Effect of climate change and eutrophication on the thrombolites and microbial mats within Lake Clifton

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It is with pleasure that I submit this thesis entitled “Effect of climate change and eutrophication on the thrombolites within Lake Clifton” as partial fulfilment of the requirements for the degree of Bachelor of Engineering (Environmental). This is to state that I carried out all the work contained herein except where indicated otherwise.

Regards,

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Abstract

Lake Clifton, a saline lake located in the southwest of Western Australia, is a RAMSAR listed lake with significant scientific and conservation value due to the large number of migratory birds and the presence of a unique community of thrombolites and microbial mats. Smith (2006) showed that Lake Clifton has undergone dramatic changes including an increase in salinity from 18 g/L (SD 1.65) in 1985 to 32 g/L (SD 4.21) in 2005 and total phosphorous values increasing since 1992 with each exceeding the eutrophic threshold (>10 µg/L). Recent climate change within the region has led to a reduction in the annual rainfall resulting in a decline in the lake’s water level. Elevated phytoplankton concentrations produced by increased nutrient concentrations and decreases in lake levels in Lake Clifton has likely led to an increase in light attenuation. Thrombolites use the light to photosynthesis for energy and for the precipitation of its calcium carbonate structure. Therefore, an increase in light attenuation may result in important changes in the physiology of the photosynthesis of the microbial community, which form the thrombolites.

The main aim of this study is to assess the effects of climate changes and increasing light attenuation on the thrombolites and microbial mats within Lake Clifton. More specifically this study used spectrofluorometry and Pulse-Amplitude-Modulation (PAM) fluorometry to:

1) Assess the water column eutrophication by comparing current measurements to historical data.
2) Measure the thrombolites and microbial mats photosynthetic efficiency.
3) Give an assessment of the health status of the thrombolites

The results show that the total phytoplankton concentration slightly declined in this study in comparison to a study done by Smith (2006). However, there was greater variability in the concentrations in this study, which often exceeded the value obtained by Smith (2006). The phytoplankton distribution was examined which found cyanobacteria was located in the top 60 centimetres of the water column while majority of the diatoms and green algae were located between 0.6 to 1.4 metres below the surface. The thrombolites and mats photosynthetic efficiencies were measured
over two transects of increasing depth. These showed that the efficiency did decline with increasing depth. However, it was a weak relationship as at some depths the efficiency increased. This means with greater depth the thrombolites had lower photosynthetic efficiencies reflecting the increase in light attenuation with depth. Thus, future predictions can be made using depth as measure for light attenuation based on predictions of increased phytoplankton concentrations, how this will affect the thrombolites and microbial mats. The health measurements of the thrombolites, which were calculated by doing dark yield measurements, show that the thrombolites health also declined with increased depth. Therefore, it seems that the increased light attenuation caused by the increase in phytoplankton is affecting the thrombolites. This means that with future changes to Lake Clifton that will likely increase in salinity and nutrients in the lake. This will lead to increase light attenuation, which will ultimately further reduce the ability of the thrombolites to photosynthesize at certain depths. This could lead to reductions in the distribution of the thrombolites as they are unable to exist anymore in certain parts of the lake due to not enough light being available for adequate photosynthesis.
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Glossary:

Benthic – from the bottom of the sea or lake

Biota – all living organisms within a certain area

Copepod - any of numerous crustaceans of the order, characterised by short, cylindrical body, with a rounded or beaked head

Hypersaline lake – lake with water that has a salinity value greater than sea water

Hyposaline lake – lake with water that has a salinity value less than sea water

Light attenuation - reduction of light irradiance in water by the scattering and absorption by water molecules and inorganic and organic particles

Microalgae – multicellular algae that are defined according to the size where the body is observable to the naked eye.

Microbialite – organosedimentary deposits formed from interactions between benthic microbial communities and detritial or chemical sediments

Multiple stressors – a combination of environmental stressors acting on a system that usually lead to interactive and dynamic effects on a system.

Photosynthesis – process that converts carbon dioxide into organic compounds (especially sugars) using the energy from sunlight

Phytoplankton – the autotrophic component of the plankton

Stratification - the division of water into layers with different temperatures and chemical characteristics

Stromatolites – microbialite exhibiting a laminated internal structure

Thrombolite – microbialite exhibiting a clotted internal structure

Abbreviations

AHD – Australian Height Datum

BMC – Benthic Microbial Mat

IOCI – Indian Ocean Climate Initiative

IPCC – International Panel for Climate Change

RLC – Rapid Light Curve
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1 Introduction

Lake Clifton is located within the Yalgorup National Park. It is a very important wetland due to the large number of migratory birds, which visit regularly each year, and the presence of thrombolites and microbial mats in hyposaline water. The importance of the thrombolites is due to their strong ancestral link to the possible first forms of life found in the fossil record. Scientists believe they can be the key to unlocking how life started.

Over a short period from the 1980’s changes have occurred in Lake Clifton from both anecdotal evidence and measurements taken over that time. One reason is likely due to an increase in population around the lake resulting in many land use changes. This has likely altered the hydrological balance of the lake particularly in reducing the amount of freshwater groundwater inputs into Lake Clifton. Due to the importance of this freshwater in regulating the salinity levels in the Lake Clifton, this decline has likely been one of the reasons for the increase in salinity in this period. Other changes to the lake include increases in nutrient inputs in the lake and the introduction of the black bream, which are all placing stress on this system.

The increase in nutrients over this period has now lead to Lake Clifton defined as a eutrophicated system. This means likely lead to an increase in the phytoplankton, which has changed the light climate within Lake Clifton. These changed conditions will restrict the ability of the thrombolites and microbial mats to photosynthesis. The importance of photosynthesis for the thrombolites is due to that it contributes to the formation of their calcium carbonate structure. If light attenuation is able to reach threshold levels, this may mean the thrombolites distribution could be reduced due to that thrombolites. The thrombolites and microbial mats adapted to low nutrient and low salinity system. Therefore, it is likely that with these changes to the lake the thrombolites and microbial mat communities are being stressed. On top of this, likely changes in the future include further land use changes and climate change, which likely lead to further stress added to the thrombolites and microbial mats to levels where these thrombolites and microbial mats perish.
This study aims to examine the effect these changes to Lake Clifton are having on the thrombolites. It uses this knowledge of these present changes since 1979 to have a look at whether the thrombolites are handling these stressors. This work will intend to indicate to some level the health status of the thrombolites and microbial mats at the present. This work will have the potential to be incorporated into future management and protection programs on Lake Clifton.
2 Literature Review

2.1 The importance and threats to saline lakes

2.1.1 Saline lakes

Salinity can be defined as the sum of all the ionic compounds dissolved in a water body. It is a measure of concentration of ionic substances, which is usually made up by a high percentage of sodium chloride (Kalff 2002). The salinity of a lake often defines the properties of that lake in terms of its productivity and abundance and diversity of biota. The salinity of seawater is at approximately 30 parts per thousand (30% or 30 g/L). In comparison, freshwater lakes are lakes with salinity less than three parts per thousand (3% or 3 g/L) (Deckker 1981; Oliva et al. 2001). Saline lakes (or salt lakes) are lakes with salt concentrations that are higher than freshwater lakes (Kalff 2002). Lakes that are hypersaline have salinity values that exceed that of seawater and often can be many times greater than this (Hammer 1986; Kalff 2002). Lakes that are hyposaline have a salinity value between that of freshwater and seawater (Hammer 1986). Saline lakes are found mostly in the arid and semi-arid regions of the world (Timms 2005). As Australia is considered 70% arid, it is not unsurprising that more than 80% of lakes and wetlands are saline (Timms 2005).

There are two types of saline lakes; the permanent lakes, which hold water all year round, and the episodic lakes, which hold water only when there is sufficient rainfall (Timms 2005).

2.1.2 Importance of saline lakes

Saline lakes do have a high significant value (Williams 2002). However, in terms of economic value there is very little value. This is because the water from saline lakes have limited opportunities for drinking, irrigating or industrial use due to the higher levels of salts. Due to usually lower numbers of fish species present in saline lake, it has limited fishing opportunities (Kalff 2002; Williams 2002). However, in some cases these salts can be precipitated out and sold (Kalff, 2002). Saline lakes have other uses including having culture, aesthetic, recreational or scientific value which far outweigh these economic factors. Saline lakes are atypical in that although they
usually lack a large amount of biological diversity they are often visited by a large number of migratory birds. The migratory birds fly between the northern and southern hemispheres to breed and/or to feed to take advantage of both summer periods. Most of this biota within saline lakes is endemic to that region and is adapted to the strict and harsh conditions of the saline lake. This means this biota is dependent on the lakes survival for their survival. This leads to the great importance in protecting and maintaining the lakes as these are considered very distinct and different environments (Williams 2002).

In particular, the importance in preserving and maintaining a healthy ecosystem is the scientific value associated with these lakes (Williams 2002). The scientific work done on salt lakes has changed its focus over time from the morphological characteristics of these ecosystems into more recently unravelling how the dynamics of the hydrological and ecological processes operate within them. Understanding how salt lakes work is important for implementing more effective management practices in protecting and maintaining these very important ecosystems (Goater 2003). Since saline lakes often respond dramatically to environmental change this consequently offers an excellent scientific research opportunity to evaluate fluctuations in water chemistry, hydrology, organic productivity, drainage basin characteristics and the climate itself. For example, from studies done in Australia and Canada, it has shown how in saline lakes both floral (including benthic algae communities) and faunal diversity decreases have occurred with increasing salinity, presumably because fewer species can tolerate the higher salinity that are common in lakes in these two countries (Melack 1988). Saline lakes can then be used as demonstrations to show the dramatic changes that will occur if a freshwater lake if it salinises including showing how declines in species abundance and changes to the community species composition occur (Kalff 2002). Thus, by visualising what happens with the increase in salinity it could help with management of other lakes as ways of motivating protection and mitigating threats to avoid the increase in salinity (Melack 1988).
2.1.3 Threats to saline lakes

Figure 1 above shows a number of threats to saline lakes which all alter or interfere with the hydrological, biological and nutrient cycles. These threats are either anthropogenic or natural in nature but ultimately cause significant changes in the saline lake by having massive impacts on the biodiversity and physical and chemical properties of the lake. In terms of the hydrological balance, the impact of climate change affects both the inputs (precipitation, runoff and groundwater) and outputs (evaporation). Other threats include land use changes, which changes the amount of nutrients, pollutants and other chemicals entering the lake and unsustainable groundwater extraction and introduced species, which interferes with native biota in the lake (Torgersen et al. 1986).
Land Use changes:

The clearance of vegetation to use for farming, housing or other purposes can cause a number of changes to an environment. One critical way land use changes affect lakes are by diversions of surface inflows (Williams 2002). This is the most significant impact on saline lakes in causing dramatic changes. By diverting the fresh water away from the saline lake for human drinking, agriculture, industrial or other uses, this alters the hydrological budget. Saline lakes immediately react with decreases in lake volume, which causes several physical and chemical changes. In particular, salinity increases which has major ecological effects with changes to species abundance and diversity observed. This is due to the salinity affecting the organisms’ ability to osmoregulate with its environment which ultimately replaces these indigenous species with salt tolerant species (Williams 1993). Most species have salinity thresholds that once exceeded puts the organism under too much stress and it perishes (Schallenberg, Hall & Burns 2003). For example, Schallenberg et al. (2003) found for its study on Lake Waihola, in New Zealand, that for a small increase in salinity it had a dramatic decrease in the abundance and diversity of the zooplankton species in the lake (Schallenberg, Hall & Burns 2003).

The removal of deep-rooted plants during clearing of vegetation has caused the groundwater in many places to rise above normal levels. This causes the mobilisation of ions in the groundwater causing the salts to rise to the lake above it. This is called secondary salinisation. Water moving over land that is salinsed often moves into a lake or other water bodies bringing with it these salts. This results in an increase in salinity of that water body, however, this increase in salinity is not due to a corresponding decrease in the volume of water in the lake. A decline in freshwater from precipitation, runoff or groundwater sources also cause an increase in salinity in a lake (Timms 2005).

Other catchment activities caused by land changes lead to greater soil erosion, increased sediments entering the lake and changes in run-off patterns, which can all have significant impacts on saline lakes in causing dramatic changes to the physical and chemical reactions and biota in the lake. Pollution, including organic and
inorganic wastes from domestic and industrial sources, also impacts on the biota if it enters a saline lake (Timms 2005).

**Unsustainable Groundwater Extraction:**

Current practices for groundwater extraction are to take increasing amounts in the rural regions to stabilise and diversify agriculture production, as landholders are requiring more water than can be supplied from rain. Often saline lakes are supplied fresh water from groundwater supplies, which regulates the salinity and keeps it at favourable levels. The over-extraction of groundwater far above the sustainable yield will cause an increase in salinity. This is due to the reduction in fresh water into the lake that would cause a concentration of the salinity. These affects to salt lakes will increase into the future with expected increases in groundwater extraction (Timms 2005).

**Nutrient loading from increased catchment activities:**

The increased nutrient use on land upstream of a saline lake can lead to these nutrients entering that lake. Human development in catchments such as farming often leads excess amounts of fertiliser being used. The excess nutrients from the fertiliser often are removed after rainfall events by runoff or by leaching down into the groundwater. The nutrients can enter a lake via the runoff directly or by then entering the lake with the groundwater inputs. The nutrients that enter the lake often exceed the normal conditions within that lake. In addition, the riparian vegetation around the lake is often altered reducing the ability of nutrient uptake and allowing more nutrients to enter the lake. This increase in nutrients then alters the natural balance within the lake system (Williams 1993). This nutrient addition, if sufficiently large, can cause eutrophication (Hammer 1981). Eutrophication has the ability to block the light to shade the benthic photosynthetic organisms such as water plants, macro algae species and benthic microbial communities. This is problem as the blocking of the light can limit how much these benthic organisms are photosynthesising. If these benthic organisms do not receive enough light, then they could possibly perish if the light attenuation is persistent (Kalff 2002).
Climatic and Atmospheric changes:

The ecosystems that will most likely impacted by climate change that are those which are limited by rainfall or temperature. Saline lakes are usually found in regions of low rainfall, high temperatures or with large evapotranspiration rates. Therefore, saline lakes will be negatively affected by climatic changes. Even small climatic changes cause dramatic changes to saline lakes, because the lakes rely on a strict hydrological balance and any deviation away from this will lead to several physical, chemical and biological changes (Gates 1993; Hammer 1986). Evaporation and precipitation are considered the two critical factors that will directly affect saline lakes. These cause lakes to dry out more frequently or become smaller and more saline (Williams 2002). Predictions of climate change are for many arid countries like Australia experience declines in rainfall. This will reduce the amount of runoff, which will cause permanent salt lakes to shrink and become more saline (Timms 2005). In addition, increases in CO\textsubscript{2} in atmosphere will change the lake dynamics such as the rate of calcium carbonate precipitation, as well as the pH level (Smith 2006). Many saline lakes are located close to the coast, such as those located in the South West of Western Australia, and with climate change there is a potential for the sea level to rise to flood these lakes (Gates 1993; Timms 2005).

If saline lakes are affected by either drying out or an increased in salinity then there will be a reduction of species diversity. Gates (2003) suggests that organisms with limited habitats would be most susceptible to climate change as would not be able to adapt to the change or move to another area of similar habitat as it would not simply exist. The organisms within saline lakes would be susceptible to climate change as they do not have the opportunity to move to another lake, have limited distribution and most likely will not be able to cope with the changes to the lake. Hence, climate change is a big potential threat to the biota within saline lakes as it may cause many of the species to perish. Migratory birds often nest or feed in saline lakes around the world. If climatic change modifies the lake in changing the species diversity or abundance or make certain physical or chemical changes that the birds cannot handle then the birds may no longer be able migrate there. This will have significant impact on these birds if they have reductions in habitats to feed or breed. This makes the migratory birds susceptible to extinction from climate change (Gates 1993).
Biological Disturbances:

Most saline lakes are usually isolated and are harsh physiological environments to live in. This usually results in unique and different species to evolve. However, many saline lakes have been affected by introduced species. For example, the brine shrimp species *Artemia pathenogenetica* has been invading saline lakes within Western Australia and replacing the native *Parartemia* species. This is likely affecting the ecological systems in these lakes by the lost of the native species (Timms 2005)

2.1.4 Environmental stressors

Stressors are defined as environmental effectors or factors, which cause or drive an ecological change in a ecosystem (Smith 2006). Stressors can be external or internal processes. Stressors are perturbations that are foreign to that system or natural to it that are applied to an excessive level. Stressors can be defined in a number of ways. One way is to distinguish between every day and natural stressors and those which are anthropogenic or infrequent. Every day stressors are those that the system can readily handle. The system can buffer against these changes as they occur regularly. It is the anthropogenic or infrequent events, which are the major problems to a system. Most man-induced stressors are similar to those which occur naturally but are often far more intense or differ in quality. However, normally a system has some resilience to change from stressors. Therefore, if a system is capable of handling change then it is likely to absorb the effect of these stressors and very little will happen to the system. However, if these stressors that are applied exceed the systems elasticity, then the system will change with many species perishing and being replaced with species adapted to these conditions (Barrett & Rosenberg 1981)

2.1.5 The scale interactions of multiple stressors on saline lakes

Environmental stressors are scale-dependent meaning its effect can vary on a range of small to large spatial and temporal scales. The nature of the stressor determines its spatial extent and temporal duration (lifetime and cycle) (Wainwright & Mulligan 2004). The scale of the stressor is only on an order of magnitude (Peterson & Parker 1998; Wainwright & Mulligan 2004). The spatial scale is the area over which the stressor will act and the temporal scale is the frequency in which the stressor will act.
Figure 2. The time-spatial scale of stressor events (Wainwright, 2004).

Figure 2 above shows a time-spatial scale distribution of different stressors of different spatial and temporal scales. Figure 2 shows that the large-scale processes are acting over vast areas (hundreds to thousands of squared kilometres) and its period over which it fluctuates varies from years to millennia. This means their temporal effects fluctuate over a longer period than other shorter stressors. However, the small-scale processes act over much more smaller spatial scales of metres and have periods of fluctuations that vary from seconds to days. Small-scale process act over much shorter periods with their effects occurring at a much frequent time intervals. Understanding how different stressors of different sizes will act according to what temporal and spatial scale will help determine what effect these will have on the system at any particular time or place (Klijn 1994).
The time and spatial scales of different stressors are important to understand and determine as they all interact with the environment and with each other in different and dynamic ways (Klijn 1994). Multiple stressors on a system combined to have various multidimensional interactions among them simply do not add together but in fact cause unknown feedbacks, nonlinearities and thresholds (Council 2007). This causes unique and unusual effects to show up in the system that are not predicted if the same stresses were applied separately or independently (Klijn 1994). Cumulative effects is where multiple stressors with unrelated effects add up synergistically on top of each other to effect the status at the endpoint in the system differently than if these were impacted separately. Cumulative effects can also be where it is only one stressor but it has a high frequency meaning the separate waves of effect is added together and the strength of the effect of the stressor builds up over time (Anom. 1988). Cumulative effect can build up in a number of different ways in a system shown in figure 3 below.

Figure 3. Cumulative effects of multiple processes (a stressor) on a generalised system showing four different pathways with these multiple stressors (Anom., 1988).
Stress in a system is often built up of the multiple stressors. A stressed system can be viewed as a system that has surpassed threshold limits in these changes, which has lead to it losing its ability to restore homeostasis. In addition, stress causes a system to become more vulnerable to further environmental change in the future. Stress places a system at a disadvantage so in response the system will use excess energy to compensate. This use of excess energy is a drain on resources on that system so it can be measured as a loss of power to that system. However, this is unsustainable and incompatible for survival. As power is lost, the community structure changes as it can no longer support the original composition. As most species have overlapping niches, once one species is no longer present or is under stress, this favours other species putting them at the advantage. Thus, a change or shift occurs with the species composition within that system. This may have dramatic effects on the system from its original state due to these new species doing different things to the original species (Barrett & Rosenberg 1981; Klijn 1994).

Smith (2006) suggested some of these stressors due to these threats outlined in 2.1.3 on saline lakes would include:

1. Increased nutrient concentration
2. Increased salinity
3. Addition of contaminants or pollutants to the lake
4. Biological disturbances
5. Changes to the available habitat

The combination of stressors put on saline lakes has greater effects on the lake than if the stressors were considered separate. The interacting effects for saline lakes, such as these just listed above, would cause vast changes in the saline lakes including changes in the biota (Folt et al. 1999). Studying the combined effects of stressors to an ecosystem such as a saline lake is complex since the effects of different stressors are often not simply combined but instead are added together in different and dynamic ways with the combined effects often occurring on non-linear time scales (Klijn 1994).
Munns (2006) suggests that multiple stressors need to be considered more environmental studies. Often experiments do not consider additive effects between different stressors to a system. It is often just easier to treat them separately and examine one stressor at a time. Experiments also do not consider the secondary effects of stressors, that stressors can have non-linear effects and that there is environmental patchiness in space or time. This is limiting and not showing the whole picture (Munns 2006). Munns (2006) suggests the reason for these limitations in experiments is it is hard to comprehend the spatial-temporal variation and interactions in the effects. Klijn (1994) agrees suggesting that it is not that hard to indentify individual stressors, as its response will be shown with some key indicator, however, when it comes to multiple stressors it is very hard to examine these and know what the effects of interacting stressors will be. Any experiment on a system with cumulative effects will be required to take an integrated approach considering all the spatial and temporal scales of these effects. This shows that there is an urgent need in expanding the knowledge base and awareness of interactive stressors on systems, especially with understanding their responses to climate change (Anom. 1988).

2.2 Light as limiting factor for photosynthetic organisms

2.2.1 Using light for photosynthesis

Visible light is part of the electromagnetic radiation spectrum that is produced from the sun that includes gamma rays, x-rays, ultra-violet, infra-red, microwaves and radio waves. Organisms (such as plants, algae or cyanobacteria) use the visible light to photosynthesise which is a process of turning carbon dioxide into carbohydrates (an energy source) to fuel the organism. The energy obtained in photosynthesis promotes growth and repair to keep the organism in a ‘healthy’ state. Growth corresponds to the difference between photosynthesis and respiration (Bainbridge, Evans & Rackman 1966). This means, as long as there is sufficient photosynthesis growth will occur. The visible light spectrum waveband is from 380nm to 770nm with photosynthetic organisms only using the range of 380nm to 710nm for photosynthesis. This range of visible light the organisms use for photosynthesis is called the photosynthetically available radiation (PAR) (Kalff 2002; Weztel. 2001). The visible light spectrum is broken down into many different colours based on different wavelengths as seen in
figure 4 below. This range of the visible light range is from blue (the lower end) to red (the upper end). The lower energy photons are found at wavelengths greater than 700 nanometres. These are perceived as heat. In the ultraviolet range, which is less than 400 nanometres, the flux of very high-energy photons may cause structural damage (Kalff 2002).

![Electromagnetic radiation spectrum](image)

*Figure 4. The electromagnetic radiation spectrum that shows the breakdown of the visible light into the different colours. The visible light range is the most important as this drives photosynthesis (Kent 2000).*

Bainbridge, Evans & Rackman (1966) states that light has always been regarded as the most significant factor within the aquatic environment due to its importance in photosynthesis. The photic zone (otherwise known as euphotic or trophogenic zone) is the vertical region within a lake where there is phytoplankton photosynthesis greater than phytoplankton respiration. The zone ends when the photosynthesis equals the respiration called the compensation zone is where one percentage (1%) of the surface or subsurface incident remains. However, it can be down to as low as 0.1% of the surface light and photosynthesis still occurring (Kalff 2002).

### 2.2.2 Light Attenuation in water

Light attenuation is the reduction of light irradiance in water by the scattering and absorption of a number of different factors including the water molecules themselves and dissolved and suspended substances in the water ((Bainbridge, Evans & Rackman 1966; Weztel. 2001). These substances include organic particles, partly by coloured dissolved organic molecules (DOM) and by sediments such as silt and clay that have
arrived from the drainage basin or reside in the water body itself. Additionally, inorganic solutes, water molecules and phytoplankton cause scattering and absorption of light (Kalff 2002). According to (Bainbridge et al., 1965) waters containing vegetable decay have yellow substances, which absorb the blue light. The light absorption capacity of any particle is a function of its abundance and mass. Total light attenuation is dependent on the size distribution of all the particles in the water with more light attenuation when all of the particles are small as opposed to fewer but larger particles. The absorption of photons by phytoplankton is largely a function of the amount of photosynthetic pigments. However, the fraction of cell volume packed with pigments decline as cell sizes increases and there is less light attenuation in lakes dominated by large algae species rather than lakes with the same biomass but smaller species (Kalff 2002).

Equation 1 reflects that the simplest way of expressing vertical light attenuation in water. It does this as a percentage reduction in light from the surface. However, this equation is only for one particular wavelength of light. Each wavelength has its own vertical light attenuation (Kalff 2002):

\[
\text{Vertical light attenuation (\%)} = 100 \frac{(I_o - I_z)}{I_o} \quad \text{EQ. 1}
\]

Where, in EQ. 1, \(I_o\) is the energy flux or photon flux density of a particular wavelength interval just below the surface and \(I_z\) is intensity at depth \(z\) (m).

![Figure 5. Transmission of light through distilled water. (Wetzel, 1975)](image-url)
The spectral composition of the light also changes with depth (Bainbridge, Evans & Rackman 1966). Within distilled water as shown in figure 5 above shows how the water attenuates the different wavelengths of visible light. Figure 5 shows that the larger wavelengths of the light spectrum get absorbed first. The only exception is that violet (~390nm) gets absorbed first before blue (~460 nm). Thus red (~750 nm) will get absorbed first while blue (~460 nm) gets absorbed last (Weztel. 2001). The amount of PAR light (quantity) and what wavelength (quality) will determine the level of biological activity in a surface water body. Thus, light will not penetrate down as far as these theoretical depths in distilled water. This is because water is always going to contain some amount of chemical or biological identities in it that will absorb the light. In clear water, water molecules much more readily absorb the red end of the spectrum than the blue end. This leads to the blue light having the maximum transmittance (transported deepest) in the water. However, blue light and ultraviolet (UV) on the other hand are particularly absorbed in turbid waters by the coloured DOM, otherwise known as dissolved organic carbon (DOC). In turbid waters short wavelengths are rapidly absorbed with the result that the small amount of down dwelling light becomes quickly dominated by long wavelength PAR (orange and red light > 600 nanometres) (Bainbridge, Evans & Rackman 1966; Kalff 2002; Weztel. 2001).

2.2.3 The quality of light is important for photosynthesis

The quality of light is just as important as quantity for photosynthesis. Bainbridge et al. (1965) states that when visible light is broken down into the different colour bands (blue, green, yellow and red) the results show that weak red light was usually more effective for photosynthesis than weak blue light. Thus, organisms that use this wavelength are maximising their ability to photosynthesis. Some forms of algae have been shown under the water to have two maximum peaks corresponding to both red and blue light. This shows they are maximising their chances of getting the maximum amount of photosynthesis they can by utilising both these two light wavelengths. These algae, however, have minimum photosynthesis through the green portion of the visible light spectrum. Other species have shown to have maximums in other regions of the visible light spectrum. Thus, the photosynthetic activity of algae, thriving at a
certain depth, therefore, depends not only of the total value of the radiant energy, but also on its spectral composition (Bainbridge, Evans & Rackman 1966)

2.2.4 Effect of increased light attenuation in saline lakes

Light attenuation occurs naturally in all lake systems. Benthic photosynthetic organisms will always be shaded by light attenuation in the above water column by water absorption and reflection, inorganic particles or organisms such as phytoplankton. Usually the response to these lower light levels are adaption’s, for instance increased chlorophyll a to cope with the lower light levels. However, the problem can occur as in many water bodies where excessive nutrients enter and cause eutrophication. This level of light blocking will change the light climate of that water body. Usually this results in a massive reduction in the amount of light reaching the benthic photosynthetic organisms. This reduction could be below the adequate levels required for these organisms to be actively growing and repairing themselves and so they perish. This is the effect of increased nutrients in reducing the ability of these benthic photosynthetic organisms (Kalff 2002).

2.3 Microbialites

2.3.1 Definition of Microbialites

Microbialites are organosedimentary structures formed from benthic microbial colonies (BMC’s) made up of cyanobacteria, diatoms, bacteria and eukaryotes interacting with detritrial or chemical sediments (Burne & Moore 1987). Most microbialites communities are dominated by photosynthetic cyanobacteria species, which are usually involved in their formation. Throughout fossil history there is the presence of BMC’s interacting with sediments forming structures similar to microbialites in the present (Burne & Moore 1987; Whitton & Potts 2000). The earliest record is from approximately 3 to 3.5 billion years ago making microbialites the earliest record of life on earth (Luu, Mitchell & Blyth 2004). It is thought that microbialites were the communities responsible for the oxygenation of the atmosphere due to oxygenic photosynthesis (Whitton & Potts 2000). Thus, these present day microbialites are considered modern analogues to these ancient communities although
not necessarily directly related. Today, microbialites are now located mostly in extreme environments such as tidal flats and hypersaline lakes. By understanding how these modern forms work can then demonstrate how these original structures formed and functioned which are considered the first forms of life (Playford 1980).

Microbialites come in various forms. Three of the most common forms include microbial mats, thrombolites and stromatolites (Burne & Moore 1987; Kennard & James 1986; Mann & Nelson 1989; Smith 2006):

**Microbial Mats:** There are many different types of microbial mats depending on the species composition. These are far more common than other types of microbialites and located in many different places around the world.

**Stromatolites and Thrombolites:** Both are laminated structure microbialites which means they form in layers. These produce large, macroscopic calcium carbonate structures that are mostly domed or conical in shape. They can grow up to about 1 metre in height. The difference in these two is mostly due to the way in which they form. Stromatolites are laminar internal-laid structures meaning as they form layers that are built up on top of each other. Thrombolites form not by layers but by unevenly growth periods which cause it to have a clotted internal-laid structure.

### 2.3.2 Locations of Microbialites

Microbialites are found usually in extreme environments around the world where there is limited or no pressure from grazers and burrowers (Y. Konishi, J. Prince & B. Knott 2001). However, in practice this idea is not always the case, with many microbialites located in areas of high metazoan activity. Example locations of microbialites include those in fresh water such as Lake Richmond in Rockingham, those in hypersaline water such as Hamelin Pool in Shark Bay or those in marine water such as in the Bahamas (Burne & Moore 1987; Feldmann & McKenzie 1998; Mann & Nelson 1989). Within Western Australia, there are many places where microbialites are found including Esperance, Rottnest Island, Rockingham, Shark Bay and Cervantes. Each of these locations have different types of microbialites constituting a different, distinct and very significant community in terms of history,
structure and morphology. One location of particular importance where microbialites are found is the thrombolites of Lake Clifton in the Yalgourup Lake system near Perth, Western Australia (Anom. 1995).

### 2.3.3 Formation processes of microbialites

Microbialites form in a number of different ways. It has been identified that most microbialites are built and dominated by cyanobacteria (Stal 1995). Microbialites are all mat like structures with some having the ability to precipitate out calcium carbonate. This can then form into consolidated rock that gives rise to the formations of distinct laminae known as stromatolites and thrombolites (Whitton & Potts 2000). For the microbialite to form the rate of excretion must exceed the rate of grazing and burrowing by the organisms and covering by sediments (Y. Konishi, J. Prince & B. Knott 2001). The net growth is at a rate of 1 mm per year meaning these are slow growing communities (Whitton & Potts 2000). Burne and Moore (1987) have suggested that certain cyanobacteria species are important in the building of the microbialite structures such as the filamentous species *Scytonema*. Since this is a key organism if they are not present then there is a strong risk that the microbial community cannot survive without them and therefore perish with the structures no longer built (Burne & Moore 1987).

Stromatolites and thrombolites can form their calcium carbonate structure through four different mechanisms, which are microbial trapping, binding of detritus, or sediment, inorganic calcification and biologically influenced calcification during photosynthesis (Burne & Moore 1987; Kennard & James 1986). These processes is strongly associated with the metabolism of the microbial community (Whitton & Potts 2000). However, stromatolites mostly form from microbial trapping and binding of detritus or sediment mechanism where they grab crystals of calcium carbonate in the water and lock it into the structure by filamentous microorganism. Conversely, thrombolites mostly form by inorganic calcification and biologically influenced calcification mechanisms where precipitating out the calcium carbonate through reactions when some of the organisms in the microflora photosynthesise (Anon. 2007; Burne & Moore 1987).
Thrombolites mostly form from the precipitation of calcium carbonate under the reaction (Whitton & Potts 2000):

\[ \text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3 \]

The concentration of \( \text{Ca}^{2+} \) in a system is usually a constant value (under a set of physical and chemical conditions) meaning that the rate of calcification is dependent on the concentration of the carbonate ion. Normally, the carbonate ion concentration in water is dependent on two reactions:

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} & \leftrightarrow \text{H}^+ + \text{HCO}_3^{-} \\
\text{HCO}_3^{-} & \leftrightarrow \text{H}^+ + \text{CO}_3^{2-}
\end{align*}
\]

However, the carbonate ion can form indirectly during photosynthesis. While the thrombolites and stromatolites photosynthesise this process fixes CO\(_2\). This process ultimately removes the CO\(_2\) from the water and reduces its concentration.

\[ 6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + \text{O}_2 \]

This reaction then causes the acceleration of the reaction below in favouring the right hand side. This then changes the concentration of carbonate ions in the water to then further favour precipitation of calcium carbonate.

\[ 2\text{HCO}_3^{-} \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{CO}_3^{2-} \]

This reactions show the dependence of the thrombolites to light in their formation process (Whitton & Potts 2000).

A microbial mat is defined as a multilayered microbial community growing on and with sediments. They are usually made up of different microorganism groups occurring in vertical biomineralogical stratified layers. The layering can be influenced by different growth periods, seasonal events, periodic events such as tides or episodic or erratic events such as storms. Lamination of mats can also occur with the growth of new layers built on top of old dead layers (Whitton & Potts 2000). Microbial mats are
mostly dominated by a top layer of cyanobacteria (Stal 1995). They cyanobacteria are at the top because they are aerobic and need light to photosynthesise. The cyanobacteria dominated mats are usually made up of halophilic filamentous and coccid species (Hurlbert 1993). However, cyanobacteria are not always dominating microbial mats with reported mats that are dominated instead by diatoms, green algae, and other eukaryote species. Cyanobacteria layers can often be sandwiched between anoxygenic bacteria and other types of eukaryotes. However, the cyanobacteria dominated mats are seen as the more successful type of mats due to the ability of the cyanobacteria to survive a number of harsh environments where few eukaryotic species are found such as tidal flats, hypersaline ponds and hot springs. As well, many species of cyanobacteria are adapted to conditions not suitable for eukaryotes such as sulphur rich environments, anoxic conditions and aerobic dark respiration (Whittion & Potts 2000). In habitats undergoing desiccation and air exposure, cyanobacteria are far the most successful group than any other organisms (Hurlbert 1993).
3 Lake Clifton

3.1 The Yalgorup Lake System

The Yalgorup Lake system is located within the Yalgorup National Park approximately 100 kilometres south of Perth in the south west of Western Australia shown in figure 6 below (Evans 1993).

Figure 6. Location of the Yalgorup Lake System (Moore, 1993)

The Yalgorup Lake System is a group of lakes and wetlands that formed between 6,000 years ago to 3,000 years ago due to the sea level rising causing coastal progradation. These lakes are located on the western fringe of the sandy coastal plain in three parallel lines. The lakes occur in depressions of the coastal plain. Shelly, marine deposits underlie the dunes and all the lake basins. The region with the lakes is dominated with Pleistocene Tamala limestone, which is seen at the shore of many of
the lakes (Y. Konishi, J. Prince & B. Knott 2001). These lakes are groundwater fed (Burke & Knott 1989). The aquifer that feeds them is the Lake Clifton Superficial Aquifer (Evans 1993). This groundwater is a fresh water lens that overlies a large (3 kilometres in length by 30 metres in depth) hypersaline groundwater aquifer beneath it (Evans 1993; Goater 2003). The lakes all have varying salinity concentrations with a lot of them much higher than seawater (Goater 2003).

Figure 7. Yalgorup Lakes (Anom., 2007)

Figure 7 above shows the different lakes in the Yalgorup Lake system. The lake system lies between three coastal sand dune barrier ridges. The Eastern barrier, which reaches a height of 70m AHD, is called the Clifton/Harvey Barrier. This separates Lake Clifton from the Peel Harvey Estuary. The middle barrier is a low, narrow ridge that separates the lake from the narrow and discontinuous chain of lakes from Swan Pond at the north end and Lake Newman at the south end. This middle barrier is 0.7 kilometres wide at the south end but very narrow at the north end that is sometimes
breached at times of high lake levels allowing exchange between Lake Clifton and Swan Pond. The third barrier separates this lake chain from Lake Preston, which lies to the southwest of these lakes (Neil, 1984).

The Yalgorup Lake system is recognised for its near pristine condition as well for its high conservation, scientific, cultural and educational values (Anom. 2007; Goater, 2003). They are important lakes for migratory birds that visit or reside in these lakes and a wide variety of unique flora and fauna including the thrombolites and benthic microbial mats (Portlock et al. 1995). The local community have developed a strong appreciation of the rare and endangered ecology to this region and are strongly interested in seeing protection for this system and in particular the thrombolites and microbial mats (Anom. a 2007; Herbert 2008; Zuks 2009).

3.2 Lake Clifton

Lake Clifton is an important wetland within the Yalgorup Lake system. Lake Clifton is defined as an endoreic lake (a closed basin lake) as it has no direct link to the ocean (Anom. a 2007; Kalff 2002). Therefore, the lake is considered a sink for the groundwater due to no direct connection to the ocean. It is 21.5 kilometres long and up to 1 kilometre wide (Neil 1984). Lake Clifton is a shallow lake with an average depth of less than 1 metre and with a maximum at 3 metres at the north section. This means every year the lake level drops around 1 metre in the northern section of the lake (Y. Konishi, J. Prince & B. Knott 2001). As Lake Clifton has a surface area of approximately 22 kilometres squared, this equates to around 22 million litres of water lost per year (Steve Dutton, personal comment). The peak lake level, which occurs during September or October, is three months later than the rainfall maximum, reflecting the groundwater influence on the system, where there is a lag time, due to the time it takes for the water to infiltrate into the groundwater (Goater 2003). Figure 8 and 9 below shows the thrombolites in Lake Clifton at the Mount John Road boardwalk. The lake level is high as this study was conducted during August to October, which is the peak water level of the lake.
Figure 8. The thrombolites of Lake Clifton at the Mount John Road Boardwalk.

Figure 9. Close up of thrombolite at the Mount John Road Boardwalk.
Lake Clifton has international recognition and as such is RAMSAR, JAMBA and CAMBA listed. It has received this recognition for two reasons. The first because it is both a resting place for thousands of migratory and non-migratory birds, such as the Pacific Golden Plover, Little Stint, Great Crested Grebe and Hooded Plover (figure 10 below). The second reason is that it is only one of two places where thrombolites are located in hyposaline water in the southern hemisphere (Anom. 1995; Anon. 2003; Anom. a 2007). The lake has a unique water cycle, which includes majority of the inputs received from a hyposaline groundwater source. It only receives very minute volumes of water from runoff and direct precipitation. Water is only lost by evaporation (Anom. 1995; Moore 1987). Moore (1987) states that Lake Clifton is the only lake within the Yalgorup Lake System to remain hyposaline throughout the whole year. However, in recent years the situation has changed and now salinity is much closer to that of seawater (Moore 1987).

Figure 10. The endangered Hooded Plover is found at Lake Clifton (Anom., 2009).

Due to recent changes in Lake Clifton up to 1995, it was assessed that the unique thrombolite community was at risk and so the lake was classed as ‘endangered’ in the threatened ecological communities list under Western Australian State Government legalisation ((Anon. 2000b; Luu, Mitchell & Blyth 2004). This was further upgraded to ‘critically endangered’ in 2000 due to recordings of higher nutrient concentrations
recorded in the lake (Luu, Mitchell & Blyth 2004). It was assessed as a critical situation for the thrombolites due to two reasons. These were that the thrombolites have a highly restrictive distribution as they only occur within Lake Clifton and that they are extremely vulnerable to known threatening processes that are likely to result in total destruction throughout its range in the immediate future (Anon. a 2007; Luu, Mitchell & Blyth 2004). Very limited protection is provided under Australian Federal Government legislation although recently a discussion has taken place to put the thrombolite community on the endangered species list (Steve Dutton, personal comment). This protection of the thrombolites and microbial mats in Lake Clifton is reflected in the local and abroad community as are very interested in the thrombolites and for them to remain in a healthy condition.

3.3 Thrombolites and microbial mats of Lake Clifton

Within Lake Clifton, it is thought that the thrombolites predominately grow by the precipitation of calcium carbonate out of the water due to the presence of the Scytonema (Anon. b 2007). The Scytonema photosynthesises which changes the carbonate ion concentration in the water, that in turn causes the calcium carbonate to precipitate in the water as stated in section 2.3.3. It is likely that the thrombolites partly form by this species and other filamentous species found trapping and binding sediments (Burne & Moore 1993; Neil 1984; Smith 2006). In addition, Smith (2006) noted that Dichotomosiphon (eukaryotic microalgae) is also critical in the formation of the thrombolites. These were suggest as critical species for the formation of the thrombolites as these are only located on the structures and not in the surrounding microbial mats. The thrombolites are thought be around 4000 years old (Luu, Mitchell & Blyth 2004).

The thrombolite and microbial mat rely on sufficient water coverage overing the growing surface of the structures. However, during summer periods the water level drops exposing many of the thrombolites and microbial mats to intense heat for a sufficient period of time. Both thrombolites and microbial mats have cyanobacteria so most likely these survive using thick mucilaginous sheaths (Lützte et al. 1995). These sheaths enable the cyanobacteria to be desiccate-tolerant and survive long
periods of time under intense radiation from the sun (Lüttge et al. 1995). *Scytonema*, in the thrombolites, most likely survives the summer during the major exposure periods to intense sunlight using a thick mucilaginous sheath. *Scytonema* also uses this sheath in the precipitation and binding of the thrombolite structure (Smith 2006). Studies by Luttge at al. (1995) on exposure of cyanobacteria to intense sunlight show how the longer time for exposure for the cyanobacteria to sunlight without water the more that they perish and that the cyanobacteria require some water during the exposure periods of excessive sunlight in order to survive. This means that during the summer exposure many of the *Scytonema* and other species in the thrombolites community will ultimately perish. However, it has been suggested that there are groundwater seeps beneath the thrombolites and microbial mats which is thought to assist the microbial community through the intense summer. The thrombolites and microbial mats in Lake Clifton are suspected to rejuvenate once submerged under water again (Luu, Mitchell & Blyth 2004).

It is suspected by the nature of the unique hydrological, biological and other physical conditions of Lake Clifton that this is primary responsible for the formation and distribution of the thrombolites and microbial mats within the lake (Sarre 1999). The thrombolites require these conditions in order to be photosynthesising and maintaining themselves and thus growing to be classified as in a healthy state (Anon (Anon. b 2007). Thus, the factors, which favour the development and formation of the Lake Clifton thrombolites and microbial mats, include (Anon. 1997):

1. Water chemistry favourable for carbonate deposition (constant source of calcium and carbonate ions)
2. Hydrological regime conductive to the development of a BMC capable of forming microbialites
3. Adequate visible light for photosynthesis
4. An ecological balance which prevents the BMC from being overly disrupted by competition and grazing pressure
5. Requirement of nutrients for sustained BMC growth although nutrient availability per se does not appear to be a limiting factor and too much will favour faster growing competitive macroalgae.
If these favourable conditions were to change to not favour the thrombolites as they should, which some of these have already changed (for example salinity and nutrient concentration have both gone up), then it could have drastic impacts on the thrombolites (Luu, Mitchell & Blyth 2004).

The thrombolite and microbial mat communities are incredibly diverse. Neil (1984) identified a number of particular organisms within these communities. These microbial communities are made up of a large number of different microorganisms. For the thrombolites Neil (1984) indentified filamentous cyanobacteria (Oscillatoria sp., Scytonema sp., Anabaena sp., Spirula sp. and Stigonema sp.), unicellular cyanobacteria (Apanocapsa sp., Chroococcus sp. and Aphanothece sp.), diatoms and other eukaryotes (Dichotomosiphon sp., Dunaliella sp., Chlorocuccos sp. and Ulothrix sp.) all in high proportions in the thrombolites sample. While for the microbial mats which are more diverse, they have many different types occur in the lake which are dependent on the exact composition of the mat. For the orange microbial mat, located in the northern section of the lake was very similar to the thrombolites except it did not contain Scytonema. Species identified included Oscillatoria sp., Anabaena sp., Stigonema sp., Apanocapsa sp., Chroococcus sp. and Aphanothece sp., Chlorocuccos sp., Ulothrix sp., Gloeocystis sp. and Stichococcus sp.. Neil (1984) shows the proportion of the thrombolites and the orange microbial mat community in figure 11 below from the northern section of the lake.
Figure 11. Species composition of thrombolites and orange microbial mats located in the northern section of Lake Clifton (Neil, 1984)
Neil (1984) details the location of the thrombolites within Lake Clifton highlighted in figure 12 above. Most of the thrombolites occur along the near shore shallow water margins of the lake between 50 to 100 metres from the high water mark (HWM). The thrombolites are located on both the western and eastern sides of the lake. The largest numbers are located along the north-eastern shoreline. On the western shoreline, the thrombolites are found on the northern section on and to the north of the sand spit, which projects into the lake south of Swan Pond. The Eastern shoreline is 21 kilometres long from the most northern tip of the lake to the southern tip. On this side, the thrombolites are located all bar the final 7 kilometres at the southern end of the lake, which is a shallow basin and which dries out during summer. For the first 6.5
kilometres from the northern tip, most of the thrombolites form an integrated reef structure consisting of a platform of coalesced tabular thrombolites in the shallow water and individual thrombolite structures in the deeper water. As the reef structure gets narrower as it moves further south. The reef is 120 metres wide at the northern end of the lake with 45 metres of that is the platform. Three kilometres south of this point, the reef is 75 metres wide with 30 to 35 metres of that is the platform. Once it reaches the island (located 7 kilometres south from the most northern tip of the lake) the reef becomes patchy and the reef platform stops. Then two kilometres south of the island there are 4 distinct sand spits that extend up to 500 metres into the lake and these are often exposed during summer. These are colonised by wide (1 to 2 metre diameters) single and composite ring structure thrombolites. Further south, discrete structures with smaller diameters wide (2 to 50 centimetres) are found less than 50 metres from the shore. The thrombolites then become increasing patchy and limited after this. At about the two-thirds point in the lake there is a narrow point in the lake, which is considerably deep (3 metres), called the deep channel. After the deep channel a few thrombolites are found on the west and east sides of the lake. The southern end of the lake (the last remaining southern 7 kilometres of the lake) is shallow and dries out in summer and no thrombolites are located in this area. As well, the thrombolites do not exist in the deeper waters in the centre of the lake (Neil 1984).

Neil (1984) noted that Lake Clifton microbialites can be divided up based on ‘zones’. The likely reasons for the differences in the structures of the different microbialites has been due to how much light they get, the composition of the species and how much access to water they have. This zonation of Lake Clifton is seen with a gradation of different morphological types of mats and thrombolites lakeward from the high water mark. Different sections of the lake have different types of microbialites growing. In particular, the thrombolites grow in large numbers in the north east section of the lake. In this location, thrombolites grow close to the shore. Surrounding it is a very thin (0.25cm) thick, flat, non-laminated, discontinuous mat that extends from high tide into the lake. Large, circular thrombolites grow in the deep water. As shown in figure 13 below the different structures present within this area of the lake. The different thrombolite structures are seen to grow differently at different depths. The different thrombolite structures is likely due to the available space while being submerged in the water. The close to shore thrombolites are unable to grow
vertical so have grown horizontally and formed this structure while the deeper thrombolite have this ability so are able to grow up rather than sideways.

Figure 13. Zonation of the different thrombolites and microbial mats in northern section of Lake Clifton. (Burne & Moore, 1993).
3.4 *Multiple stressors to the thrombolites and microbial mats in Lake Clifton and their consequences*

![Diagram](image)

*Figure 14. This diagram illustrates the important threats acting on Lake Clifton and the thrombolites. The different size arrows indicates their significance.*

All of the threats affecting Lake Clifton are highlighted in figure 14. These threats include; large scale land use changes causing increased fertiliser use causing excessive concentrations of nutrients to enter Lake Clifton and increased groundwater extraction, introduced species, and climate change, which influences the hydrological balance of Lake Clifton. This induces a number of stressors on the lake which will ultimately impact on the survival of the thrombolites and microbial mats. These stressors effect or reduce those conditions stated in section 3.1.3 that are critical in the formation and maintenance of the thrombolites.
These stressors include:

- Biological disturbances by introduced species
- Salinity increases
- Nutrient increases
- Climate changes

**Biological disturbances by introduced species**

The impact of introduced species into Lake Clifton is seen as a minor factor due to the limited research into the impact of their effect. Black Bream (*Acanthopagrus butcheri*) in figure 15 below were introduced into Lake Clifton during the 1960’s for recreational fishing (Sarre 1999). Although according to Sarre (1999) they do not directly feed on the thrombolites, but they do feed on the small invertebrates living around the thrombolites, which still causes impact by it ripping into the microbial material. In 1996, it was thought after the discovery of the large size Black Bream (up to 40 centimetres) that this may have been a new species (Sarre, 1999). However, this large size was due to very limited pressure placed on this fish species by predators allowing them to grow to these proportions (Smith 2006). The genetic differences in these fish in Lake Clifton to others from nearby populations can be explained by the allele frequencies for the larger size fish being favourably selected (Norriss et al. 2002). The introduced black beam has been suggested to have a direct influence on the reduced metazoan diversity that has been seen in Lake Clifton (Goater 2003). Along with the black bream, snails have been introduced into Lake Clifton. Both species are known to graze on the thrombolites and microbial mats as well consume some of the endemic metazoan species. These species may have impacts on the other endemic fauna of the lake as well (Luu et al. 2004).
Salinity increases in as seen in figure 15 below is a critical stressor in Lake Clifton due to the significant impact it has on the biota. It has been shown that from the period during the 1980’s to 2006 that there has been a steady increase in salinity. This has been an increase from 17g/L to 32g/L in this period. The data recording the salinity has been patchy and inconsistent over this period, however, the trend is present (Knott et al. 2003). It shows that the concentration of salinity in the 2001-2005 averages slightly below seawater (32g/L).

Knott et al. (2003) suggested that there was a relationship between the amount of rainfall, the water level of Lake Clifton and the salinity, which reflects the results obtained by Smith et al. (2009). Knott et al. (2003) showed how the lake salinity changed at the same time there has been a decline in the rainfall and the lake water level. The rainfall pattern for the Lake Clifton area is shown in figure 17 below. Smith (2006) showed that there was a significant change in the rainfall during the periods between 1981 to 1991 and 1993 to 2006, corresponding to this change in salinity reflecting the strong link between declines in rainfall and increases in salinity.
Figure 17. Rainfall from 1981-2006 approximated for Lake Clifton (Smith et al., 2009)

Figure 18 below is showing the relationship between rainfall and salinity. Although there is a relationship between both salinity increasing and rainfall decreasing during this period of 1985 to 2006 the $R^2$ value is equal to 0.38. This shows in fact there is only a weak relationship between the rainfall and salinity. This is surprisingly low and suggests that the increase in salinity during this time is due to another cause.
Figure 18. Relationship between rainfall and salinity in Lake Clifton (Smith et al., 2009).

The decline in rainfall will ultimately have two effects. One will be less direct precipitation and runoff entering Lake Clifton. This change is likely to be very negligible due these being small inputs initially. The second effect is that it will result in less groundwater recharge. Examining the water level of the lake over this same period, which would be predominately determined by the amount of groundwater input into the lake, shows that there had been a slight decline in lake level. This is likely due to the reduction in rainfall during this same period. In comparing the salinity to the lake water level, the salinity is found to be higher at lower lake levels. This is shown in figure 19 below, which clearly shows this trend. At higher lake levels (4.4m to 4.6m), the salinity is between 15g/L to 20 g/L. However, once the lake level drops below 4.3 m salinity is shown at some periods that exceed that of seawater (35g/L). The trend line plotted shows that there is a relationship present. The $R^2$ value is 0.39. Although this shows the relationship as weak, there is a general increase in salinity with the decreasing lake level.
However, it was suggested by Knott et al. (2003) that rainfall alone was not the only driver for the salinity. This seems contradictory as if there are declines in the amount of freshwater inputs into the lake from rainfall or infiltrated groundwater sources then the salinity will rise. This is expected to be the most reasonable explanation. However, if rainfall was the sole reason then the increase in rainfall in the late 1990’s should have decreased the salinity. Nevertheless, the salinity did not decline meaning that rainfall is not the only effect to the salinity levels in Lake Clifton. Another likely reason to further increased salinity increase could be attributed to increased and unsustainable groundwater extraction due to recent increases in population in the region. Unsustainable water extraction will lead to the groundwater level further declining resulting in less freshwater inputs into Lake Clifton (Luu, Mitchell & Blyth 2004).

Salinity increases in lakes cause major ecological effects with changes in species abundance and diversity usually occurring due to the salinity affecting the organisms’ ability to osmoregulate with its environment. Most species have salinity thresholds.
that once exceeded puts the organism under too much stress and it perishes (Schallenberg, Hall & Burns 2003). This increase in salinity may result in a change in the dominant species in the microbial communities in the thrombolites and microbial mats in Lake Clifton. In particular, Scytonema, a key component of the thrombolites is adapted to low salinity levels (Neil 1984). Thus, an increase in salinity will most likely result in the Scytonema perishing due to passing its threshold levels. It is expected that the level of impact of the increased salinity may not be that severe in the northern section of the lake, due to fresh water input from the groundwater, where the thrombolites are mostly occurring (Smith 2006). However, with increasing changes to the lake including increased groundwater extraction and decreasing rainfall and infiltration, this could result even in this section of the lake going above critical threshold levels for the thrombolites and microbial mats communities and they perish.

*Increased nutrient concentrations changing the light climate of Lake Clifton*

The thrombolites and the microbial mats in Lake Clifton are able to recycled and conserve most of the nutrients that they gain from the environment. This enables them the live in low nutrient environments (Luu, Mitchell & Blyth 2004). Western Australian waterways ecosystems, in particular, have evolved to cope and deal with low nutrient inputs (particularly phosphorous) from the land before human arrival. Upon arrival into Western Australia, humans have applied fertiliser to this low nutrient land to grow crops, which has seen a dramatic increase in the amount of nutrients entering most waterways (Anon 1999). Eutrophication is common in a lot of the waterways in Western Australia due to this increased in nutrients (Anon. 2000a). It is likely Lake Clifton used to have low nutrient loading, meaning that the lower light attenuation enabled the thrombolites and microbial mats to flourish (Smith 2006).

The nutrient inputs into Lake Clifton have changed and increased due to land use changes in the Mandurah region, which included clearing native vegetation for housing and agricultural practices. These land changes have ultimately reduced the vegetation buffer from the specified requirement of 200m that is considered necessary for the removing majority of the nutrients in the groundwater and surface runoff to be removed in the loose sandy soils around the lake. As well, weeds are prevalent in the
vegetation buffer further reducing its effectiveness (Luu, Mitchell & Blyth 2004). The nutrient (nitrogen and phosphorous) levels have been recorded sporadically in Lake Clifton since 1979 as shown in figure 20 and 21 below. The nutrients concentration were obtained from studies done by Moore (1993), Rosen at al. (1996) and Smith (2006). Both the total nitrogen and phosphorous values are shown to fluctuate over the period. The total nitrogen appears to show an increase in concentration from 1979 to 2006. For the total phosphorous, it appears that the concentration declines over this same period as recordings taken from 1991 onwards are lower than the recordings in 1979 and 1988. However, the value obtained in 2006 far exceed the values from 1991-1992. Overall, this nutrient data sparse and is not enough data to conclusively show that there has been a trend in an increase in the nutrient concentrations. It is likely however, that nutrients were far below this until very recently when land changes in the Lake Clifton region occurred.

![Figure 20. Nutrient concentrations of total nitrogen over time in Lake Clifton.](image)
Figure 21. Nutrient concentrations of phosphorous over time in Lake Clifton.

However, most of the concentrations recorded from 1979 to 2006 show they exceed the eutrophic lake threshold in the Australian/New Zealand Guidelines. The threshold values determined by these guidelines for a freshwater lake are for total phosphorous 10μg P/L and for total nitrogen 350μg N/L (Anon. 2000a). This means Lake Clifton is a eutrophic system with sufficiently high productivity, which would mostly be phytoplankton. This confirms that the lake has a high level of phytoplankton which is likely shading the thrombolites (Luu, Mitchell & Blyth 2004).

As mention in section 2.1.3 increased nutrients into a saline lake like Lake Clifton, will increase the phytoplankton concentration. Particular species within the phytoplankton react strongly to the nutrients causing mass growth. This increase in phytoplankton would change the limit climate of Lake Clifton. As well, certain benthic algal species such as Cladophora have been observed in recent years to be growing on the thrombolites (Steve Dutton, personal comment). This recent event is likely due to the with increase nutrients in the water. These are smothering the thrombolite community below restricting the available light to it. Both the
phytoplankton and benthic macroalgae are reducing the available light to the thrombolites and microbial mats to photosynthesis. As the thrombolites use photosynthesis for not only for gaining energy but also in building of its structure it reflects the importance of them having access to light. This increase in nutrients is likely influencing the thrombolites and microbial mats. If the phytoplankton were to reach a concentration where the threshold of light attenuation levels where the thrombolites and microbial mats are unable to photosynthesise at adequate levels anymore this would result in these microbial communities perishing (Luu, Mitchell & Blyth 2004).

*Future Climatic changes*

Further climate change impacts on Lake Clifton could likely see the salinity increase. This is due to the predictions made for the south-west of Western Australia which includes Lake Clifton and the other Yalgorup Lakes, that there will be a decline in rainfall, an increase in temperature and evaporation, and an increase the average wind speed (Anon. 2009; Garnaut 2008). Therefore, this makes Lake Clifton very susceptible to climate change as these will alter the hydrological balance of the lake. One additional side to climate change is that it will likely change the pH of the water, which will the rate of calcium carbonate precipitation due to higher CO₂ concentrations in the water (Smith 2006).

The fresh groundwater predominately flows into Lake Clifton on the north-eastern side of the lake and is most likely the reason for the thrombolites growing there. Exposure of a large number of thrombolites and microbial mats occurs regularly during summer when the lake level drops. At the present summer exposures limits the ability of the thrombolites to grow. Usually, *Scytonema* survives using its sheath to protect itself. It is during these low lake levels that the thrombolites and microbial mats have access to groundwater seeps at the base to help them survive. However, with further reductions in groundwater, due to reductions in rainfall, this could lead to longer exposure periods for the thrombolites and mats. With longer exposure periods, more thrombolites and microbial mats will more likely perish under these conditions as well longer periods. Climate change predictions are for higher temperatures, which will mean higher water temperatures. These increases may allow higher concentrations of phytoplankton to exist year round, as these organisms prefer higher
water body temperatures. The Lake Clifton is a shallow lake so would change more rapidly than other deeper lakes. It will still have a large volume, so will not change a great deal but will still change. With climate change, there will be higher water body temperatures. Anneville et al. (2005) mentions the potential for climate interacting with the phytoplankton, as the higher water temperatures will drive great phytoplankton concentrations. This potentially leads to greater shading in the lake over the thrombolites and microbial mats. This highlights another impact of climate change that could occur on Lake Clifton (Anneville, Gammeter & Straile 2005).

Multiple stressors on the thrombolites and microbial mats

The interaction of these multiple stressors will add up on each other and cause different and unanticipated effects to occur. The stressors of salinity increase and the increase nutrients will combine their effects on thrombolites and microbial mats communities as they are adapted to low salinity and low nutrient environments. Changing both of these conditions in the lake will increase the impact on the thrombolites and microbial mats and add further stress to them. If this stress reaches a threshold, ultimately the thrombolites and microbial mats will perish. One particular threat to Lake Clifton is climate change, as this will bring with it unknown changes to the lake. These future climate changes will interact with these present changes of salinity, nutrients and introduced species to have further negative impacts on the microbial community of the thrombolites and microbial mats.

One interesting point in regards to multiple stressors is the interaction of black bream with salinity. Although the black bream can tolerate some increases in salinity, it has been seen in large numbers to perish during high peaks of salinity. This means the problem of dealing with the introduced black bream may be solved by the increases in salinity in the lake. Often normally multiple stressors will act synergistically, however, in this case the increase in salinity if sufficient is able to reduce the effect of the black bream on the thrombolites and microbial mats (Norriss et al. 2002).

Luu et al. (2004) suggested that if there are sufficient changes in Lake Clifton to the physical parameters of the lake then it might cause a shift or change in the microbial community in the thrombolites from those now, which dominate the thrombolites and
which precipitate out the calcium carbonate, to a community that no longer does this. Smith (2006) did a comparison by a percentage of the total flora making up the microbial community of the thrombolites between 1984 and 2006 and found that in fact there had been a shift in the thrombolite community as shown in figure 22 below. The composition of the thrombolite community has significantly changed during this period. Of particular importance is the decline in filamentous cyanobacteria and in particular Scytonema, which are responsible for the formation of the thrombolite structures. Smith (2006) found a massive reduction in the Scytonema from approximately 20% of the thrombolite microbial community in 1984 down to less than 1% in 2006. This species is critical in the formation of thrombolites. The likely low percentage of Scytonema in this study was concerning for the future of the thrombolites in Lake Clifton. Other changes in the thrombolite community include a dramatic increase in unicellular cyanobacteria (16% to 50%) and diatoms (8% in 1984 to 28% in 2006). The likely reason for these changes in species composition is these stressors affecting the thrombolites community. It is likely these changed conditions in the lake are now favouring the diatoms and unicellular cyanobacteria over the filamentous cyanobacteria (Smith et al. 2009).
Figure 22. Species composition comparing Neil (1984) and Smith (2006) in thrombolites in the green-black thrombolites (located in the northern section of Lake Clifton). Filamentous bacteria did not include the species Scytonema. This was considered separate.
4 Thesis research Aims

Lake Clifton provides a good opportunity to study a site where vast changes have occurred and now looking to see what these changes are doing to the biota within the lake. This study aims to:

1. Understand how increased phytoplankton may affect the light climate in Lake Clifton
2. Assess how the changed light climate is affecting the ability of the thrombolites and microbial mats to photosynthesise
3. Assess the health status of the thrombolites and microbial mats
4. Predict how future climatic changes will impact the thrombolites and microbial
5. To increase understanding, knowledge and raise awareness of the rapid changes to Lake Clifton and the effect this is having on the thrombolite and microbial mats communities.

The significance of this work will be to give an idea to the level of health of the thrombolites and microbial mats in a lake that has undergone a large number of changes. This has never been done before on the thrombolites and microbial mats in Lake Clifton, highlighting its significance and innovation. Thus, using the photosynthesis rates, measurements of stress and the species composition of the thrombolites and microbial mats can be used as an assessment for their health.
5 Methodology

The following approach was employed to achieving the research aim using spectrofluorometry and Pulse-Amplitude-Modulation (PAM) fluorometry to:

1. Assess the water column eutrophication by comparing current measurements to historical data.
2. Looking at a change in the photosynthetic efficiencies of the thrombolites over depth.

5.1 Site

The boardwalk at the end of Mount John Road at the north-west section of Lake Clifton was the sampling site as shown in figure 23 below (approximately 32°44.717S, 115°39.245E). Sampling with the fluoroprobe, water samples were taken at the end of the boardwalk. This sampling took place from August until the end of October 2009, on a weekly basis, meaning the results are reflective of winter and spring time conditions in the lake. Measurements of the health status and the photosynthesis efficiencies of the thrombolites and microbial mat were taken in two transects alongside the boardwalk. This sampling was done over two days over a period of two weeks (11th of October and 18th of October). Along these two transects, thrombolites were measured at approximately 1 metre apart. The two transects were of slightly different depths which went from the shoreline of the lake to approximately 1 metre deep. The microbial mats were measured only at three positions along one of the transects. Microbial samples of the thrombolites and microbial mats were collected sporadically during the sampling visits.
Figure 23. Close up of the section of Lake Clifton showing where sampling was completed.

5.2 Chlorophyll a

The phytoplankton concentration, composition and distribution in the water column was measured using the concentration of chlorophyll a as an approximate value due to this being the dominant phytoplankton pigment. The fluoroprobe was used to calculate this. This was done at the boardwalk platform where it was lowered into the lake at 1 metre intervals along the boardwalk for 36 drops per sampling visit (figure 24 below). The total concentration of chlorophyll a and the concentration in terms of chlorophyll a of the four different groups in the phytoplankton are measured. The fluoroprobe records the concentrations of four groups in the phytoplankton: green algae, cyanobacteria, diatoms and cryptophyta. The concentrations were recorded over the depth of the lake. Averages of the total chlorophyll a were made and of the four different groups the fluoroprobe is able to distinguish over depth on each sampling visit. Collecting was done over a period of weeks to also see if there were changes in concentration and distribution from winter into spring.
The phytoplankton total concentration was also measured by taking 1 L water samples at the sampling site in Lake Clifton and then using the soaking-extraction method to calculate chlorophyll a. This was done as a comparison to the concentrations of the fluoroprobe. Water samples were collected at mid-depth of the lake using the Van Dorn Sampler. Four 1 L water samples were collected on each sampling visit. The water bottles were rinsed three times with the sample water prior to collection (figure 25 below).
The water was put into a dark container and then transported back to the laboratory where the water was filtered in a filtration manifold using 25 mm glass microfibre filters. Two filter papers were collected per sample bottle. The filter paper was then refrigerated. The filter paper were then were added to 8 ml of 90% acetone. The samples were then put into the freezer to steep for 24 hours after this. The tubes were shaken once during this period. The filter paper was removed from the tube before it was placed into the Allegra X-12 Centrifuge (at 2500 RPM for 5 minutes) to concentrate the chlorophyll a to the bottom. The tube was placed into the fluorometer to calculate the chlorophyll a concentration. An initial value was recorded after stabilisation which is before the acid is added value (R_b). After which, 3 drops of 1M HCl are then added to the tube. The tube is capped and then shaken for 60 seconds. The extracted solution is then run through the fluorometer again to record the after the is acid added value (R_a). Then the following equation was used to calculate the chlorophyll a concentration:

\[
\text{Chlorophyll a (\mu g/L)} = \frac{(r/r-1)(R_b - R_a)}{v/V}
\]

EQ 2

\( r \) = the before to after acidification ratio of a pure chlorophyll a solution. This value is standard for the fluorometer machine and is 1.78.

\( R_b \) = fluorescence of a sample prior to acidification

\( R_a \) = fluorescence of a sample after acidification

\( v \) = volume of the extract (ml) (i.e. the volume of acetone added to the sample)

\( V \) = volume of the filtered sample (ml)

The chlorophyll a concentrations obtained were averaged for each sampling visit. These values obtained in the soaking-extraction method were then compared to the fluoroprobe concentrations as a check and validation to see if they were both giving similar results.

5.3 Photosynthetic efficiency of the thrombolites and microbial communities

The photosynthesis efficiency of the thrombolites was recorded using the Diving PAM fluorometer at the sampling site. The photosynthetic efficiency is a
measurement of Two transects of thrombolites were measured from the shore into the lake. In order to calculate efficiency of photosynthesis a number of Rapid Light Curves (RLC’s) are measured on each thrombolite. This included taking measurements on the top surface of the thrombolites and on the sides of the thrombolite. The surrounding microbial mats were assessed as well. An example of the PAM connected to a thrombolite is shown in figure 26 below.

![Figure 26. Photograph of thrombolite with the adaptor and fibre optic cable connected to the thrombolite](image)

RLC’s provide detailed information on the saturation characteristics of electron transport, as well as the overall photosynthetic performance of a plant. RLC emit light of different intensities to measure the potential activity (electron transfer rate). The electron transport rate (ETR) was found to be closely related to the photosynthetic activity when measured by oxygen evolution or CO2 uptake. This can then provide information of the saturation characteristics of electron transport and the overall photosynthetic rates of the organism, by an approximation of the rate of electrons pumped through the photosynthetic chain. At a certain intensity the rate will reach a maximum. After this point photoinhibition begins in the microbial community and begins to reduce the amount of photosynthesis it can do. The value of ETR where the RLC plateaus is the measure of efficiency of photosynthesis (Ralph & Gademann 2005). Figure 27 below shows how the efficiency of photosynthesis was calculated at each RLC. Over the RLCs generated per thrombolite an average efficiency was calculated. A graph was made comparing depth of which the thrombolite was measured to the average
photosynthesis efficiency of the thrombolites. Similarly, a similar plot was completed with the microbial mats in testing their efficiency of photosynthesis against depth.

![Diagram showing ETR vs. Irradiance](image)

**Figure 27. Sample RLC’s from one of the thrombolites measured. The lines drawn show where the curve is plateauing and so is the values for the efficiency of photosynthesis at that particular location on the thrombolite.**

### 5.4 Thrombolites and microbial mats ‘health’

The measure of health of the thrombolites is by the dark yield (DY) measurement. This is a measure of how much stress the thrombolite and microbial mats are in. This uses the Diving Pam Fluorometer. The diving Pam Fluorometer is able to measuring the ability of photosynthesis of the thrombolite under the saturation of light by measuring the fluorescence returned. The DY is calculated by the difference in two fluorescence measurements after thrombolite or microbial mat has been saturated with light. The first fluorescence (F) is measured on the thrombolite after it is saturated in light under normal ambient light conditions. The thrombolite or microbial mat is then dark adapted for a period of 10 minutes using the Surface Holder DIVING-SH, saturated with light and then the fluorescence is measured (F’).
(PAM manual). Figure 28 below show waiting the 10 minutes for the dark adaption. The dark yield calculation is measured as:

\[ \text{DY} = \frac{(F' - F)}{F} \quad \text{EQ 3} \]

This dark yield value is then used a value of health of the thrombolite. A more ‘healthier’ thrombolite will be photosynthesising relatively more (as using more light in the photosynthesis process so will emit less as fluorescence) while a less healthy thrombolite will not be as they will be stressed (not using as much light so emitting more light as fluorescence).

![Figure 28. Dark adapting the thrombolites for 10 minutes.](image)

### 5.5 Water Column biological samples

Microbial composition of the phytoplankton in the water column was examined under the Ziess Optical microscope. Water samples (100mL) were taken at Lake Clifton using the Van Dorn Sampler. These were then placed into the dark container where they were brought back to the laboratory and placed in the refrigerator for 24 hours. 15 ml water subsamples were first placed into the Allegra X-12 Centrifuge (at 2500 RPM for 5 minutes) to concentrate the organisms to the bottom of the tube. The water subsamples were taken to the Centre of Microscopy and Material Analysis (CMCA) at the University of Western Australia to examine under the Ziess Optical
Microscope. Multiple view fields were used under examination of the microscope include; 2.5X, 5X, 10X, 20X and 40X magnification. Only preliminary identification of the species present were made using those indentified by Smith (2006) and Neil (1984) as guides. High magnification photographs were taken using a mounted colour CCD digital camera attached to the Ziess Optical microscope as seen in figure 28 below. In addition, fluorescent microscopy using the Ziess Fluorescence microscope was used to indentify species fluorescence under UV, blue and green light with photographs taken of organisms fluorescing under these lights. The fluorescence is used to show up what the different absorption and fluorescence light back different organisms have. For example, under UV light chlorophyll a fluoresces red. Thus, any organism that fluoresces red under UV is photosynthetic.

Figure 29. Ziess optical microscope with mounted CCD digital camera used to obtain images

5.6 Thrombolites and microbial mats microbial biological samples

Samples of both the thrombolites and microbial mats were taken from the sampling site. A small scraper instrument was used in removing the thrombolite mat samples as well as taking the microbial mat samples. Approximately 60 grams of thrombolite and microbial mat material was taken overall from a number of small, thin samples. Samples were then placed into a 100 ml glass bottle filled with lake water. The sample is stored in a dark container until it was transported back to the laboratories at the University of Western Australia. The samples were refrigerated for
a period of 24 hours. The samples were then looked at using the Ziess Optical microscope at CMCA. The samples under the microscope were examined to identify some of the different microorganisms identified in the thrombolites and microbial mat microbial communities. Both light and fluorescence microscopy were used to examine the thrombolite and microbial mat samples. Fluorescence microscopy was used to find photosynthetic organisms in the thrombolite or microbial mat samples. This was used to get out a proportion of the amount of *Scytonema* and other important species present in the thrombolites and microbial mats communities. Photographs were taken of interesting results.
6 Results

6.1 Chlorophyll a concentrations in the water column

The average total chlorophyll a concentrations measured from each sampling visit to Lake Clifton by both the fluoroprobe and by taking water samples and doing the soaking-extraction method as presented in figure 30 and 31 below respectively. The concentrations of chlorophyll a is shown to vary in both methods fluctuate in time. The chlorophyll a concentrations were higher in the soaking-extraction method than the fluoroprobe method. There did not appear to be any seasonal pattern in the total chlorophyll a concentrations. The values of the fluoroprobe seem to match closely to that from doing the soaking-extraction method.

A comparison of the chlorophyll a concentrations between the two methods: using the fluoroprobe to measure in-situ and the soaking-extraction method to measure in the ex-situ in the laboratory is shown in figure 32 below. A linear regression analysis was performed comparing the values measured on the same sampling days. There is a strong relationship with a $R^2$ value equal to 0.8424. In all the samples, the concentration of the soaking-extraction method was always greater than the fluoroprobe measurements.

![Figure 30. Total chlorophyll a concentrations of the water column over time using the fluoroprobe over sampling period](image)
Figure 31. Total chlorophyll a concentrations of the water column over time using the soaking-extraction method over sampling period.

Figure 32. Comparison of the fluoroprobe chlorophyll a concentrations to the chlorophyll a concentrations over time.
6.2 **Comparison of average total chlorophyll a to previous data.**

The comparison of the average concentration of chlorophyll a in the soaking-extraction method against measurements taken by Smith (2006) using the same method are shown in figure 33 below. This figure shows that the concentration of chlorophyll a in 2009 is less than the concentration from 2006 (2.27µg/L to 2.67µg/l respectively). The standard error bars show that there is greater variability in the values in 2009 than in 2006. Therefore, even though the average was smaller it means that the values did fluctuate around more.

![Figure 33. Average chlorophyll a concentration from 2009 compared to that from Smith (2006). This is comparing concentrations measured using the soaking-extraction method.](image-url)
6.3 Phytoplankton distribution in the water column

Figure 34 below shows the concentration of chlorophyll a in the water column. This plot shows over depth the concentration of chlorophyll a. The graph shows the concentration does increase slightly with depth. The maximum concentration of approximately 1.6μg/L is consistent from 0.6m to 1.2m depth. The large standard error bars show that for most of the depths these overlap each other showing that the concentrations remains consist with depth.

Figure 34. Total chlorophyll a concentration over the water column
Figure 35 below shows the chlorophyll a concentration of four groups in the phytoplankton (green algae, cyanobacteria, diatoms and cryptophyta) over the water column. The figure shows that the cyanobacteria are mostly located in the top layer of the water column to a depth 0.4 m after which the concentration rapidly declines. For green algae and diatoms in this surface layer are found in low concentrations. These two groups peak in concentration at approximately 0.6 m in the water column. These two groups consistently remain at this concentration until a depth of approximately 1.2 m. Cryptophyta was found throughout the water column. However, its concentration was always low in comparison to the other groups reaching a maximum at 0.12μg/L at 0.4 m depth.

Figure 35 Concentrations of chlorophyll a for the four different groups of microorganism in the phytoplankton identified by the fluoroprobe over depth of the water column.
6.4 Efficiency of photosynthesis for the thrombolites and microbial mats

The measurements of efficiency of photosynthesis were calculated for each of the RLC’s that were measured on each thrombolite along the two transects. An example of the rapid light curves produced from the thrombolites is shown below in figure 36. This was of a thrombolite at 40 cm depth. Further examples are in Appendix 10.3. The photosynthesis efficiencies were averaged for each thrombolite.

![Example rapid light curves from thrombolite of 40 cm depth](image_url)

Figure 36. Example rapid light curves from thrombolite of 40 cm depth
Figure 37 below shows the average photosynthesis efficiency of the thrombolites over depth in the two transects. The pattern observed is not clear but shows there is a weak association with the efficiency declining for either transect. As the figure 37 shows transect 1 does decline from 0.3m to 0.9m in transect except for 0.4m and 0.7m. The large standard error bars do show there is wide variability in the 0.7m recording meaning there is considerable overlap with the efficiency measurement of the depths above. For transect 2, which had much lower photosynthetic efficiencies, the trend is similar to that of transect 1. Therefore, overall there appears to be a relationship between increasing depth and decreases in photosynthetic efficiency.

![Figure 37. Average photosynthetic efficiency over two transects for the thrombolites over depth in Lake Clifton](image-url)
Figure 38 below shows the RLCs for the microbial mats. For the three microbial mats sampled, only one RLC measurement was taken. It shows that mat one located only in 30 cm in water had a much greater ETR before photosynthetic inhibition occurred compared to the other two mats curve from the RLC.

![Figure 38. Rapid light curves for microbial mats at three different depths.](image-url)
Figure 39 below of is the efficiency of photosynthesis of the different mats against depth. This shows clearly for the microbial mats that with increasing depth there is decreasing efficiency in the photosynthesis, which means lower amounts of photosynthesis are occurring with mats of increasing depth. Since only one measurement was taken at each depth for the microbial mats there was no standard error bars.

Figure 39. Average photosynthetic efficiency of microbial mats over depth
6.5 Health assessments of the thrombolites and microbial mats using

The values of dark yield overall were found to be low. The dark yields were measured over two sampling days. On the first sampling day the dark yields were all measured as 0. This would suggest that the thrombolites were under tremendous stress and are no longer photosynthesising. On the second sampling day the average dark yield was measured at 0.20 (SD 0.29). This shows that the values between all the measurements are highly variable. These low values, suggest that the thrombolites are stressed. This means the health of the thrombolites could be threatened.

Figure 40 below shows the plot of dark yield measurements over depth. It was hypothesised that with increasing depth the health of the thrombolites could be reduced due to increased stress placed on it due to higher levels of light attenuation. The dark yield values oscillated between increasing and decreasing with depth. The dark yield values over depth increased from 0.2m to 0.3m and 0.4 to 0.5. It declined during 0.3m to 0.4m and 0.5m to 0.6m. This makes it hard for any pattern to be deduced. However, it appears likely that it would decrease with depth. The standard error bars associated show how much they vary. Although the average DY value at 30 cm was the highest recorded, the large standard error bars do show it fluctuating to be even lower than the average DY value at 0.2m.
Only one measurement of dark yield for the microbial mats was taken at 30cm depth. The measurement 0.12. Without other values to compare from same location or from other depths no further analysis can be done. However, it is a low number suggesting the microbial mat is not in a healthy state.
6.6 Water Column biological samples

Biological sampling of the water column showed that a number of different species were in the water column. The exact species could not be identified, however, the preliminary analysis found that eukaryote algae, cyanobacteria species and pennate diatoms were the dominate species. There were several unknown or unidentified structures, which occurred frequently. Fluorescence microscopy was used to determine photosynthetic organisms. Except for a high abundance of Oscillatoria, very few other photosynthetic organisms were found. Figure 41 below shows the images of the different organisms. Included are some pictures taken under UV light to show up chlorophyll a pigmented organisms which fluoresce red under this light. Further images are in appendix A.
Figure 41. Microscope images of specimens found in the water column of Lake Clifton
6.7 Microbial composition of the thrombolites and microbial mats

Images of both the thrombolites and microbial mats are shown in figure 42 and 43 below. The thrombolite samples, which were examined, were found to have large amounts of Scytonema. It was thought that the amount examined in the samples was closer to the amounts observed by Neil (1984) at approximately 20% of the material rather than Smith (2006) who found very little. Only one small sample of microbial mats was analysed and very little biological material was identified. The images are seen in figure 41 below. Further images of the thrombolites are in Appendix A.
Figure 42. Images of the thrombolite microbial community. A large amounts of organisms found were identified as the cyanobacteria *Scytonema* due to it fluorescing red under UV light.
Figure 43. Images of the most common specimens in the microbial mats located at the boardwalk in Lake Clifton
7 Discussion

7.1 Phytoplankton concentration and composition in the water

Understanding the phytoplankton of Lake Clifton was one of the aspects of this study. By furthering knowledge on the total concentration and composition of the phytoplankton in Lake Clifton this will contribute and be able to help with management of the thrombolites. Species composition can be used as an indicator of changes in the environment, notably the changes in nutrient loading into the lake. How the phytoplankton concentration changes is also important for management as it a key concern of regulators to determine the presence and abundance of nuisance species which could go into eutrophication and cause other environmental problems. This is important in knowing the species composition of the water (Anneville, Gammeter & Straile 2005). The total concentration will be used in understanding how much the thrombolites are being light attenuated which will reduce the ability of the thrombolites to photosynthesis.

With the increase of nutrients into Lake Clifton, it has likely led to an increase in the phytoplankton. The total chlorophyll a concentration was slight below the concentration measured in 2006. However, there was a greater standard error bars in these results which is likely due the longer sampling regime undertaken in this study. In some of the measurements, the measurements of chlorophyll a exceeded those from the 2006 concentrations. This could mean that the phytoplankton concentration had in fact increased between 2006 and 2009. The sampling period in this study was much longer than period than Smith (2006). The concentration was seen to fluctuate more which may be common feature in Lake Clifton that Michael Smith failed to find. It is also possible that these values of chlorophyll a obtained by both Smith (2006) and in this study could be representative of the spring bloom effect (Klaff 2002). This could mean during the rest of the year the concentrations could be lower and do not cause as much light attenuation onto the thrombolites over the rest of the time.
The composition of the phytoplankton found that the cyanobacteria was located in high concentrations (approximately 1 μg/L) in the top layer of the lake (approximately top 40 cm). Klaff (2002) suggests that over 70% of the cyanobacteria accounts for the phytoplankton during the summer periods. These measurements were measured in spring and not summer. However, the conditions during spring are ideal due to the increase in temperatures and the amount of sunlight after winter is lower however, there still was a high proportion of cyanobacteria present. The diatoms and green algae had concentrations of approximately 0.4 μg/L and 1 μg/L. These were found in higher concentrations at depth between 0.6 to 1.2 m.

There are a number of other reasons for the phytoplankton distribution. One of these reasons is due to the active movement on behalf of the organism to go to particular parts of the water column to be at light levels that they are adapted to. These phytoplankton like light of certain frequency and intensity, so they pick a spot in the water column Phytoplankton is able to do this during periods of stratification or low turbulence in the water. Stratification could occur in Lake Clifton due to the freshwater inputs from the groundwater having an lower density than the saline water of the lake. This would cause a freshwater layer overlaying the higher saline water from the lake. This could explain the cyanobacteria being located in the top 40 cm of the water column and the diatoms and cyanobacteria from the depth of 60 to 120 cm of water column. These could show the two layers of the water column with the cyanobacteria in the top layer and the diatoms and green algae in the bottom layer. For example, with high concentration at the top the cyanobacteria could like these conditions of light and position itself here. Often cyanobacteria have large cyclic blooms at the surface. As well, cyanobacteria can use a gas vacuole as an effective means of remain buoyant and close to the surface (Klaff 2002).

Another likely reason for this distribution is because of the settling out of the phytoplankton due to their size. Bigger and denser size objects will sink faster than smaller and lighter objects. If the system is well mixed then the distribution will be dependent on size of particles as the turbulence will be far greater than any ability in the organism to move itself in the water. Diatoms often contain silica which means that they are often more denser than other phytoplankton groups. The larger species
are often prone to sinking in periods of low turbulence. This could explain the reason for the diatoms having higher concentration at greater depth. The larger size of diatoms in comparison to cyanobacteria is a possible reason for the diatoms located deeper in the water column as they sink deeper. Winds can cause this turbulence in the water. Even at low wind speeds (3m/s to 5m/s) it can induce enough mixing to exceed any movement by the phytoplankton (Klaff 2002). At almost every sampling visit to Lake Clifton it was noticed that there were at least some moderate winds experienced. This would assume that Lake Clifton then undergoes some level of mixing regularly. This likely means the phytoplankton to some level are distributed due to settling upon their size.

7.2 Light levels within Lake Clifton and impact of future changes to the lake

This thesis was to further understand the how the changing phytoplankton concentrations was altering the limit climate in Lake Clifton and then to determine the effect this had on the thrombolites and microbial mats. This was done by assessing the thrombolites and microbial mats in their ability to photosynthesis over a range of depths as with increasing depth there is increasing light attenuation. Thus, the photosynthetic efficiency was assessed. The efficiency of photosynthesis is a measure of the effectiveness or the ability of an photosynthetic organism to photosynthesis. The results from the efficiencies against depth found that overall it appears that the efficiency did decline with depth, however, this relationship was weak due to the efficiency actually increasing at certain depths. The depth values will be used as a proxy to the amount of light attenuation rate. The depth values will relate to how much light reduction there is and how this relates to photosynthesis efficiency. Similar studies have been done with seagrass using depth as a measure of the level of light attenuation showing that it can be used as an effective proxy value (Anon. 2008).

This depth value will be used as a value for the amount of light attenuation which can be used in future predictions on how these changes will impact the thrombolites and microbial mats. Future changes within the Lake Clifton region include an increases in the human population which will possibly lead to greater land use resulting in greater inputs of nutrients (particularly phosphorous) into Lake Clifton. Therefore, the likely
increases in nutrients leading to increased phytoplankton which will lead to greater levels of light attenuation in the lake. This will result in restricting the thrombolites and microbial mats distribution as the level of light attenuation at certain depths will exceed the ability of the thrombolites to photosynthesise. The thrombolites and microbial mats in Lake Clifton must exist in this photic zone region otherwise they will not have enough light for adequate photosynthesise (Klaff 2002). Predicted future change would most likely reduce the available area of the thrombolites habitat due to exceeding the threshold levels of light attenuation at certain depths.

7.3 Thrombolite and microbial mats health status at present

To examine the health of the thrombolites and microbial mats it required assessing both the thrombolites and microbial mats. Light is critical for the thrombolites and microbial mats as it is very important in terms of photosynthesis to gain energy. In particular, for the thrombolites, it is also the formation of their calcium carbonate structures. The results from the dark yields were all relatively low values meaning that the thrombolites likely to be stressed. However, no previous data from previous studies is available to confirm such values. When these measurements were taken over depth, it found that the DY values fluctuated with depth. Initially the DY values increased from 20 cm to 30 cm depth, after which it declined to 40 cm. It increased again then decreased again with depth. There are large standard error bars that overlap each other could mean that the dark yield values does not change between thrombolites over with depth. This is surprising since with increased depth there will be more light attenuation, which means the thrombolite would most likely be more stressed as it will be closer to reaching the threshold of not enough light reaching it for photosynthesis. This would likely stress the thrombolite out as it has limited access to light.

This result from the DY’s measuring health shows the thrombolites are likely to be stressed from all the changes to the lake. The pattern of photosynthetic efficiency against depth shows that there is a weak relationship between the efficiency and depth although there were depths where it was found to be an increase in efficiency rather than a decrease. However, overall there was a pattern of it decreasing. This is showing how the deeper thrombolites are being reduced in their ability to photosynthesise by
the reducing light climate. Similar results were found from the microbial mats photosynthetic efficiencies. Only 1 DY measurement was taken from the microbial mat.

With both of these results, they suggest that with depth the thrombolites and microbial mats are less healthy and have lower photosynthesis efficiency. This means the thrombolites are being affected by the light attenuation. It is likely that an increase in phytoplankton resulting from the increase in nutrients has occurred in Lake Clifton and has resulted in increase in light attenuation. This is likely resulting in this pattern to form due to the deeper thrombolites being restricted to limited light levels causing the thrombolites to become stressed. This paints a dire situation for the thrombolites.

Contrary to the evidence from these results, where it seems to indicate the thrombolites are stressed, the analysis of the thrombolite samples themselves show that *Scytonema* and other filamentous species were present in relatively high proportion in the thrombolite samples. This would indicate that the thrombolites, at least during the sampling period of September-October, had the right constituents within them. This could likely infer that the thrombolites had the capability to photosynthesise and grow to form their structure. The higher amount of *Scytonema* found in these results, leads to the idea that the thrombolites are in similar proportions to those collected by Neil (1984) rather than where Smith (2006) suggested very little *Scytonema* were present in the thrombolites. Other filamentous species were identified in the samples which are also important in the formation of the thrombolite structure. Having the right species in the thrombolite community could indicate that in fact the thrombolites are not in such a dire situation after all, as the results from the dark yields and photosynthesis suggested.

### 7.4 Lake Clifton under threat from multiple stressors

Lake Clifton is under a number of different threats and each of these cause a number of different stressors added to the lake. These stressors have the potential for synergistic and unique interactions causing changes to the lake. Stressors such as increases in salinity, nutrients and introductions of Black Bream within Lake Clifton
will have a number of impacts on the thrombolites and microbial mats. Individually, these stressors have a number of effects the thrombolites microbial mats. These stressors act simultaneously and their effects combine with each other on the thrombolites and microbial mats. These stressors will effect the thrombolite in ways that are unknown or different than expected. The consideration of the multiple stressors within Lake Clifton means dealing with all of them at the same time, which is much harder. If treating each threat or stressor alone, this will not cover the whole effects on the lake. These stressors ultimately add up on each other causing unknown and unusual side effects. In addition, future climate change predictions indicate further reductions in rainfall. This ultimately will lead to reductions in groundwater recharge and lower amounts of freshwater entering Lake Clifton. This will likely influence the salinity, although as the recent change in rainfall showed only 38% of the change was due the rainfall decline.. However, this reduction of rainfall will most likely increase the salinity in lake and will put further pressure on the thrombolite and microbial mat communities. Other changes are higher temperatures leading to high water temperatures, which could have higher phytoplankton concentrations year around, and as well causing further declines in water table to expose the thrombolites for longer periods testing the ability of thrombolites to survive exposure. Increases in nutrients are predicted in the future, likely due to further human development within the region, allowing more nutrients to be able to enter the lake via the groundwater and runoff.

These stressors will cause changed in the microbial communities of the thrombolites and microbial mats. Most likely, the species in the communities will perish particularly the low saline adapted species. *Scytonema*, a critical species in the thrombolite community is one species that is adapted to low saline water. If the salinity was to rise as this will perish. However, at the same time the amount of nutrients has change resulting in a change in the light climate. The increased light attenuation could lead to the *Scytonema* perishing at lower salinity levels as it is not getting enough light for energy and growth. This species is critical for the formation of the thrombolites so if it perishes, ultimately it will lead to the thrombolite community not being able to survive. The changes to the lakes are not independently affecting the thrombolites separately. They are occurring simultaneously causing greater effects than when the different factors affect them than when kept separate.
(Folt, 1999). Results from Smith (2006) showed there was a lack of *Scytonema* species, a key former of the thrombolites, found when examining the thrombolites. This would suggest that these thrombolites have already been affected by these changes to the lake and changes in the thrombolite community as predicted have occurred. However, examination of the thrombolite mats in this study however show there was greater proportion of *Scytonema* than what Smith (2006) found. In fact, the proportion of Scytonema may have been closer the 20% of the thrombolite mat that Neil (1984) found. This may reflect a change in the conditions in the lake since that time or that could be some cyclical variations in the conditions in the lake or in the thrombolite communities themselves.

The results from this study show that there seems to be a relationship between depth and photosynthetic efficiency and the health of the thrombolites and microbial mats. This means that thrombolites and microbial mats are effected by light attenuation in the water column. This is increasing the stress on the thrombolites with this change in the light climate. This stress on the thrombolites is likely due to the effect of the changed light conditions but also partly due to the other stressors on the lake. With the greater nutrient inputs into the lake this would likely affect the deeper growing thrombolites as the most amount of light attenuation will occur to them. Thus, there could be strong pressure placed on the thrombolites and overall reducing their available distribution. This health measurement is a combination of the effects of the rising salinity, reductions in freshwater and available ions, effect of introduced black bream and snails, and increased nutrients causing reduction of the light. It is important to be able to understand and appreciate the synergistic and unknown effects and try to predict how they will end up affecting the thrombolites in the end.

The thrombolites and the microbial mats are adapted to a number of conditions including low saline water and they are adapted to low nutrient levels to avoid competition with faster growing algae species to exist in a low light attenuated system. The thrombolites require the calcium ions from the groundwater. Now that Lake Clifton has changed, these requirements of the thrombolites and microbial mats are not being met. Also, with further changes predicted for Lake Clifton likely in the future, the prosperity of thrombolites and microbial mats looks unlikely. Smith (2006) suggested the condition of the thrombolites is critical and urgent action should be
done in order to protect the thrombolites with high recordings of salinity and nutrient concentrations likely to have had an effect over an extended period. For if the thrombolites diminish, this will ultimately mean the rest of the lake’s biota and physical and chemical properties have changed. Thus, the thrombolites can be seen as an indicator for Lake Clifton’s general health. If they are still growing under all these stressors and threats posed to it, then the lake is still in reasonably good health. However, if the thrombolites are not found to be growing then it seems as if the changes to the lake are having an effect and the lake is not in good health.

7.5 Comparing fluoroprobe to the soaking-extraction method

The results show there is a strong relationship with an $R^2$ equal to 0.8424 between the measurements taken of total chlorophyll a using the fluoroprobe as compared to the water samples taken and using the soaking-extraction method. This was done as a validation check between the measurements undertaken by the fluoroprobe to see if these matched closely to values obtained in the soaking-extraction method. In all the sampling visits the chlorophyll a concentrations were found to be greater in the soaking-extraction method than in the fluoroprobe method. In comparison to other studies which have collected chlorophyll a measurements using both these methods and did comparisons, they showed that they get similar results. One study by Chiuchiolo et al. (2006) found that on average the fluoroprobe total chlorophyll a concentrations were 25% less than the concentrations measured using the soaking-extraction method. The $R^2$ value in comparison of the values was calculated to be 0.64 (Chiuchiolo, 2006). In comparison to this study, it shows that there was not as strong relationship between the values obtained between the two different methodologies. However, in a study done by Gregor et al. (2005) comparing chlorophyll a concentrations between these two methods show that the $R^2$ value was higher at 0.91. This shows that there is some level of variability with the fluoroprobe chlorophyll a values in the comparison to the soaking-extraction method with the values from this study similar to these other studies.

However, in comparison of the two methodologies the fluoroprobe is found to be the easiest to use and allowing for a large amount of sample points to be taken in a short
period of time in-situ. The extraction method is ex-situ but is able to produce highly consistent results. However, it is a slow method (requiring a period of over 48 hours). The fluoroprobe enables a fast and effective measurement of the chlorophyll a concentration over a depth profile. It is even able to break down the chlorophyll a into rough proportions of four major groups of the phytoplankton. This would allow for greater spatial and temporal recordings that are considered necessary for future management practices in Lake Clifton.

7.6 Accuracy of results

There were certain results from this study that had a number of uncertainties.

The Diving PAM in the first sampling visit in measuring the dark yield produced values that were all zero. Although this result could indeed mean that the thrombolites are essentially not photosynthesising and are dead, this is unlikely to be the case. It has been suggested that the light intensity in flashing the thrombolites may have been too high resulting in this error. Most likely, it was the result of not using the right calibration of the Diving PAM. In examination of the RLCs it does show that these are measuring the thrombolites photosynthesis efficiencies which are non-zero. This means the thrombolite mats are photosynthesising. As well, thrombolite samples were examined which showed the presence of Scytonema and numerous other microbial community species. Thus, it seems like these results suggesting that the thrombolite community have perished under these conditions is in fact not completely accurate. However, the Diving PAM uses fluorescence as the proxy value to the measure of photosynthesis, and so does not actually measure the photosynthesis rate. Therefore, this can then be influenced by other processes such as non-photochemical quenching (NPQ). This means that the interpretation of the results is tricky, complex and ambiguous (Rosenberg & Loya 2004).

In terms of how many thrombolites are sampled, it is likely that the more thrombolites that are sampled the better it would be in understanding and completing the composition of the thrombolites. It will also show the natural variation that exists between different thrombolites. Smith (2006) mentions only a limited number of thrombolites were examined and suggested that more analysis needed to be done on
them to determine if the thrombolite community really is changing. This study examined more thrombolites. However, more still could have been examined. Only shallow thrombolite samples were obtained. This could significantly change the results as the shallow thrombolites are the healthier organisms with higher photosynthesis rates. Thus, had samples been taken over a greater variation with depth, it would allow comparisons between examining the thrombolite communities and taking photosynthetic efficiencies. This comparison could show whether these deeper thrombolites had different thrombolite communities to the shallower ones. This difference could be a reduction in the amount of the important photosynthetic *Scytonema* in the deeper thrombolites.

Fluoroprobe measurements are affected by the ambient light conditions as depending on how much light there is, this will determine how much it interacts with the amount of fluorescence the phytoplankton will emit. This means that although there is that strong relationship between the fluoroprobe and the soaking-extraction methods, in terms of the chlorophyll a concentrations, this could explain the reason for them not being the same. On days measured, where it was cloudy and so had lower light levels, the chlorophyll a concentrations in the fluoroprobe measure are higher and closer to the values obtained by the soaking-extraction method. Also, it was the sampling visits with the most sunlight, where the fluoroprobe recorded their lowest concentrations and which differed most from the soaking-extraction method.
8 Suggested future work

The aims of this thesis were to examine the effect of increased phytoplankton concentrations on the thrombolites and microbial mats. Although the thesis was successful in somewhat showing that the thrombolites are less healthy and have lower photosynthesis efficiencies with increased depth resulting from increased light attenuation, further evidence is still required to show this. The other aim of this thesis was to examine how climate change and other likely changes to Lake Clifton in the future will affect the thrombolites.

It is hopeful with further studies done on Lake Clifton, that further work can be completed to assess the thrombolites in a similar manner as conducted in this study, as well as figuring out further how the thrombolites will be impacted by further changes to the lake. In particular, future studies could examine some of these suggested recommendations:

1. To measure the circulation dynamics of Lake Clifton. In particular, measuring how much the water is mixed around due to wind induced currents and waves and the distribution in salinity in the water column. By understanding if, the water stratifies on a seasonal basis or if it mixes continuously will be able to help with identifying how fresh water and particular ions are transported in Lake Clifton. This will hopefully gain more understanding of the circulation dynamics within Lake Clifton and why the thrombolites are located in the north-eastern section of the lake. This will also help in the understanding of the phytoplankton distribution in the water column as to whether the individual organisms are moving to those positions or if it due to them settling out due to size and density.

2. Determine and map the bathymetry of Lake Clifton and the locations of the thrombolites. This will help with future predictions on increasing light attenuation in the lake on how it will reduce the distribution of the thrombolites and microbial mats. By knowing where the thrombolites are and what depth they are at will help determine which thrombolites will be lost if there is an increase in light attenuation as they will be unable to
photosynthesis. It will also be useful to find out the number or areas of thrombolites that will be lost.

3. Greater spatial and temporal sampling of the chlorophyll a concentration over a greater time period (year or more) and over a greater number of sites to get a more extensive understanding of the phytoplankton concentration, distribution and composition within Lake Clifton. There is a strong relationship that exists between fluoroprobe and the soaking-extraction method. It is the writer’s suggestion that future recordings of chlorophyll a of the water should use the fluoroprobe. As well, measure the nutrients (nitrogen and phosphorous) in Lake Clifton over this same time period. Do comparisons between the nutrient concentrations to the chlorophyll a concentrations. There is limited data on this so building up a record at set regular time intervals is important in assessing whether there are significant increases.

4. Build up a record over a period of time of the microbial composition of the phytoplankton of Lake Clifton to see if can find any nuisance species which will cause large eutrophication. Then can do an analysis of the water samples and get out these organisms below the group level measured by the fluoroprobe.

5. To record the light intensity at the same time as the thrombolites dark yields were measured could help in determining whether low light levels are in fact having an effect on the health of the thrombolites. Light measurements taken along a transect at a number of thrombolites and microbial mats at the boardwalk in Lake Clifton. This also can be used to make future predictions on the thrombolites and microbial mats in Lake Clifton if there are future changes to the light climate, which could reduce the ability of thrombolites to photosynthesise in certain areas.

6. More study needs to be focused on the microbial mats within Lake Clifton. These are important and highly significant in their own right. There are many different types of microbial mats in Lake Clifton making them all unique microbial communities. Very little work has been done on them previously.
The orange mat around the thrombolites in the northern section of Lake Clifton was included in this study but with very limited samples taken of it is hard to deduce the same results as were obtained from the thrombolites.
9 Conclusion

The aim of this study was to assess the thrombolites and microbial mats to see how healthy they are and how much they are photosynthesising. This was to be assessed on the recent changes in Lake Clifton where there has been an increase in nutrients which has increased the phytoplankton in the lake and the salinity has increase mostly due to recent climatic changes. The thrombolites and microbial mats were found to have lower health and photosynthesis efficiency measurements with increasing depth. With increasing depth means higher levels of light attenuation. This means that the thrombolites and microbial mats are influenced by the light climate within Lake Clifton. If there was increased light attenuation by increases in phytoplankton this will further reduce the health and ability to photosynthesise of the thrombolites and microbial mats. However, these relationships with depth for the thrombolites were found to be weak. The phytoplankton concentration slightly decreased within Lake Clifton since 2006, although the variability in the results indicated that the concentration did in fact increase substantially above 2006 concentrations on some of the sampling visits. These values of chlorophyll a are compared to increasing nutrients concentrations (nitrogen and phosphorous) since 1979. However, this nutrient data on Lake Clifton is sparse. This limits the level of certainty which to definitely imply that the thrombolites, microbial mats and other biota in this lake are going to be impacted by these increased phytoplankton concentrations. However, the preliminary work done herein and from the previous studies like Smith (2006) show that there is some evidence of impact on the thrombolites from these stressors.

This thesis was to examine how future climatic and other changes in Lake Clifton will impact on the thrombolites. Further land changes are expected within the Yalgorup Lakes region will increase in the future. This along with climate change will ultimately bring more changes to the lake. Fresh water, low nutrient levels and adequate light are seen as some of the key variables required for the thrombolites and microbial mats to grow. These future threats that likely will increase the salinity and nutrient levels further in the lake which will further impact on the thrombolites and microbial mats in limiting their ability to photosynthesis. This will ultimately change
the species composition of these microbial communities resulting in many of the thrombolites no longer growing. As a result, it is predicted that the thrombolites microbial community will be put to further stresses which will most likely result in reductions to the ability of the thrombolites to photosynthesise and grow. This will require further studies to show this. Overall, the future of the thrombolites and microbial mats looks bleak unless all the stressors can be identified and then reduced.
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10 Appendix A: Further images of the thrombolites and phytoplankton in the water column

Figure. Additional water column images. a) copepod feeding (40X view); b) aggregate with Oscillatoria; c) aggregates including diatoms; d) aggregate including diatoms under UV showing the photosynthetic organisms; e) unknown f) unknown fluorescing blue under UV light both at (20X magnification).
**Figure.** Additional water column images. a) b) c) d) Pennate diatoms al at 20X view; e) pennate diatom under UV showing chlorophyll a in red at 20X view; f) unknown g) unknown object under UV light shows chlorophyll a photosynthetic organisms in red; h) aggregate of unicellular and filamentous cyanobacteria; i) aggregate under UV light shows the red fluorescence (chlorophyll a) emitted by the cyanobacteria.
Figure. Additional thrombolite images. a) green-black mat indentified as thrombolite in northern section of Lake Clifton; b) green mat under UV light showing chlorophyll a photosynthetic organisms; c) close up view (40X) of green material covering thrombolites d) green material under UV light at 40X showing chlorophyll a; e) thrombolite mat showing Scytonema; f) unidentified shell structure.
Figure. Additional thrombolite images. a) thrombolite mat showing diatoms and other unidentified organisms; b) UV fluorescence showing the chlorophyll a photosynthetic organisms; c) Scytonema in thrombolite microbial mat material using green light fluorescence; d) aggregate with unknown organism on the outside; e) unknown organism blue under UV light. Most likely a photosynthetic organism using a different pigment.
Figure. Additional thrombolite images. a) Unknown organism at 10X view; b) unknown organism is red under UV light shows that it contains chlorophyll a at 10X view; c) 2.5X view of thrombolite sample showing the large amount of filamentous Scytonema;
**Figure. Additional thrombolite images.** a) Diatoms, unicellular and filamentous cyanobacteria clump of thrombolite mat; b) UV light showing the chlorophyll a photosynthetic organisms in red.
11 Appendix 2: Chlorophyll a concentration data

11.1.1 Fluoroprobe data

Table: Fluoroprobe data for total chlorophyll a concentrations

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<td>0.852</td>
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Table: Fluoroprobe data for green algae chlorophyll a concentrations

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<thead>
<tr>
<th>Depth in water column</th>
<th>0-0.19</th>
<th>0.20-0.39</th>
<th>0.40-0.59</th>
<th>0.60-0.79</th>
<th>0.80-0.99</th>
<th>1.00-1.19</th>
<th>1.20-1.39</th>
<th>1.40-1.59</th>
<th>1.60-1.79</th>
</tr>
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<tbody>
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<td>28/08/2009</td>
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<td>0.089</td>
<td>1.555</td>
<td>2.243</td>
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<td>2.223</td>
<td>2.252</td>
<td>2.365</td>
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</tr>
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<td>0.939</td>
<td>0.915</td>
<td>0.992</td>
<td>1.393</td>
<td>0.000</td>
</tr>
<tr>
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<td>0.263</td>
<td>0.223</td>
<td>0.301</td>
<td>0.467</td>
<td>0.358</td>
<td>0.418</td>
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</tr>
<tr>
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<td>0.972</td>
<td>0.962</td>
<td>0.982</td>
<td>0.797</td>
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<td>0.000</td>
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<td>0.324</td>
<td>0.397</td>
<td>0.400</td>
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</tr>
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<td>0.334</td>
<td>0.450</td>
<td>0.391</td>
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<td>0.000</td>
</tr>
<tr>
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<td>2.032</td>
<td>2.053</td>
<td>2.219</td>
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Table: Fluor probe data for cyanobacteria chlorophyll a concentrations

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<tr>
<th>Depth in water column</th>
<th>0-0.19</th>
<th>0.20-0.39</th>
<th>0.40-0.59</th>
<th>0.60-0.79</th>
<th>0.80-0.99</th>
<th>1.00-1.19</th>
<th>1.20-1.39</th>
<th>1.40-1.59</th>
<th>1.60-1.79</th>
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<tbody>
<tr>
<td>6/09/2009</td>
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<td>0.025</td>
<td>0.039</td>
<td>0.023</td>
<td>0.008</td>
</tr>
<tr>
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<td>1.312</td>
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<td>0.144</td>
<td>0.164</td>
<td>0.117</td>
<td>0.115</td>
<td>0.209</td>
<td>0.000</td>
</tr>
<tr>
<td>20/09/2009</td>
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<td>0.362</td>
<td>0.088</td>
<td>0.033</td>
<td>0.021</td>
<td>0.014</td>
<td>0.017</td>
<td>0.023</td>
<td>0.000</td>
</tr>
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<td>0.031</td>
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<td>0.003</td>
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<td>0.043</td>
<td>0.042</td>
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<td>0.174</td>
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<td>0.057</td>
<td>0.036</td>
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Table: Fluor probe data for diatoms chlorophyll a concentrations

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<th>0-0.19</th>
<th>0.20-0.39</th>
<th>0.40-0.59</th>
<th>0.60-0.79</th>
<th>0.80-0.99</th>
<th>1.00-1.19</th>
<th>1.20-1.39</th>
<th>1.40-1.59</th>
<th>1.60-1.79</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/09/2009</td>
<td>0.000</td>
<td>0.024</td>
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<td>0.411</td>
<td>0.482</td>
<td>0.487</td>
<td>0.516</td>
<td>0.558</td>
<td>0.386</td>
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<td>0.047</td>
<td>0.101</td>
<td>0.309</td>
<td>0.201</td>
<td>0.331</td>
<td>0.312</td>
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<td>0.458</td>
<td>0.430</td>
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<td>0.180</td>
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<tr>
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<td>0.156</td>
<td>0.176</td>
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<td>0.190</td>
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Table: Fluor probe data for cryptophyta chlorophyll a concentrations

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<th>0.80-0.99</th>
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<th>1.20-1.39</th>
<th>1.40-1.59</th>
<th>1.60-1.79</th>
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<td>0.064</td>
<td>0.068</td>
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<td>0.000</td>
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<td>13/09/2009</td>
<td>0.000</td>
<td>0.131</td>
<td>0.408</td>
<td>0.241</td>
<td>0.157</td>
<td>0.141</td>
<td>0.150</td>
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<td>0.062</td>
<td>0.063</td>
<td>0.050</td>
<td>0.053</td>
<td>0.067</td>
<td>0.000</td>
</tr>
<tr>
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<td>0.094</td>
<td>0.053</td>
<td>0.041</td>
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<td>0.000</td>
<td>0.000</td>
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<td>0.122</td>
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<td>0.066</td>
<td>0.068</td>
<td>0.083</td>
<td>0.022</td>
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<td>0.000</td>
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<td>0.017</td>
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11.1.2 Soaking-extraction method

Table: Soaking-extraction method from boardwalk

<table>
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<tr>
<th>Bottle</th>
<th>Sample</th>
<th>Rb</th>
<th>Ra</th>
<th>r</th>
<th>v (ml)</th>
<th>V (ml)</th>
<th>Chl a (μg/L)</th>
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Table: Soaking-extraction method from boardwalk

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<tr>
<th>Bottle</th>
<th>Sample</th>
<th>Rb</th>
<th>Ra</th>
<th>r</th>
<th>v (ml)</th>
<th>V (ml)</th>
<th>Chl a (μg/L)</th>
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Table: Soaking-extraction method from boardwalk

<table>
<thead>
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<th>Bottle</th>
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<th>Rb</th>
<th>Ra</th>
<th>r</th>
<th>v</th>
<th>V</th>
<th>Chl a (μg/L)</th>
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<td>6/09/2009</td>
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11.2 Rapid Light Curves for the two transects

11.2.1 Transect 1

Figure. Thrombolite RLC at depth 15 cm.

Figure. Thrombolite RLC at depth 30 cm.
Figure. Thrombolite RLC at depth 40 cm.

Figure. Thrombolite RLC at depth 50 cm.
11.2.2 Transect 2

Figure. Thrombolite RLC at depth 60 cm.

Figure. Thrombolite RLC at depth 30 cm.
Figure. Thrombolite RLC at depth 40 cm.
Figure. Thrombolite RLC at depth 50 cm.
Figure. Thrombolite RLC at depth 60 cm.
Figure. Thrombolite RLC at depth 70 cm.

Figure. Thrombolite RLC at depth 80 cm.
Figure. Thrombolite RLC at depth 80 cm.

Figure. Thrombolite RLC at depth 90 cm.
## Appendix C: Dark Yield Numbers

Table. Table of the health values of the thrombolites. These values are all relatively low showing that the thrombolites are stressed.

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