyanin extraction efficiency

Extracting chlorophyll pigments from glass fiber filter is a well-established method known to be efficient; however extraction of phycobiliproteins with this method is less common. To ensure that the extraction and analysis from a GF/C filter provided accurate values for phycocyanin, a cyanobacteria culture of *Microcystis* strain 299 was used to test that efficiency. Varying volumes of culture were used to represent a concentration gradient of 100%, 50%, 25% and 12.5% of phycobiliproteins. The representative volume was centrifuged (10 min. 5000g) down to a pellet and also filtered onto a GF/C filter. The filter was prepared as described in Section 2.21 and both the filter and pellet were extracted according to the methods outlined in Section 2.22. A comparison of the phycocyanin concentrations measured from the pellet and the filter are below (Figure 7.1).

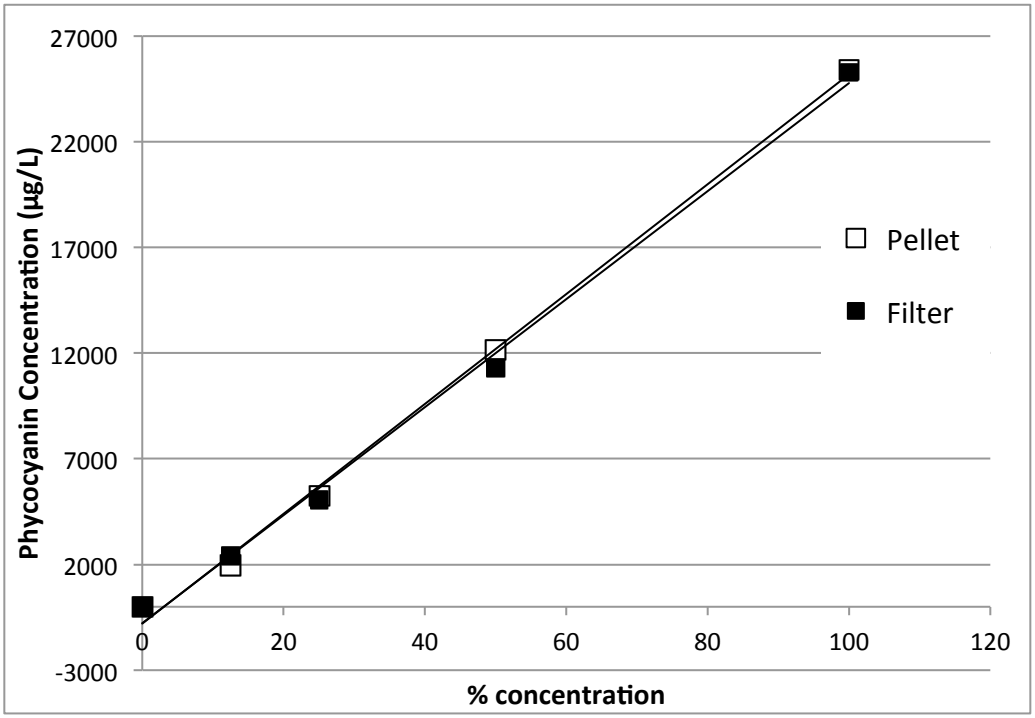


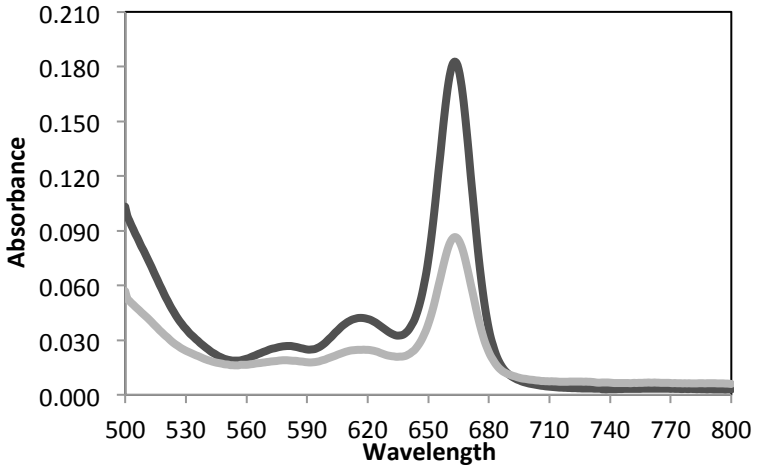
Figure 7.1. Phycocyanin extraction of *Microcystis* strain 299 from a pellet and filter.

Absorbance wavelength peaks and scans

Table 7.1. Absorbance wavelengths for determination of pigment concentration. Absorbance values were corrected for turbidity/light scattering by subtracting the background absorbance at 750 nm.

Wavelength (nm)	Importance
750	Turbidity of sample
664	Absorbance peak for chl *a*
647	Absorbance peak for chl *b*
630	Absorbance peak for chl *c*
620	Absorbance peak for PC
545	Absorbance peak for PE

(a)



(b)

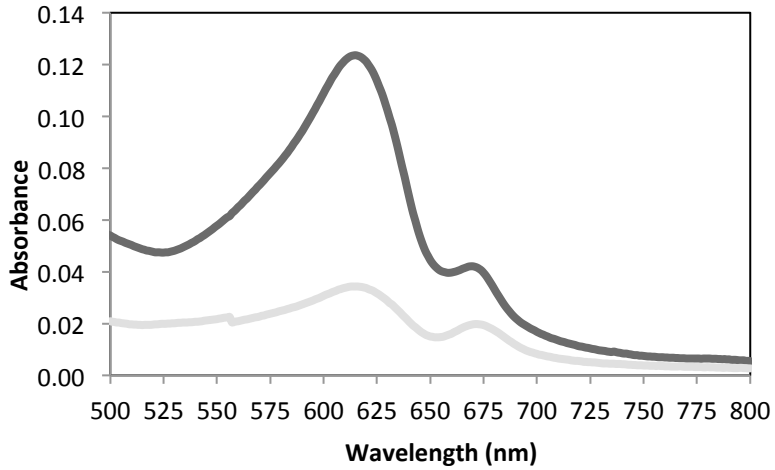


Figure 7.2. Absorbance scans for the (a) Chlorophylls and (b) Phycobiliproteins.

7.2 Secchi depth, *in situ* fluorescence and vertical profile data

In addition to the methodology described in Section 2.0, additional Secchi depth, *in situ* fluorescence and vertical profile data were collected throughout the study. These parameters are important in understanding water turbidity, overall algal biomass and their distribution throughout the water column. Due to specific characteristics of Lake Naivasha that impact the accuracy of the measurements, such as its shallow nature and frequent mixing, these parameters could not be utilized for specific comparisons to extracted pigments and microcystin production. Nevertheless, they were still valuable in interpreting some of the conditions in Naivasha at the time and therefore the results are described below.

Visibility was quantitatively inferred by measuring the degree of transparency of the water with a Secchi disk—a black and white circular weighted plate that is 20 cm in diameter. The disk was lowered into the water column, on the shady side of the boat, and the average of two depths was taken: the depth where the disk was longer visible upon descent and then by lowering the disk even further and slowly ascending, the depth where it reappeared again (Lind, 1979; Wetzel and Likens, 1991). The Secchi depth was generally measured in the same consistent manner between the hours of 10:00 and 15:00 to minimize variability due to non-biological factors (Lind, 1979). In productive lakes, the Secchi depth can also help estimate the density of phytoplankton (Wetzel, 2001); however, turbidity can also be present due to factors other than algal biomass (Lind, 1986), so other observations and measurements are important in understanding and analyzing changes in the Secchi depth.

In situ fluorescence of surface waters was measured with the YSI multi-parameter water-sonde as described in the methods section of the thesis. In addition, during Field Season 1, vertical profiles were taken at Site A (Middle of Crescent Island basin) and J (Hippo Point off shore). The sonde was slowly lowered down the water column and it recorded measurements at the various depths while it descended. During Field Season 2, a vertical profile was taken at each site visited.

Results and discussion

Secchi depth and in situ fluorescence

Secchi depth measurements for both the Main Lake and Crescent Island basin are displayed in Figure 7.3. The Secchi depths overall are shallow in Lake Naivasha, never reaching greater than 0.7 m in the Main Lake basin and 1.1 m in the Crescent Island basin (Figure 7.3a). The Secchi depth was always deeper in the Crescent Island basin compared to the Main Lake basin, and on any given day, the range of values measured in the Main Lake basin never overlapped with measurements in the Crescent Island basin.

In general, for both basins, the Secchi depth was shallower during the period of September to mid-November when chl-*a*/biomass was greater, and was relatively deeper in December (Figure 7.3a). After December the Secchi depth remained fairly stable in the Main Lake, and yet became shallower, even though algal biomass decreased over that time. In the Crescent Island basin the Secchi depth fluctuated, although chl-*a* remained stable, but generally remained deeper than when algal biomass peaked (Figure 7.3a).

During Field Season 2, more shoreline sites were visited (these sites could have greater sediment disturbance that would impact the Secchi depth), and therefore sites that were only sampled during this period are displayed in a different colour in Figure B-1a. Although no algal bloom was present and algal biomass was less than Field Season 1, the Secchi depths in both the Main Lake and Crescent Island basins were generally shallower than the previous months of sampling, and were actually more comparable to when algal biomass was highest in Sept-Nov 2010 (Figure 7.3a). This is evident even if only the sites sampled consistently throughout both field seasons are considered. Qualitative observations also indicated more turbid waters during Field Season 2 (Table 3.2) therefore other processes besides algal biomass may be impacting the Secchi.

The surface *in situ* chlorophyll fluorescence measurements (represented as a concentration of chlorophyll in μg/L) also indicate that some other processes or features of the lake may be affecting the penetration of light through the lake water (Figure 7.3b). While it is known that the absolute measurements of *in situ* chlorophyll will not accurately represent extracted chlorophyll-*a* biomass, the trend in *in situ* chlorophyll from October to December generally decreased which was also observed with the extracted pigments. However, beginning in December 2010, the *in situ* fluorescence started to

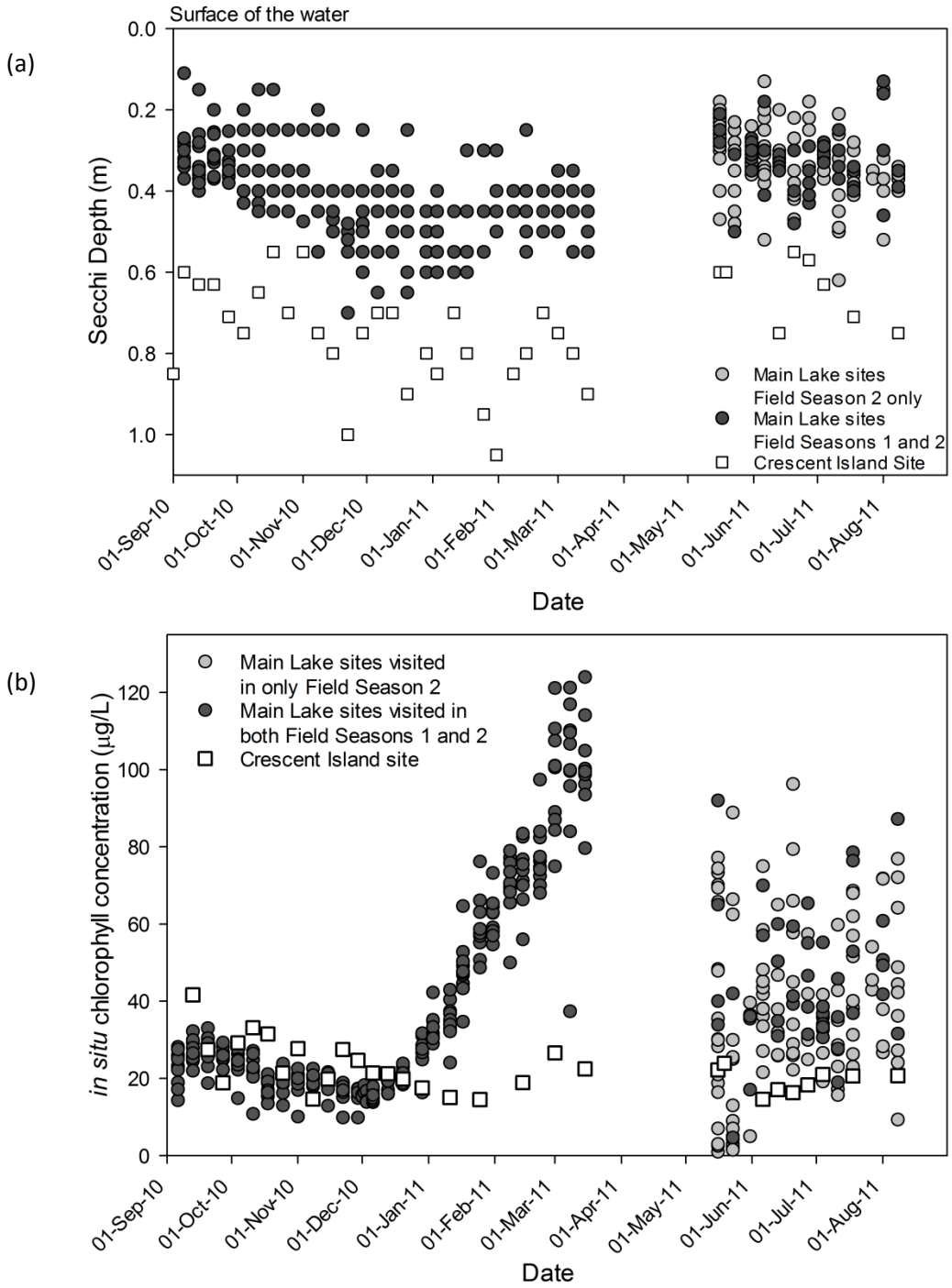


Figure 7.3 (a) Secchi depths in the Main Lake and Crescent Island basin of Lake Naivasha. In the Main Lake basin each point represents a different sampling site on a given day (ranging from N=3 to N=15). Note the reversed y-axis to better represent lake depth. (b) *In situ* chlorophyll concentrations (determined with fluorescent probe) in the Main Lake and Crescent Island basin of Lake Naivasha. Each Main Lake point represents a different sampling site on a given day (with N = 9 or greater).

gradually increase each week and also become more variable at the different sites around the lake (Figure 7.3b), despite this being the time of decreased chl-*a* concentrations and ranges (Figure 3.11a). This was also a period where there was an increase in chl-*c* (Figure 3.11c) and dominance of *Aulocoseira* (Figure 3.4a) and lake level decline (Figure 3.1).

From May to August 2011, the *in situ* fluorescence ranged even more than in February and March 2011 on a weekly basis (Figure B-1b). It should be noted that by just focusing on the sites consistently sampled in both Field Season 1 and 2, less variability is observed, however overall the ranges of *in situ* chlorophyll measured are still large and values are high considering no bloom was present and the extracted chl-*a* concentrations were relatively lower than the previous year (Figure 7.3b).

Vertical Profiles of in situ fluorescence

Although the accuracy of the surface water measurements of *in situ* chlorophyll fluorescence may not be reliable or completely indicative of the algal biomass in this lake, the vertical profiles of in situ fluorescence can identify where there is a relatively greater amount of algae throughout the water column.

At the Hippo Point site in the Main Lake basin, the in situ fluorescence generally remained very stable throughout the water column, indicative of a well-mixed system, although near the sediment there was almost always an increase in fluorescence due to the accumulation of detrital algal cells (Figure 7.4).

In the Crescent Island basin, which is a much deeper location than any spot on the Main Lake, there were some instances where in situ fluorescence was greater in certain areas of the water column (Figure 7.5). In particular, October 11, 2010 was when chlorophyll-a and phycocyanin concentrations were extremely high in Crescent Island (Figure 3.14 a, b). Although no surface observations gave indication of this elevated biomass, the vertical profile indicates that there may have been a relatively larger amount of algae approximately 1 m down the water column. Given that the Secchi depth was shallow at 0.65 m, this mass of algae dominated with *Anabaena* and *Ceratium* (Figure 3.4b), may not have been visible from the surface waters. Additionally, compared to the Main Lake basin, surface in situ fluorescence measurements tended to be more stable (Figure 7.3b) and so the shift/decrease seen November 8-22 in the vertical profiles (Figure 7.5) may be indicative of the changes in biomass in Crescent Island.

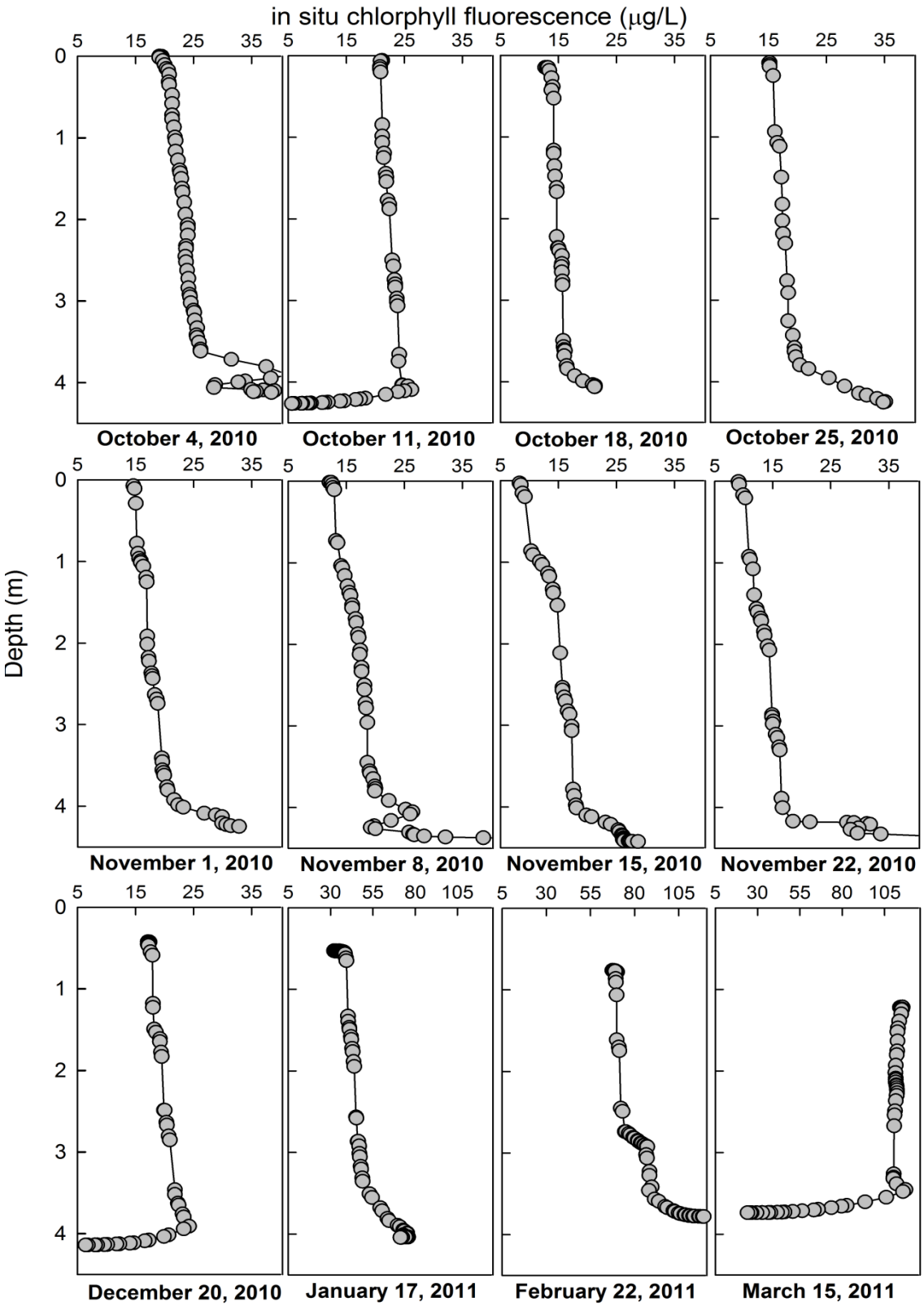


Figure 7.4. Vertical profiles of *in situ* chlorophyll fluorescence at Hippo Point of the Main Lake basin of Lake Naivasha. Note that all axes scales up to December 20, 2010 are identical, but the in situ chlorophyll fluorescence scale changes from Jan-March.

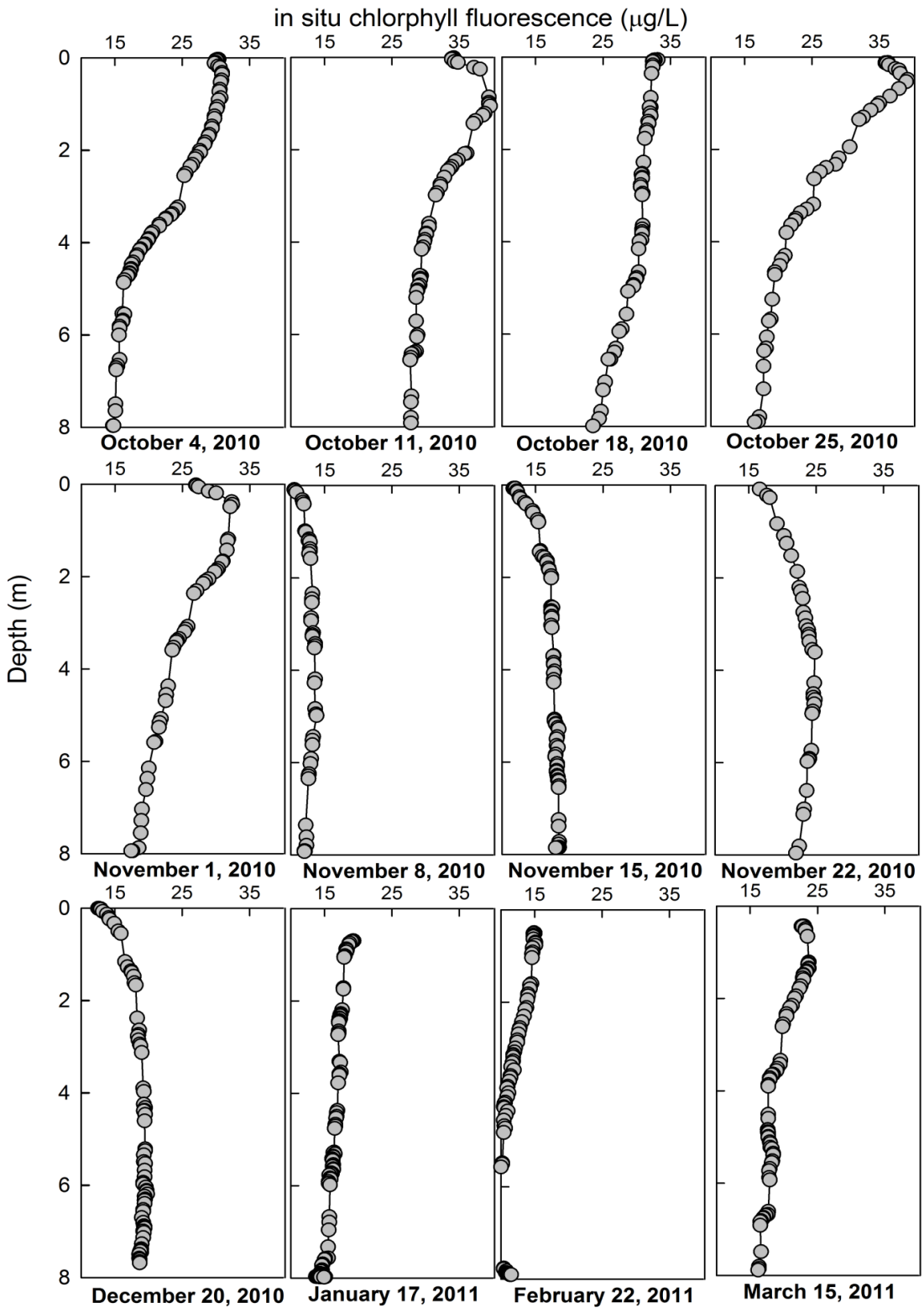


Figure 7.5. Vertical profiles of *in situ* chlorophyll fluorescence in the Crescent Island basin of Lake Naivasha. Note that all axes scales within the figure are identical, but they differ from Figure 7.4.

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7.3 Algal blooms, dissolved oxygen and pH

The presence of the cyanobacterial bloom studied in this thesis and the corresponding high biomass of all algae (represented by chlorophyll-*a* levels) allowed the opportunity to assess how the bloom impacted the dissolved oxygen dynamics in Lake Naivasha. In situ DO, pH and extracted chl-*a* data from Field Season 1 (September 2010 to March 2011) was examined to determine whether any relationships existed.

Introduction: Algal blooms and Dissolved Oxygen (DO) changes

Decomposition of an algal bloom often causes declines in the DO concentration of the water due to an increase in the organic material the senesced algae provide for bacteria to rapidly consume (Reynolds and Walsby, 1975; Paerl et al., 2001). This rapid breakdown of organics causes substantial consumption of oxygen through bacterial respiration, and with no considerable photosynthesis occurring since the bloom has died, DO may remain low for a prolonged period of time until it can be replenished from the atmosphere or from an increase in productivity. This can have considerable effects on aquatic organisms, in particular large fish, which have the greatest demand for oxygen. A general guideline is that DO levels from 2-4 mg/L can cause fish distress, so when levels like this are widespread in a lake for an extended time, a fish kill event could occur (Francis-Floyd, 1992). Fish kill events are commonly reported within tropical lakes that support cyanobacterial blooms, for example, Lake Victoria (Ochumba, 1990).

DO changes in water from algal or cyanobacterial bloom decomposition obviously do not impact drinking water and human health directly; however the indirect consequences are important to consider because DO changes impact fisheries. For many communities living near lake ecosystems, especially those in developing countries, fish represents a major source of food and protein (Otiang'a-Owiti and Oswe, 2007). The nutritional benefits of fish for both children and adults is very important and if access to that source of food is diminished due to a fish kill there may not be an affordable or suitable alternative available. In communities that are very dependent on that economic resource, fish kills could have major repercussions not only for the industry and economic gains of a region as a whole, but also for the individuals whose employment and livelihood are reliant on being able to perform their job (Otiang'a-Owiti and Oswe, 2007).

Results

Main Lake basin

When data for the dissolved oxygen (DO) from the 10 Main Lake basin sites were compared, it was clear that they fell into two groups: a group where dissolved oxygen was generally higher and less variable (6 sites; Figure 7.6) and a group where the dissolved oxygen was generally lower and had large fluctuations (4 sites; Figure 7.7). These groups of "High DO sites" and "Low DO sites" are distinguished in the results.

There was only one point within the High DO group, in wich DO was less than 4 mg/L (Figure 7.6). This occurred at site K (Oserian Bay) on November 8, 2010, when that area of the lake was densely covered with water hyacinth. This macrophyte cover was substantial enough to inhibit light pentration and therefore decrease photsynthesis and dissolved oxygen. This point was still included in all subsequent analyses.

Figure 7.8a shows that the low DO sites only rarely represent the highest biomass concentration(s) measured on a given day. Dissolved oxygen varies around the lake on a given day and from September to December the difference from the maximum to minimum was usually around 2 mg/L (Figure 7.8b). During this same period dissolved oxygen concentrations remained fairly stable between 6-8 mg/L.

A decline in chl-*a* concentrations occurred on approximately November 22, but there was no major corresponding decline in dissolved oxygen on that date which could be because biomass levels were still relatively high, and there was still presence of the cyanobacterial bloom on the lake (Figure 3.24a, Table 3.2). However after this point in time a clear change in the variability of dissolved oxygen levels was observed. An intense storm and heavy rains occurred on November 30, 2010, and while chl-*a* concentrations remained relatively stable at both the High and Low DO sites (when compared to previous months), a major difference was seen between these sites in terms of dissolved oxygen concentrations (Figure 7.8a,b). The High DO sites remained high, within a small range and even increased, whereas the Low DO sites started to diverge, the range of concentrations increased and overall concentrations slightly declined (Figure 7.8b). Some Low DO sites on a given day were comparable to the High DO group of sites, but there were usually measurements of 4 mg/L or less at multiple sites from December 2010 to March 2011 (Figure 7.8b).

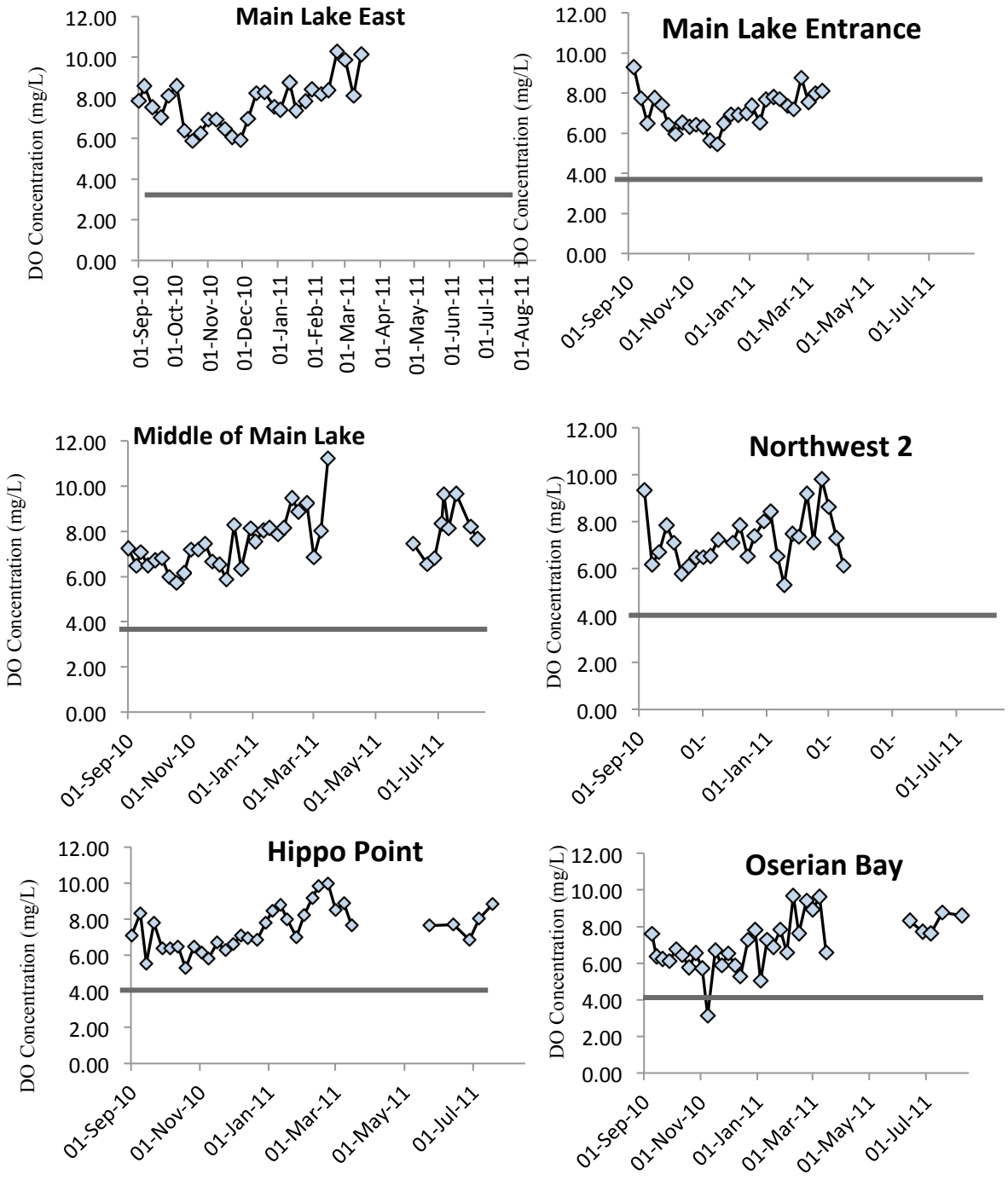

Figure 7.6. High DO sites.

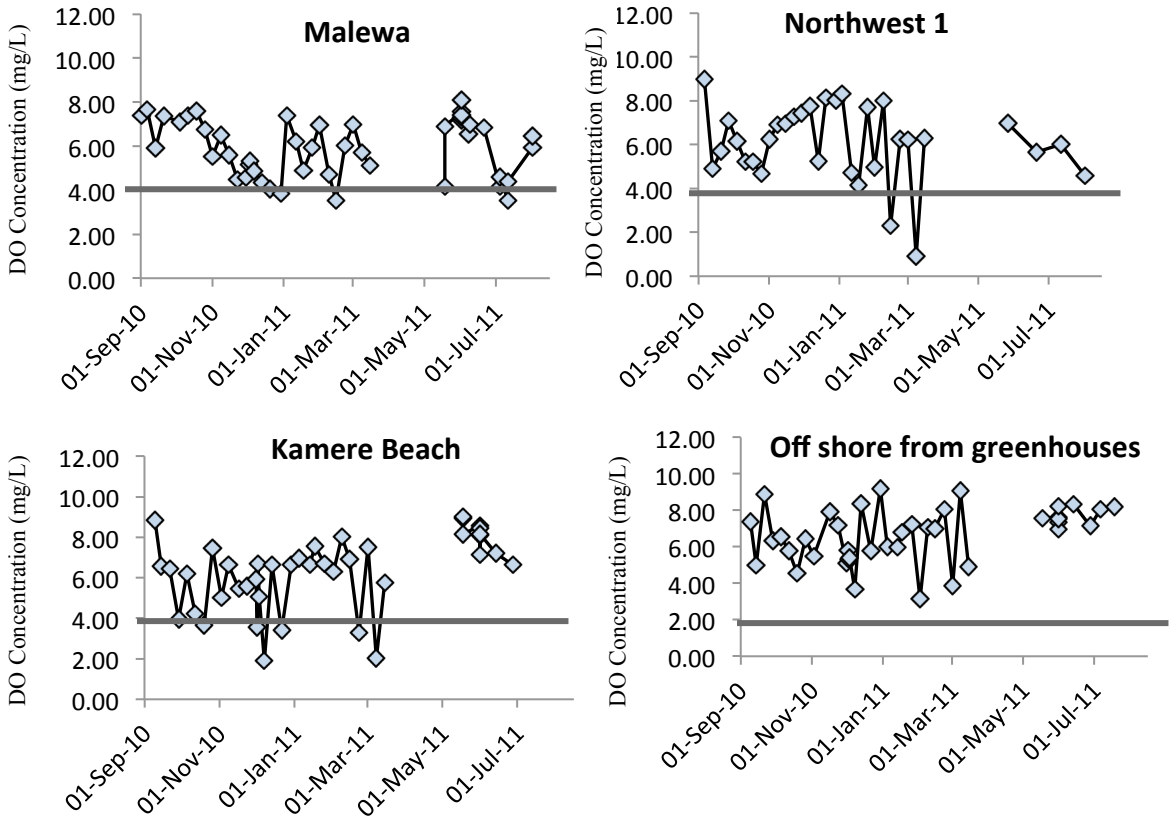

Figure 7.7. Low DO sites

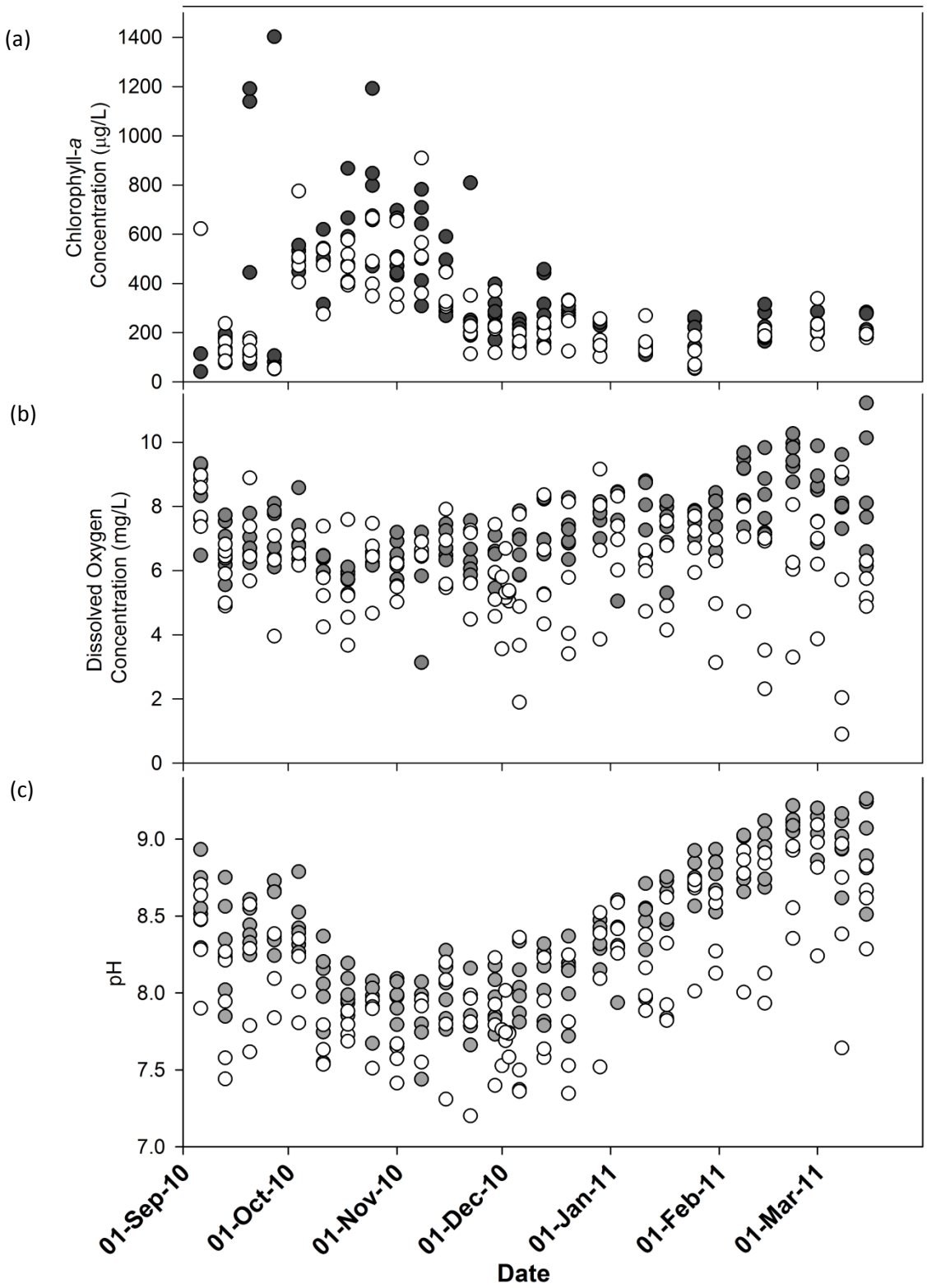

Figure 7.8. (a) Chlorophyll-*a,* (b) Dissolved Oxygen, and (c) pH from September 2010 to March 2011 in the Main Lake basin of Lake Naivasha. For each graph the darker symbols represent the group of high DO sites and the white symbols represent the low DO sites.

pH can be used to infer photosynthesis—higher pH can indicate greater photosynthesis—as this process utilizes CO_2, thereby limiting the formation of carbonic acid and hydrogen ions in the water, which decreases pH. pH measurements were lowest when chl-*a* was highest in October and November 2010 (Figure 7.8c). pH steadily increased weekly starting in December 2010 until March 2011. In general throughout the entire study period, the range of pH at most sites on a given day was a relatively stable 0.5 pH units. Each day there was always about 2-3 sites with a much lower pH compared to most of the other sites and these were often from the Low DO group (Figure 7.8).

While Figure 7.8 looked at the overall changes over the study period, the variables were plotted against each other to determine if there were any strong relationships (Figure 7.9). No linear relationships could be statistically determined for DO vs. chl-*a* and pH vs. chl-*a* (Figure 7.9a,b). There seemed to be a relationship between pH and DO, where, as pH increased so did DO, although with all points considered the relationship was not statistically linear (Figure 7.9c). The subset of the data representing the peak bloom period up until Nov. 15, 2010 did show a statistically linear relationship albeit not very strong ($R = 0.69$, $r2 = 0.48$, $p < 0.001$, $N = 112$), and is highlighted by the white symbols in Figure 7.9c).

Crescent Island basin

In the Crescent Island basin, the highest chlorophyll-*a* concentrations, and therefore phytoplankton biomass were observed for three weeks in October 2010. On November 8, after 2 weeks of declining phytoplankton biomass, a very large drop in both dissolved oxygen and pH was observed (Figure 7.10a,b,c), marking the lowest surface DO measured in Crescent Island during this study. Separate to this major instance of a biomass change however, there does not seem to be any overall relationship between chlorophyll-*a* levels and DO, which is also evident in the lack of significant relationship between the two variables in Figure 7.11a. Furthermore no significant correlation was seen between chlorophyll-*a* and pH (Figure 7.10b), but it is clear that the DO does fluctuate a lot from week to week in the Crescent Island basin (Figure 7.10b). Some of these fluctuations do tend to correspond to the changes in pH (Figure 7.10 b,c), which translated to a weak regression between DO and pH in the data measured (Figure 7.11c; $R = 0.62$, $r^2 = 0.39$, $p < 0.001$, $N = 30$).

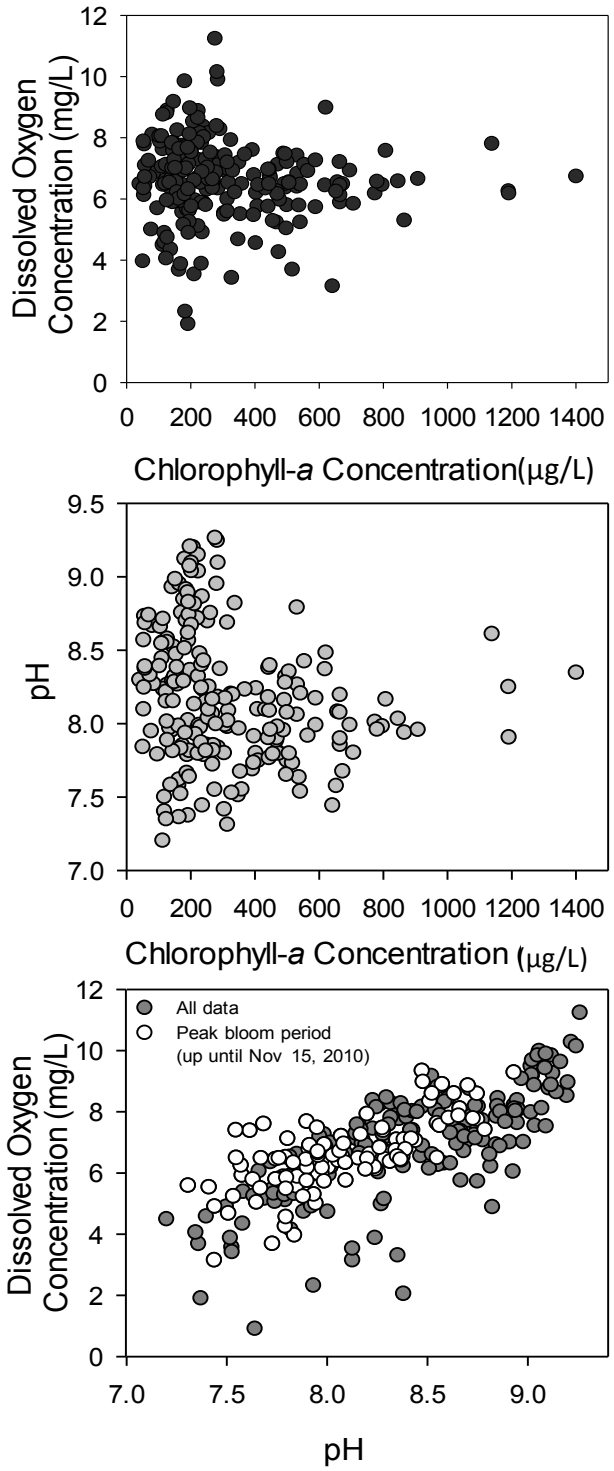

Figure 7.9. (a) DO vs. chl-*a* (N = 213), (b) pH vs. chl-*a* (N = 213) and (c) DO vs. pH (N = 290) from September 2010 to March 2011 in the Main Lake basin of Lake Naivasha.

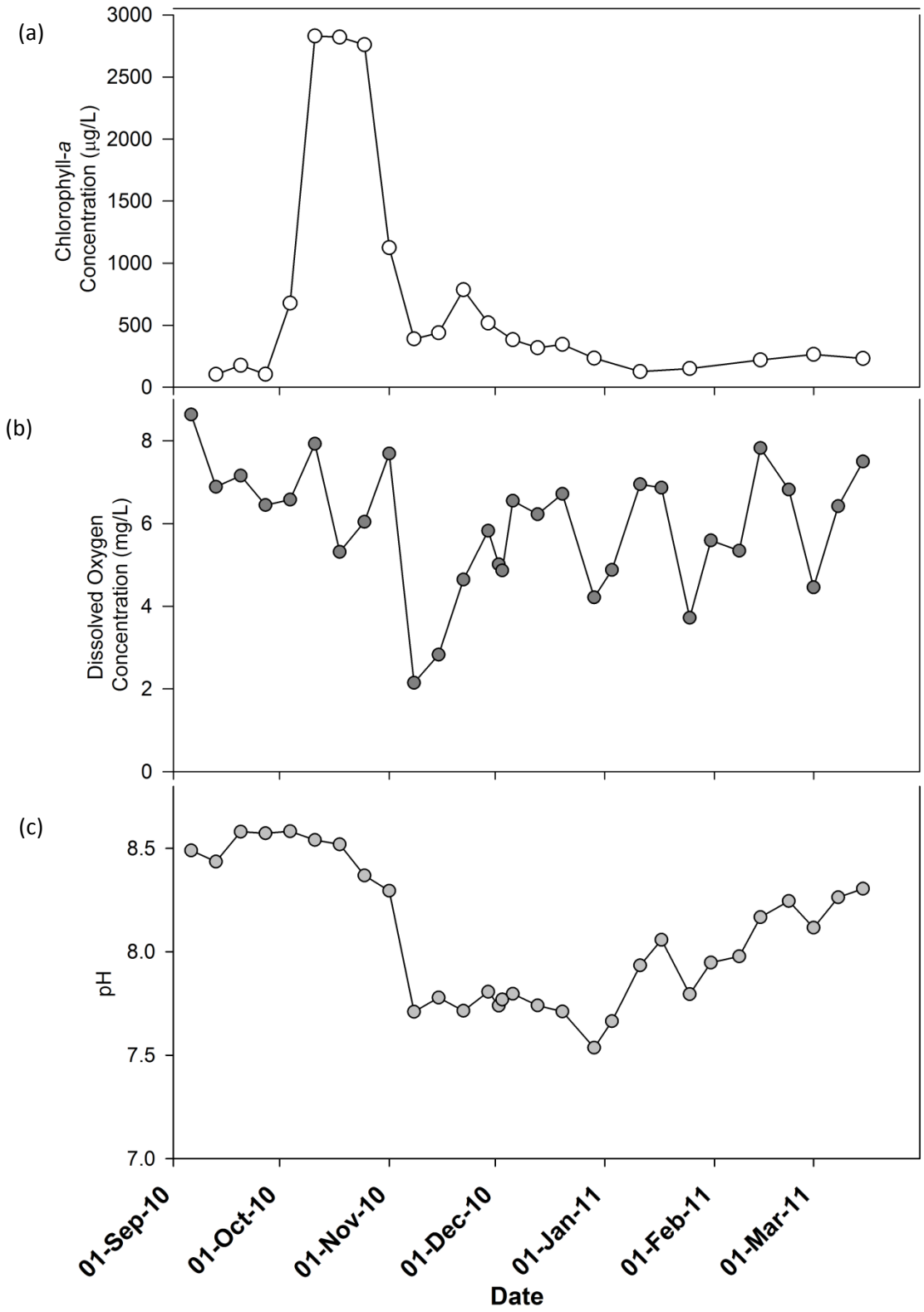

Figure 7.10. (a) Chlorophyll-*a,* (b) Dissolved Oxygen, and (c) pH from September 2010 to March 2011 in the Crescent Island basin of Lake Naivasha.

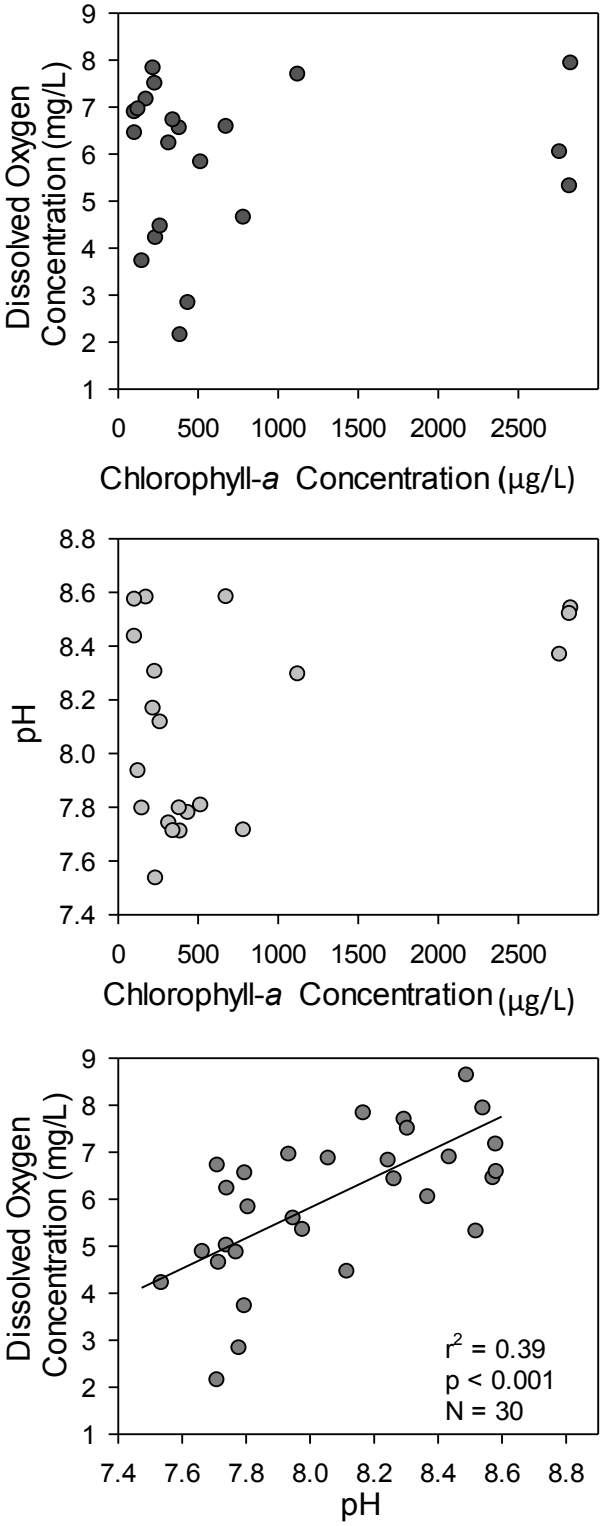

Figure 7.11. (a) DO vs. chl-*a* (N = 21), (b) pH vs. chl-*a* (N = 21) and (c) DO vs. pH (N = 30) from September 2010 to March 2011 in the Crescent Island basin of Lake Naivasha.

Discussion

In Crescent Island there was a clear decline in both DO and pH when the algal biomass decreased in November indicating a strong association between increased biomass decomposition following photosynthesis and potential fish kills (Figure 7.10).

In the Main Lake basin there was not a clear decline in dissolved oxygen when the biomass decreased. However what is clear is that when bloom biomass was highest, there was less variability in dissolved oxygen concentrations at all sites, and levels were fairly high, indicating that the bloom was tightly controlling the photosynthesis and respiration changes (Figure 7.8b). However when the bloom became less dominant, there was a change in dissolved oxygen variability: some sites (the 'high DO sites') still remained high, while the 'low DO sites' largely fluctuated and there were multiple instances where it was lower than 5 mg/L. It cannot be determined whether this was solely due to the change in bloom dominance because a major storm event also occurred on November 30 and it likely could have been a combination of both. The storm could have been what prevented the bloom from recovering, and the changes in DO show which areas of the lake may be more susceptible to organic loading and unable to recover from productivity changes. One reason why we perhaps did not see a sharp decline in DO as expected was because this bloom persisted. There were periods where the biomass did decline, but it was not an instance of total bloom collapse within a short period of time.

Additionally, it's interesting to note the stronger relationship between DO and pH, but the weak relationships between DO and chl-*a* and pH and chl-*a* (Figure 7.9). When the biomass was highest, the pH was not high, indicating a potential disconnect between growth (and respiration) and the amount of photosynthesis (Figure 7.8). This suggests that there was may have been more than enough CO_2 in the water to satisfy algal needs and also maintain that relatively lower pH. The buoyant cyanobacteria may have also been utilizing atmospheric CO_2 just as it was diffusing into the water as a carbon source rather than any of the dissolved inorganic carbon presenting the water column (Paerl and Paul, 2012).

In summary, algal blooms do seem to impact the dissolved oxygen dynamics, and as further analysis on other controls to dissolved oxygen in the lake are completed, a better understanding of the magnitude and role that algal blooms play will be clarified.

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7.4 Climate Change, cyanoHABs and Lake Naivasha

There has been a growing body of literature focusing on the impacts of regional and global climate change on the proliferation of cyanoHABs in eutrophic systems (Paerl and Huisman, 2009; Paerl et al., 2011; El-Shehawy, 2012; O'Neil et al., 2012; Pearl and Paul, 2012). While there are many complex interactions for each of the key issues, they will briefly be discussed here in the context of Lake Naivasha.

Temperature

Increased water temperatures favour the growth of cyanobacteria over eukaryotic algae (Paerl and Husiman, 2011). Whereas diatoms cannot withstand temperatures greater than 25 °C, the growth rates of cyanobacteria are maximal at temperatures from 25-35 °C. The water temperature during this study was fairly consistent between 20-23 °C and we saw that both cyanobacteria and diatoms were able to thrive in Lake Naivasha. If these temperatures rise, this could further benefit the growth and dominance of cyanobacteria such as *Microcystis*, but then also prevent greater dominance of the diatoms which have always been a major component of the phytoplankton in the lake. If this occurs, the issue of cyanoHABs will be of even greater concern in Lake Naivasha.

Vertical Stratification

The density of water declines as temperatures increase and therefore the warming of surface water will promote greater stratification. This should not impact the shallow Main Lake basin of Naivasha in which wind patterns are strong enough to influence frequent mixing. However, the deeper Crescent Island basin—which currently only stratifies occasionally—may see increased stratification that will allow for greater proliferation of cyanobacteria, particularly the genus *Anabaena* which is already dominant in the basin and is known for its potential to produce neurotoxins.

Carbon Dioxide and pH

Cyanobacteria and all algae have a high demand for CO_2, therefore dissolved inorganic carbon can be limiting for photosynthesis and growth (Paerl et al., 2011). Increased CO_2 in freshwater (due to increased atmospheric CO_2 and its subsequent dissolution) could then be advantageous for all algal growth and its rapid utilization could increase pH. However in these more alkaline and dissolved CO_2-limited scenarios, cyanobacteria would then have an advantage because of their ability to use bicarbonate as

their carbon source (Kaplan et al. 1991), and because their buoyant properties allow them to also utilize atmospheric CO_2 as it is diffusing into the water. Therefore overall, atmospheric CO_2 increases may favour cyanoHAB proliferation over other algal bloom species. The carbon dynamics of Lake Naivasha need to be further examined as this is potentially another avenue that could lead to greater cyanoHAB production.

Hydrologic Changes

While Naivasha has already experienced dynamic weather patterns of drought and heavy rains, these changing patterns are expected to continue and potentially become more intense. Given what we have learned about the drivers to the cyanoHAB event in this study, the potential for dramatic fluctuations in precipitation and drought should be concerning for the future of Lake Naivasha with respect to cyanobacteria.

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CURRICULUM VITAE

EDUCATION

University Of Western Ontario, London, ON, Canada 2010-2012
M.Sc. Candidate (Biology)

University Of Western Ontario, London ON, Canada 2005-2009
Honors Bachelor of Science (B.Sc.) in Biology
- Dean's Honor roll 2005-2009

RELATED EXPERIENCE

University Of Western Ontario, London ON, Canada 2010-2012
Teaching Assistant
4th Year Ecosystem Health (Jan- Apr 2011, 2012)
2nd year Research Methods in Biology-Writing Unit (Oct 2010-Apr 2011)
1st year Biology Tutorial Leader (Jan-Apr 2010)

University Of Western Ontario, London ON, Canada May to Dec. 2009
Research Assistant
"Population genetic survey of termites in Point Pelee National Park"

Lawson Health Research Institute, London, ON, Canada 2007-2009
Research Assistant, Mental Health and Homelessness

Shopper's Drug Mart, Leamington, ON, Canada 2003 - 2008
Front Store Supervisor, Merchandiser and Cashier

AWARDS

3 Minute Thesis Competition Finalist, University of Western Ontario 2012

Earth Day Colloquium Best Presentation, University of Western Ontario 2011

Western Graduate Research Scholarship, University of Western Ontario 2010-2012

Undergraduate Continuing Admissions Scholarship, University of Western Ontario 2005-2009

PUBLICATIONS

Referred Publications

Raffoul, M.H., S.J. Hecnar, S. Prezioso, D.R. Hecnar, and G.J. Thompson. (2011) Trap response and genetic structure of Eastern subterranean termites (Isoptera, Rhinotermitidae) in Point Pelee National Park, Ontario, Canada. *Canadian Entomologist* 143(3): 263-271.

Non-referred Publications
Raffoul, M.H. (2009) Water and human health in a rural village of Tanzania. Undergraduate Honors Biology Thesis, University of Western Ontario.

ORAL PRESENTATIONS

Raffoul, M.H, Creed, I.F., Trick, C.G.(2012) *Harmful cyanobacteria blooms in Lake Naivasha, Kenya: Causes, health risks, and the way forward*. Transcending Borders Towards Global Health, Schulich School of Medicine and Dentistry, Western University, London Convention Centre, London ON, Canada, April 27-29, 2012

Raffoul, M.H. (2012) *Harmful cyanobacteria blooms in Lake Naivasha, Kenya: Causes, health risks, and the way forward.* Earth Day Colloquium, Western University, London, ON, Canada, April 13, 2012.

Raffoul, M.H. (2012) *Harmful cyanobacteria blooms in Lake Naivasha, Kenya: Healthy environments, healthy people.* 3 Minute Thesis Competition, School of Graduate and Postdoctoral Studies, Western University, London, ON, Canada , April 26, 2012. *(Finalist in the competition)*

Raffoul, M.H. (2011) *The decline of ecosystem services in Lake Naivasha, Kenya: the role of cyanobacteria harmful algal blooms*. Earth Day Colloquium, Western University, London, ON, Canada, April 11, 2011. *(Awarded Best Presentation)*

Raffoul, M.H., and R.C. White (2010) *Don't drink the water: Ecological responses to human influences on Lake Naivasha, Kenya*. 7th Annual Earth Day Colloquium: University of Western Ontario, London, Ontario, April 23, 2010.

Raffoul, M.H. (2009) *Water and human health in a rural village of Tanzania.* UWO Biology Day: University of Western Ontario, London, Ontario, April 4, 2009.

Raffoul, M.H. (2009) *Water and human health in a rural village of Tanzania.* Ontario Biology Day: University of Windsor, Windsor, Ontario, March 21-22, 2009.