The biota of the Swartkops Solar Saltworks and their potential for producing biofuels

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2012

The biota of the Swartkops Solar Saltworks and their potential for producing biofuels

by

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Submitted in fulfilment of the

requirements for the degree of

Magister Scientiae

in the faculty of Science at the
Nelson Mandela Metropolitan University

December 2012

Supervisor: Prof. Eileen Campbell

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Signature……

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To My Father and Mother

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Abstract

The Swartkops and Missionvale salinas in Port Elizabeth on the east coast of South Africa are surrounding by large informal settlements. The runoff from these settlements contributes largely to the eutrophication of the solar saltworks which in turn has an effect on the biotic functioning of the systems, ultimately affecting the quantity and quality of the salt produced. Inorganic nutrients and organic composition, as well as important biological groups were examined within the brine with the aim of comparing the current condition of the same salinas to their condition twelve years ago. Comparisons between inorganic nutrient concentrations and biological groups showed significantly higher inorganic nutrients, with chlorophyll *a* concentrations in the Swartkops salina in 2011 being significantly higher than in 2012 and the 1999 and 2011 chlorophyll *a* concentrations being significantly higher than 2012 in the Missionvale salina.

Microalgae found in the salinas were cultured in four different growth media. Cells were stained with Nile Red fluorescent dye in order to estimate the extent of lipids production. Five of the most promising lipid producing species were isolated into a monoculture and grown at different salinities to establish the growth and lipid production in response to salinity. *Halamphora coffeaeformis* and *Navicula* sp. were found to be the best candidate species. They grew best at salinities between 50 and 70 psu and produced lipid vesicles consuming approximately 10% of the cell.

Acknowledgements

There are many people who provided me with support, advice and finance throughout my study and I am indebted to them all.

My sincere thanks to my supervisor Prof. Eileen Campbell for her time, help and valuable comments.

This research was funded by the Nelson Mandela Metropolitan University and the Swartkops Seesout Edms. Bpk.

Thank you to the staff at Marina Seesout for all their help and support when I was on their salt pans.

A huge thank you to the staff and students of the NMMU Botany Department for the help in the field and in the laboratory; especially Cynthia Tobias, Chan Visage, Elizabeth Milne, Eric van Onslen, Nadia Barnado, Tim Vink, Pascal Tabot and Nuette Gordon.

Thank you Prof. Michael Kleyer and the landscape ecology department at Oldenburg University in Germany for your thought-provoking discussions, help to think outside the box and help with statistics.

A special thanks to my brother, Alfons de Lauwere, for helping me sample and venturing into the salt pans with me especially when it was not your field of study.

To my family and friends for all the love, support and continual prayer, without you I would not have been able to conquer this challenge. A huge thank you goes to my parents for believing in me and loving me no matter what.

To Dean for all your love, support and faith in me and helping me through my low of lows, I will always be indebted to you.

To God.

1. Introduction

One of the natural chemical compounds that can be harvested from the Earth is salt or sodium chloride (EU-China, 2009). Salt is a white, crystalline compound that has low toxicity, is non-flammable and is used in everyday households to flavour foods and contribute to the daily dietary requirement of humans (EU-China, 2009). It is commonly used to de-ice roads and pavements and plays a big role in the production of consumer-related end-use products such as PVC (polyvinyl chloride), a plastic used in the building industry (EU-China, 2009).

Salt can be classified into different types according to the method of harvesting (EU-China, 2009): The first type is rock salt – this type of salt is mined either at the surface or underground. The second is solar salt coming from salinas where seawater is evaporated from shallow ponds. The third type is brine that comes from the solution mining of underground halite.

A solar saltworks (salina or saltern) is a series of concentrating ponds that are interconnected allowing seawater to flow from one pond to the next with the seawater being continually evaporated by the sun and wind (Davis, 2001). The salinity increases in the succession of the ponds. Calcium carbonate is precipitated out once the brine has reached three times the salinity of seawater (Davis, 2001). Calcium sulphate (gypsum) is present once the water reaches four times the salinity of seawater (Davis, 2001). The brine is allowed to flow into the crystallizer ponds where the brine is removed periodically to increase the rate of evaporation in order to accumulate approximately 20 cm of salt on the base (Davis, 2001). The salt is harvested, thoroughly washed to remove contaminants and then stockpiled for packaging later (Davis, 2001). In a solar saltworks that is functioning well and managed properly the above process can produce salt with a purity potentially exceeding 99.7% dry mass (Davis, 2001).

In the ponds of the solar saltworks, biological systems composed of planktonic and benthic communities develop (Davis, 2001). The energy from the sun and inorganic nutrients are used by plants, algae and bacteria to manufacture organic matter through photosynthetic processes (Davis, 2001). Consumers found in the ponds then oxidize these substances (Davis, 2001). The salt production can either be enhanced or harmed by the biological system (Davis, 2001).

Biological systems may enhance salt production if they (Davis, 2001):

- Develop and maintain appropriate planktonic communities that consist of a wide range of species well adapted to different salinity ranges.
- Form benthic, mat-like communities that consist of a wide range of species that cover the pond floors with the appropriate quantity of organic matter.
- Provide organic nutrients for the whole salina.
- Colour the water to aid evaporation.
- Minimize the increase of organic substances and gypsum on the pond floors of high salinity ponds.

By contrast, biological systems may harm salt production if they (Davis 2001):

- Produce communities that are comprised of a few species well adapted to survive in wide ranges of salinities.
- Form inadequate benthic communities.
- Produce damaging amounts of mucilage.
- Are incorrectly managed i.e. ponds are too deep or brine is turbid.

The role of phototrophic organisms in salinas is not only scientifically interesting, but they have an important function in the ponds (Rocha et al., 2012). The benthic cyanobacteria form a mat on the bottom of the ponds sealing them and preventing brine leakage (Rocha et al., 2012). The potential for some Cyanobacteria to produce mucilage in sufficient quantities to degrade salt is the most important biological factor in a salt works, as it has a catastrophic impact on salt production (Davis and Giordano, 1996, Oren, 2009). The mucilage is mainly produced when there has been an environmental disturbance and causes the viscosity of the brine to change, thus interfering with salt crystal formation resulting in the production of fragile crystals (Coleman, 2009). These crystals are costly to harvest with the final product containing many impurities (Coleman, 2009). The positive biological attributes of a saltern are that the soluble metals, nutrients and suspended solids are removed from the brine and deposited onto the sediments (Coleman, 2009). Solid crystals with low impurities are produced in solar saltworks that have their biology in equilibrium (Coleman, 2009).

Salterns harbour diverse prokaryotic and eukaryotic organisms (Oren, 2002) that belong to communities that may have high salinity adaptations, however these adaptations are not always reflected if the target organisms are cultured (Sørensen et al., 2005). For decades light, temperature, nutrients, water movements and the growth rate of the organisms have been investigated as the factors involved in governing the aquatic environments biochemical composition (Abid et al., 2008). The halophilic organisms that are found in these environments have specific characteristics in order to function (Elevi Bardavid and Oren, 2008, Abid et al., 2008). The protein and lipid composition of the halophilic Archaea and Bacteria (Oren and Mana, 2002), the phytoplankton and brine shrimps (Moraiti-Ioannidou et al., 2007) have been shown to vary with changes in salinity (Abid et al., 2008).

The information available on the biology of South African solar saltworks is limited to the private sector, that is, people who are involved in designing and developing solar saltworks. Previous studies by Du Toit (2001) and Difford (2008) provide a solid foundation for further research. Du Toit (2001) found that changing the water levels in the ponds of the Swartkops salina reduced macroalgae and increased phytoplankton significantly. In the same study found that nutrients had a great effect on the biota of the Swartkops and Missionvale salinas. A study by Difford (2008) found that there are benefits in moving the seawater extraction site for the Swartkops salina downstream from the current position, as salinity is higher and nutrient concentrations are lower. Difford (2008) showed that brine shrimp (*Artemia salina* L.) only survived above a salinity greater than 65 psu, and that the use of decomposing barley straw to control macroalgal blooms had adverse effects on the structure and function of the benthic mats and therefore should not be used in this salina.

Since conventional fuel sources are becoming limiting, much emphasis is placed on biofuel and finding alternative fuel sources (Singh and Gu, 2010). Some microalgae produce lipids which, in theory, can be harvested and used for fuel (Singh and Gu, 2010). Solar saltworks contain halotolerant microalgal species that have potential to produce lipids. The focus of this study was to determine if the salinas contain species that could be economically viable to grow for biofuel production. An ideal candidate species would be one that has a high growth rate and produces a substantial percentage of its biomass as lipid.

The aim of this study was to evaluate the biota of the Swartkops and Missionvale solar saltworks and to culture salina microalgae at different salinities in order to determine their growth rate and potential for lipid production.

Urban saltworks are particularly susceptible to nutrient loading due to their proximity to human settlements. Evidence indicates that over the past twelve years the source water for the urban saltworks has had an increase in nutrient concentrations (Swartkops and

Missionvale) close to Port Elizabeth (MacKay, 1994, Du Toit 1998, Du Toit, 2001, Difford, 2008). Estuarine ecosystems and coastal waters (from which the urban salinas derive their brine) are particularly vulnerable to pollution (Winter, 1990).

The following hypotheses were tested for the Swartkops and Missionvale solar saltworks:

Hypothesis 1:

Eutrophication remains a problem at both Swartkops and Missionvale salt pans.

Hypothesis 2:

Algae cultured from the high salinity ponds will have a higher lipid yield than those cultured from lower salinity ponds.

Hypothesis 3:

Some phytoplankton species will have sufficient lipid content and sufficiently high growth rates to be used for harvesting.

Salinity has a variety of terms and units, for the purpose of this study, practical salinity units (psu) are used throughout. The terms “salina”, “saltern”, “solar saltworks” and “systems” (implying saltworks systems) are used interchangeably throughout this dissertation.

2. Literature Review

2.1 The physical environment

The physical components of a solar saltworks include water temperature, pH, salinity, water colour and the depth of the ponds. These components are important for the overall functioning of salinas as they can affect the biological components in each pond.

2.1.1 Temperature

The importance of having optimal temperature (warm and dry conditions) to increase the salinity from one pond to the next has been known since before Christ (BC), but only recently has physical condition behind the requirement become clear – as the water salinity increases the specific heat decreases (See, 1960, Copeland, 1967). When the salt concentration of water increases the specific heat decreases (See, 1960, Copeland, 1967), meaning that the water in the salt pans takes longer to heat up, thus increasing evaporation time. The halophilic algae and bacteria that are found in the ponds are responsible for the red colour of the brine as these organisms retain the beta carotene, carotenoid and retinal based proteins (Jones et al., 1981, Litchfield, 1991). This increases the rate of heat absorption, thus increasing the temperature of the brine (Javor, 1989, Jones et al., 1981). Year-round salt production is possible when there are high temperatures combined with low rainfall as this will result in high evaporation rates (Sammy, 1983).

2.1.2 pH

PH is an important physical factor in many marine systems as it may regulate algal abundance and distribution (Chen and Durbin, 1994). Algal growth can be affected by a variation of pH in a number of ways (Chen and Durbin, 1994). The carbon dioxide species and availability of carbon can be changed, and physiological effects can occur at a high pH (Chen and Durbin, 1994). The relative concentrations of CO_2^-, HCO_3^{2-} and CO_3^{2-} of the carbonate system and the pH of seawater are closely linked (Chen and Durbin, 1994). Carbonate increases and bicarbonate and molecular CO_2^- decreases with an increase in pH (Chen and Durbin, 1994).

Most of the studies that have been conducted on pH show that pH plays a role in regulation marine algal growth and distribution (Yoo, 1991, Hinga, 1992, Riebesell, et al., 1993, Chen and Durbin, 1994). However, there are studies showing that the pH in marine systems can significantly change despite the strong buffering capacity (Chen and Durbin, 1994). Generally correlations are found between pH levels in a marine system and changes in the

abiotic environment as well as between changes in phytoplankton production (Chen and Durbin, 1994).

2.1.3 Salinity

The water fed into salinas comes from various sources. The most common are estuarine- and seawater, while underground saline aquifers may also be used (Jones et al., 1981).The brine that is pumped into the salinas vary widely in salinity (it either comes from the sea or an aquifer) (Du Toit, 2001). Campbell et al. (2001) reported a constant brine salinity of 35 psu for a study on Tankatara (a South African salina), while Carpelan (1957) recorded 20 psu and 30 psu salinities for the brine entering the Alviso Salina from an estuary. In Port Alma, South Australia the water is pumped from an underground source and has an approximate brine salinity of 80 psu (Jones et al., 1981) and in Velddrift on the west coast of South Africa water is pumped from an underground aquifer with a brine salinity of approximately 100 psu (Du Toit, 2001).

Algae can be grouped either as halophilic (require high concentrations of salt for optimum growth) or halotolerant (have mechanisms that allow survival in saline environments) (Ranga Rao et al., 2007). A good example of unicellular, green microalgae that survives well under salt stress is *Dunaliella* spp*.* (Ranga Rao et al., 2007). Salinas require a stable salinity gradient for optimal biotic diversity (Davis, 1990). A study conducted by Litchfield et al. (2008) on a salina in Israel found that the lower salinity ponds had the greatest microbial diversity.

It is most important to maintain the correct salinity in each pond of a salina in order to obtain an abundance of high quality salt (Davis, 1990). In order to keep the salinity maintained in a narrow range the flow rates from pond to pond should to be adjusted according to the rate of evaporation and rainfall (Davis and Giordano, 1996).

2.1.4 Colour

The different colours of the ponds in solar saltworks are recorded as they assist with evaporation (Campbell and Wooldridge, 1994). *Dunaliella salina* (Dunal) Teodoresco) and *Halobacterium* (Elazari-Volcani) spp. colour the brines in the crystallizer ponds orange to red and pink to red respectively (Jones et al., 1981). The solar radiation entering the crystallizer ponds is absorbed by algae and bacteria (Jones et al., 1981). This reduces reflection from the white salt pan floors (Jones et al., 1981) thus increasing solar absorption. Brines are clarified by the brine shrimp that are found in the ponds and they (and algae) provide proteinaceous nutrients to red halophilic bacteria found commonly in the crystallizers (Jones et al., 1981). The addition of green or black dyes to crystallizers increases solar absorption

of brines, thus elevating salt production (Jones et al., 1981). Jones et al. (1981) found that even though brine has a high absorbance capacity, dyes do further enhance absorbance. Campbell and Wooldridge (1994) found that the higher salinity ponds at Swartkops had an orange colour and that most of the Missionvale ponds were brown indicating that Swartkops system is more stable than Missionvale.

2.1.5 Pond depth

The balance between macroalgal and microalgal biomass in the evaporation ponds of solar saltworks is influenced by pond depth (Du Toit, 2001). Two saltfields in Australia , the larger field is Dry Creek in Southern Australia and the other is Port Alma in Queensland were studied by Jones et al. (1981). The mean depth of the Dry Creek evaporation ponds was 1.25 m to 1.5 m and for Port Alma the evaporation ponds were 0.5 m deep (Jones et al., 1981). The mean depth of the crystallizer ponds for both saltfields was 0.15 m (Jones et al., 1981).

Pond seepage is a significant problem and the factors that affect it are pond depth and the substrate type (Jones et al., 1981). Jones et al. (1981) noted that where the substrate was made up of a mixture of clay and shell grit and the pond depth varied between 1.25 and 1.5 m is where seepage occurred in a South Australia saltworks. In the same paper by Jones et al. (1981), they noted that in a similar saltworks where the pond depth was 0.5 m the seepage was negligible.

The depths of the solar salt ponds should be adjusted to allow the benthic communities to receive light (Davis and Giordano, 1996). This suppresses the planktonic community and allows the benthic community to develop (Fong et al., 1993).

2.2 Nutrients

There are a variety of parameters that control the nutrient concentrations in a solar saltworks (Javor, 1989). The geographic location of a saltworks plays an important role in understanding what factors could influence the nutrients, such as proximity to rivers, urban areas, nutrient status of the incoming seawater and climatic conditions (Javor, 1989). Nutrient concentrations are also largely influenced by the extent and nature of the fauna and flora in the area, and by management practices (Javor, 1989). High levels of nutrients are associated with high rainfall (MacKay, 1994). The Swartkops estuary has higher nutrient levels in comparison to other unpolluted estuaries (Emmerson, 1985). Seawater from the Swartkops estuary (high pollution) is pumped directly into the Swartkops solar saltworks and from there is pumped into the Missionvale solar saltworks.

2.2.1 Inorganic Nutrients

Ammonium, nitrate and phosphorus are dissolved inorganic nutrients that are not essential for the salt production, but they do have an important role in salinas (Difford, 2008). They are a good indicator of the brine quality (Difford, 2008). It is important that there are sufficient nutrients in the salt-works as they promote cyanobacterial growth allowing microbial mat formation (Difford, 2008). This is important to the system as the microbial mats seal the concentrator ponds and limit the amount of brine that is lost from the system (Difford, 2008). Inorganic nutrients promote phytoplankton growth in the water column, which colours the ponds, thus increasing solar absorption and increases the temperature of the pond, ultimately increasing the rate of evaporation from the system (Javor, 1989).

Difford (2008) found that the primary controlling factor of biota in the Swartkops Solar Saltworks ponds was the balance between inorganic nitrogen and phosphorus. However when nitrogen is abundant water clarity becomes the controlling factor (Difford, 2008). Campbell and Wooldridge (1994) found that the two most important nutrients that algae in the Swartkops and Missionvale salinas require are nitrogen (N) and phosphorus (P).

Ammonium

High levels of ammonium can be toxic to algae (Campbell and Wooldridge, 1994). The water ammonium concentration at Missionvale has been historically been high in some ponds (Campbell and Wooldridge, 1994). The salina is separated from informal human settlements by a narrow road and Campbell and Wooldridge (1994) surmise that the runoff from the informal settlements is entering the ponds.

Out of the four major run-off sources that enter the Swartkops Estuary, MacKay (1994) found that the Chatty River was the most significant contributor of inorganic nitrogen than any of the other sources, including the discharges of treated sewage. Similarly Difford (2008) found that the main input of inorganic nutrients in the Swartkops salina does not come from the main input waters, but rather from within the estuary. Mackay (1994) found that the ammonium concentrations taken at two sites in the Chatty River varied between 70 µM (in dry weather) and 600 µM (in wet weather). The Swartkops and Missionvale salinas are negatively affected by these high ammonium and nitrate levels as the Chatty River bisects the Swartkops system and discharges less than 500 m from the inlet pipe (Du Toit, 2001).

Campbell et al. (2001) found that the nitrogen pool in the Tankatara salina (a South African salina) was equally contributed to by ammonium and nitrate, except in winter when there was less ammonium.

Nitrate

It was found by Sammy (1983) that the nitrate levels in the Dampier salt fields in Australia have low reactive nitrate levels, meaning that the utilisation of nitrate by the organisms in the system is less rapid than the utilisation of phosphate. At the end of winter and the beginning of spring in Patos Lagoon estuary (Southern Brazil) the nitrogenous nutrient (nitrate + nitrite and ammonium) concentrations peak, which is related to the rainy season adding large volumes of freshwater into the estuary (Abreu et al., 1995). It is useful to know the nutrient concentrations of estuaries as they can serve as the source water for solar salt works. A high nitrate concentration indicates that there is an imbalance between the inorganic nitrogen supply and biological utilisation (Campbell and Wooldridge, 1994).

Generally it is believed that the rate at which phytoplankton utilise nitrate is reduced in the presence of ammonium (Dortch, 1990). This is known as either preference for ammonium or inhibition of nitrate uptake (Dortch, 1990). In extreme circumstances nitrate will not be taken up above an ammonium threshold of ca. 1 µM. In earlier studies by Losado & Guerrero (1979) and Syrett (1981) it was thought that nitrate uptake and reduction were inseparable and therefore ammonium must inhibit the uptake of nitrate. It is now known that the uptake and reduction of nitrate are uncoupled in marine phytoplankton (Dortch et al., 1979).

Phosphate

Reactive phosphorus is often the growth-limiting nutrient and is used for protein synthesis in microorganisms that live in saline environments (Davis, 1978). It is important for their growth and survival (Davis, 1978). The phosphate is also removed from the water by the microorganisms that live on the pond floors (Davis, 1999). Salina productivity and the establishment of biological communities that are important for the production of salt are enhanced by sufficient phosphate concentrations (Davis, 1978).

Orthophosphate in the water column of the Salin de Giraud salina was practically absent over most of the salinity range and was only found in the low salinity ponds, where the maximum concentration was 0.04 µM (Britten and Johnson, 1987). Even so, the salina, the largest in Europe, had an efficient biological system (Britten and Johnson, 1987). Another biologically highly productive Indian saltworks had phosphate concentrations varying between 0.5 µM to 3.5 µM (Rahaman et al., 1993). Even though the recommended phosphate level for salinas is 1 µM (Davis and Giordano, 1996), salinas can function effectively with elevated phosphate concentrations.

The Tankatara salina on the east coast of South Africa had soluble reactive phosphorus concentrations varying between 1 µM and 4 µM over a six year period, with the evaporation ponds having approximately 10 500 kg of phosphorus added into the ponds in the first two years (Campbell et al., 2001). In the same study it was also found that fluctuations in the phosphorus concentrations were not seasonal (Campbell et al., 2001). Management tools are important to ensure that the optimum production is reached, and Campbell et al. (2001) noted the inorganic N: P ratio as a useful management tool. When this ratio is stable it will indicate a stable species composition and as and when necessary can be adjusted by the addition of these nutrients (Campbell et al., 2001).

2.3 Chlorophyll *a*

Water quality is influenced by a variety of biological factors (Du Toit, 2001). Chlorophyll *a* is not only the primary photosynthetic pigment; it also gives an estimation of algal biomass (Binning, 1999). Phytoplankton biomass is an integral part of a salina's ecosystem (Campbell et al., 2001). Apart from changing the salinity of the ponds, evaporation of the brine also changes the biological features of each pond in series (Coleman, 2009). The types of algae, plants and animals found in each pond are determined by the salinity, temperature and nutrients availability (Coleman, 2009). Cyanobacteria and algae (macroalgae and microalgae) use the sunlight during the daytime consuming the carbon dioxide and producing oxygen in order to make carbohydrates (Coleman, 2009). In addition to producing oxygen the cyanobacteria and algae also use oxygen in their metabolism, however the production of oxygen during the day far exceed their use of oxygen (Coleman, 2009).

A study by Javor (1983) showed that chlorophyll *a* concentrations of two salinas correlated with their nutrient concentrations. The ESSA Salina in Mexico had low chlorophyll *a* concentrations (below 10 µg l^{-1}) which reflects the low nutrient concentrations in that salina, while the Western Salt Salina in California had higher nutrient concentrations, which resulted in the chlorophyll *a* concentrations being high (nearly 200 µg l^{-1}; Javor, 1983).

2.4 Biota

2.4.1 Bacteria

Dense halophilic microorganism communities characterize the crystallizer ponds worldwide (Elevi Bardavid and Oren, 2008). However, these salt-saturated ponds have a limited microbial diversity (Elevi Bardavid and Oren, 2008). The only primary producer of the crystallizer ponds is *Dunaliella salina*, a β-carotene rich green alga (Elevi Bardavid and

Oren, 2008). *Haloquadratum walsbyi* A.E Walsby, a square-shaped, gasvacuolate archaeon generally dominates the heterotrophic prokaryote community (Bolhuis et al., 2004) in these ponds. *Salinibacter ruber*, a rod-shaped bacterium, is also present in large numbers (Elevi Bardavid and Oren, 2008). The three above-mentioned microorganisms have orange, pink or red pigments (Elevi Bardavid and Oren, 2008). Thus, because of their high abundance they impart this colouration to the brine in the pond (Elevi Bardavid and Oren, 2008). The high concentrations of red halophilic bacteria promote heat absorption that in turn accelerates evaporation and reduces the concentrations of dissolved organics (Sorgeloos and Tackaert, 1991).

2.4.2 Macroalgae

Some solar saltworks are associated with natural wetlands and macroalgal species in some way (Du Toit, 2001). The macroalgae commonly occur in the initial ponds. *Cladophora* Kützing*, Rhizoclonium* Kützing*, Ulva* Linnaeus*, Batophora* J.Agardh*, Valonia* C.Agardh and *Enteromorpha* Link in Nees were genera that Davis (1990) found most common in the lower salinity ponds. The macroalgae are only found on the periphery of the low salinity ponds and are not prolific, however if there are high nutrient concentrations this results in rapid production and infestation in lower salinity ponds (Du Toit, 1998, Difford, 2008). Stable communities of macroalgae develop on the water surface and benthos of solar saltworks if the incoming nutrients are low and balanced (Javor, 1983, Sammy, 1983). Difford (2008) found that a micro-phytoplankton dominated system can shift to become macroalgae dominated in the water column is too stable and if the brine becomes too fresh. The increase in suspended solids and eutrophication is however likely to cause macroalgal blooms (Coffaro and Bocci, 1997, Du Toit, 2001). Eutrophic environments are advantageous to macroalgae, epiphytes and phytoplankton as they outcompete the seagrass for light (Coffaro and Bocci, 1997).

2.4.3 Zooplankton

Zooplankton forms an integral part of the biology of solar saltworks, from the plankton communities that inhabit the lower salinity ponds to the *Artemia salina* (Linnaeus), or brine shrimp populations that inhabit the pre-crystallizer ponds (Du Toit, 2001). *Artemia* spp. is the most important (and often dominant) component of the macrozooplankton in hypersaline environments (Wurtsbaugh and Gliwicz, 2001). The genus *Artemia* Leach is often the dominant consumer of hypersaline food webs, controlling the water clarity by its grazing activities (Mohebbi, 2010) and minimizing algal blooms (Sorgeloos et al., 1986). Brine shrimp are filter feeders feeding on the detritus that is suspended in the water column, or the unicellular microalgae and other plankton higher up in the water column (Mohebbi, 2010).

Artemia naturally occurs in water bodies at salinities of at least 100 psu (Sorgeloos and Tackaert, 1991). Population densities are indirectly limited by the low level of nutrients in the intake waters (Sorgeloos and Tackaert, 1991). Salt ponds that have high densities of *Artemia* are found near human population centres as they contribute to nutrient input (Sorgeloos and Tackaert, 1991). The presence of *Artemia* is important in the salt production process not only because they control the algal blooms, but because they provide nutrients as suitable substrates for the development of *Halobacterium* in the crystallizer ponds (Sorgeloos and Tackaert, 1991).

2.4.4 Plants

Nutritional and physical parameters determine the plant communities that are found in the early concentrator ponds of a solar saltworks (Du Toit, 2001). Stable ecosystem structure and high productivity are characteristic of seagrass meadows (Coffaro and Bocci, 1997). Seagrass meadows are also particularly efficient at nutrient cycling and provide food and shelter for many organisms (Coffaro and Bocci, 1997). However, the persistent, slower growing species can be outcompeted by the opportunistic species that have high surface to volume ratios because of eutrophication in the enclosed body of water (Du Toit, 2001). This is advantageous for macroalgae (e.g. *Ulva rigida* and *Gracilaria* spp.) because when the nutrients in the water column are high the macroalgae replace the seagrass (Coffaro et al., 1997).

2.4.5 Avifauna

Solar saltworks make for a good resting, feeding and breeding place for migratory birds worldwide (Lopez et al., 2010, Masero, 2003). Many non-breeding shore species also feed in salina ponds, indicating the importance of this habitat and is based entirely on the number of birds that frequent the ponds (Rocha et al., 2012).

The avifaunal studies conducted on South African salinas over a seven-year period showed that there were different feeding trends depending on the season (Campbell et al., 2001). In summer and spring, carnivorous and scavenging birds were more abundant, in autumn, herbivorous birds were most abundant and throughout the year, except for summer, omnivorous birds are abundant (Campbell et al., 2001). The low salinity ponds had the highest abundance of birds (Du Toit, 2001). Birds are a good indication of any changes occurring in the ecosystem (Du Toit, 2001) as evidenced by an increase in herbivorous birds when phytoplankton biomass was highest (Campbell et al., 2001).

2.5 Marine Culture Media

Seawater is very complex medium containing many known elements and organic compounds (Harrison and Berges, 2005). It is seldom acceptable to use natural seawater directly for culturing algae, without any additional nutrients, trace metals and vitamins (Harrison and Berges, 2005). Unenriched natural seawater provides an algal yield that is too low for laboratory experiments and culture maintenance (Harrison and Berges, 2005). There are two groups of marine culture media: natural and artificial seawater (Harrison and Berges, 2005). Both natural and artificial seawater are unenriched media having the same problems with meeting the culture requirement (Harrison and Berges, 2005). To obtain a substantial algal yield enrichment solution can be added into the media. Once the mass cultured algae's environmental conditions are ideal for optimal growth, mixing becomes the most important factor in order to obtain high algal biomass (Richmond and Becker, 1986, Grobbelaar, 2004). Nutrients and metabolites can easily be transferred as this turbulence increases diffusion gradient (Grobbelaar, 2004) allowing the algae to efficiently utilise the nutrients in the culture media.

The culture medium used greatly influences the growth rate of the microalgae being cultured. The culture medium that is used for culturing microalgae on a large-scale is suitable for laboratory use, with some minor adjustments (Borowitzka, 2005). There are several factors that determine the type of medium that should be used including the algal growth requirement and how composition of the medium may affect the quality of the final product and the cost of the medium (Borowitzka, 2005). High grade medium, with food grade chemicals, are used when culturing microalgae for health food and the nutraceutical markets and cheaper industrial grade chemicals can be used in the culture medium where the microalgae are cultured for animal feeds (Borowitzka, 2005).

Enrichment cultures are used as the first step in obtaining single-cell isolations (Andersen and Kwachi, 2005). They provide a suitable environment for the microalgae to grow and reproduce as well as inhibiting the growth of unwanted organisms (Lee and Shen, 2004). In order to establish enrichment cultures nutrients are added to a natural sample, enriching the sample (Andersen and Kwachi, 2005). Culture medium, soil water extract and macronutrients (i.e. nitrate, ammonium and phosphate) are common enriching substances, with trace metals being the limiting factor in some cases (Andersen and Kwachi, 2005). If good quality soil can be obtained, the soil-water extract can be used and it the simplest and most successful enrichment culture method (Andersen and Kwachi, 2005). The enrichment culture can become anoxic or toxic if the bacterial growth is too high, thus causing the algal cells to die (Andersen and Kwachi, 2005). Enrichment is important and very necessary for r-

selected species and less added nutrients are required for the k-selected species (Andersen and Kwachi, 2005). These conditions allow for k-selected species to dominate (Andersen and Kwachi, 2005).

2.5.1 Macronutrients

The macronutrients used when culturing are generally nitrogen (N), phosphorus (P) and for diatoms, silicoflagellates and chrysophytes, silicon (Si) (Harrison and Berges, 2005). The ratio in which these macronutrients are usually required is 16 N: 16 Si: 1 P (Brzezinski, 1985, Harrison and Berges, 2005). Nitrogen is an important macronutrient as it promotes algal biomass production (Grobbelaar, 2004). When nitrogen is limiting, discoloration of the algal cells can be observed due to the decrease in chlorophyll concentrations and the increase of carotenoid concentration (Becker, 1994, Grobbelaar, 2004). Phosphorus is often a growth-limiting nutrient as it can easily bind to other ions precipitating out thus being unavailable for the algae (Grobbelaar, 2004). Algae may store excess phosphorus in their cells so that when the external phosphorus becomes limiting they can use the stored phosphorus (Grobbelaar, 2004). The relative concentrations of macronutrients in most media are unbalanced, and media such as f/2 (reference for f/2) have ratios of nitrogen: phosphorus greater than 16:1 (Berges et al., 2001). The N: P ratio is important as it determines algal productivity and maintains the selected species' dominance (Grobbelaar, 2004). For most diatoms N: Si ratios are 1:1 requirement, but in most culture media the N: Si ratio is not near 1:1 (Berges et al., 2001). Working stock solutions can be made up containing various chemical compounds that serve as a nutrient source (Harrison and Berges, 2005). Nitrate and phosphate can be added as $NaNO_3$ and $NaHPO_4.H_2O$ respectively where ammonium and silicate can be added in as NH_4Cl and $Na_2SiO_3.9H_2O$ respectively (Harrison and Berges, 2005). The stock solutions should be kept free from bacteria and fungi either by filter sterilization or the solution must be autoclaved (Harrison and Berges, 2005).

2.5.2 Trace Metals

Only small quantities of trace metals and vitamins are required in culture medium (Harrison and Berges, 2005). Therefore "primary" stock solutions of high concentrations need to be made up to permit the weighing of reasonable amounts (Harrison and Berges, 2005). Working solutions are made from the primary stock and are then used to make the final medium (Harrison and Berges, 2005). Evaporation may occur if the stocks are kept for a very long time, so it is advised to store it in a fridge wrapped in clingfilm (Harrison and Berges, 2005).

The trace metal stock solutions can typically consist of chloride or sulphate salts of zinc, cobalt, manganese, selenium and nickel kept in an EDTA chelator solution (Harrison and Berges, 2005). Iron can precipitate and is therefore kept in a separate solution chelated in 10^{-2} M HCl^- (Harrison and Berges, 2005). Natural seawater contains sufficient concentrations of boron and it should only be added if artificial seawater is used (Harrison and Berges, 2005).

2.5.3 Natural Seawater Media

There are both broad spectrum and species-specific natural seawater enrichment seawater that are used when culturing microalgae (Harrison and Berges, 2005). Erdschreiber medium, ESNW (enrichment solution of natural seawater) and f/2 medium are the most cited broad spectrum culturing media (Harrison and Berges, 2005). There has been a medium developed specifically for oceanic species called the K-medium (Keller et al., 1987, Harrison and Berges, 2005). Another medium used for oceanic species is Provisoli. A study by Fryxell et al. (1991) used Provisoli to culture a sample taken from the Antarctic, and they found that growth occurred after one week.

2.6 Techniques of Isolating Microalgae

By the late 1890's the traditional methods of isolating microalgae into culture was well established thanks to Beijerinck (1890) and Miquel (1890-1893) (cited in Andersen and Kwachi, 2005). Not all species are easily cultivated and the "weed" species often grow the best and the fastest (Andersen and Kwachi, 2005). In order to be successful in isolating the microalgal species, the natural environment needs to be fully understood and recreated with optimal conditions (Andersen and Kwachi, 2005). Certain environmental factors need to be considered when isolating algae for example when isolating marine algae the temperature and salinity are of importance and when isolating the oceanic phytoplankters the metal toxicity and water quality are of concern (Andersen and Kwachi, 2005). Each species of algae growing in different natural environments has its own set of requirements that should be taken into consideration when isolating.

Then next important step in isolation is to ensure that all contaminating species are eliminated, especially those that can easily outgrow the target species (Andersen and Kwachi, 2005). Some of the methods that are widely used for isolating microalgae are dilution, single-cell isolation by micropipette, density gradient centrifugation and filtration (Lee and Shen, 2004, Andersen and Kwachi, 2005).

Continual growth upon sub-culturing is the last step in the isolation procedure (Andersen and Kwachi, 2005).

2.6.1 Sample collection

The method in which the sample is collected is critical for the success of the isolation as dead or damaged cells lead to failure (Andersen and Kwachi, 2005). It is always helpful when studies have already been successful to use these as a guide for future sample collections (Andersen and Kwachi, 2005). All factors need to be taken into consideration when collecting samples for example samples that are collected at different depths, for example in salina ponds, can be sensitive to light or temperature changes (Andersen and Kwachi, 2005). Regardless of the collection, viable cells can be collected if whole water samples are collected in sterile containers and are kept cool (Andersen and Kwachi, 2005). Caution is to be taken to ensure that samples are not contaminated or lost and multiple methods used if sampling in an environment where knowledge is lacking and not much research has been conducted on the target organism/s (Andersen and Kwachi, 2005).

Samples that are collected from natural environments are most likely to contain zooplankton that feed on the algae (Andersen and Kwachi, 2005) thus they should be eliminated. Gently filtering the sample will remove any larger, unwanted organisms (Andersen and Kwachi, 2005). Care must be taken not to damage or desiccate the target species (Andersen and Kwachi, 2005). Another important factor when isolating algae is time (Andersen and Kwachi, 2005). Some organisms die quickly while others may survive for a few hours (Andersen and Kwachi, 2005). It is best to proceed to the isolation quickly and cautiously as the success of the growth of the isolated cells depends on the state of the cells at the time of collection (Andersen and Kwachi, 2005).

It is important to use a good sterile technique when collecting samples (Andersen and Kwachi, 2005). To prevent unwanted contamination the sampling equipment needs to be clean and sterile as cysts of other organisms can remain for substantial periods of time (Andersen and Kwachi, 2005). This is especially important when sampling unknown species (Andersen and Kwachi, 2005).

2.6.2 Sterilization and sterile technique for culturing microalgae

In order to establish an environment that is aseptic and free from unwanted microorganisms, all equipment and workspaces should be sterile thus reducing the risk of contamination and giving more precise results (Kawachi and Noël, 2005).

Unless purchased sterile, all glass and plastic vessels should be properly sterilized before use (Kawachi and Noël, 2005). This sterilization process should involve washing the vessels in an acid bath for a week, then rinsing them several times under running tap water and lastly rinsing with distilled water before air drying (Kawachi and Noël, 2005).

If glassware is to be reused it is very important ensure that culture vessels thoroughly sterilized (Kawachi and Noël, 2005). This can be done by autoclaving the vessels should be to kill any living organisms that could cause contamination (Kawachi and Noël, 2005). The standard cleaning procedure requires vessels to be immersed into a detergent bath overnight, then cleaning with a brush and sponge (Kawachi and Noël, 2005). The vessels must then be rinsed under running tap water and finally rinsed with deionised or distilled water (Kawachi and Noël, 2005). This same procedure is used when cleaning glass petri dishes, watch glasses and microscope slides (Kawachi and Noël, 2005).

Pasteur pipettes are reusable and therefore need to be immediately rinsed after use (Kawachi and Noël, 2005). These pipettes can be immersed in a bath with detergent and left for one to two days (Kawachi and Noël, 2005). Pipettes are then either wrapped in tinfoil or placed in a special canister and autoclaved (Kawachi and Noël, 2005).

The most effective and popular way to sterilize heat resistant materials and liquids is to autoclave them (Kawachi and Noël, 2005) at 120 °C.

2.6.4 Single-Cell Isolation by Micropipette

The most common method of isolating single cells from a culture is using a micropipette (Andersen and Kwachi, 2005). A Pasteur pipette or a glass capillary is used for this technique (Andersen and Kwachi, 2005). The micropipettes can either be bought or made from Pasteur pipettes by heating a Pasteur pipette over a flame, removing it from the heat and immediately gently extending the tip with forceps (Andersen and Kwachi, 2005). The tip is carefully broken off at its narrowest. If properly done the broken tip should be smooth and rounded, if the tip is broken the pipette should be discarded, as the cells will not be properly drawn up (Andersen and Kwachi, 2005).

The aim of micropipette isolation is to pick up single cells from a sample and deposit them in a sterile droplet without causing damage to the cell (Andersen and Kwachi, 2005). This process produces monospecific, clone cultures of microalgae (Andersen and Kwachi, 2005). The advantage of this technique is there is complete isolation of species, but the disadvantage is that excessive handling can cause cell damage (Andersen and Kwachi, 2005).

In order to pick up the cells, a flexible latex tube is place on the wide end of the pipette, and a sterile droplet is loaded at the other end (Andersen and Kwachi, 2005). Use of this equipment requires the operator to place his/her tongue over the mouth piece. The micropipette is then placed near the target cell; the tongue is gently removed and the cell is

drawn into the micropipette (Andersen and Kwachi, 2005). The cell is ejected into the culture medium by gently blowing on the mouthpiece.

2.6.5 Dilution techniques

For many years the dilution technique has been used (Andersen and Kwachi, 2005). This technique was developed by Allen and Nelson (1910 in Droop, 1954). It is especially effective for abundant organisms in a sample, but ineffective for rare organisms because competing species often occur in far greater numbers (Droop, 1954, Andersen and Kwachi, 2005). The dilution technique's goal is to deposit a single cell into a test tube, flask or multiwell plate (Andersen and Kwachi, 2005) in order to obtain a monoculture as the end product.

2.7 Culture monitoring

Continuous monitoring is required in order for culturing to be successful (Borowitzka, 2005). Regular microscopic examination of the cultures is the simplest method to detect any culture changes or abnormalities or if there is any contaminations (Borowitzka, 2005). If any contamination does occur, early detection will allow control measures to be implemented timeously (Borowitzka, 2005). To avoid nutrient deficiencies the nutrient concentrations need to be replenished regularly and regularly monitored. This can be done using pH and oxygen indicators (Borowitzka, 2005). Pulse amplitude modulated fluorometry (PAM) is a new technique that is able to determine the physiological state of microalgal cultures for any signs of deterioration or starvation (Torzillo et al., 1998, Lippemeier et al., 2001).

2.7.1 Measuring Microalgae growth rates

Growth can be interpreted as any form of accumulation of the biomass of algae (Wood et al., 2005). Algal growth will be optimal when the culture environment is favourable and nutrients are not limiting (Lee and Shen, 2004). In continuous cultures, it is important for growth to remain exponential therefore fresh medium is added continuously (Wood et al., 2005). The selection of a dilution rate that maintains an appropriate cell concentration can be used to determine the requirements of cells (Wood et al., 2005).

In a steady state culture, the dilution rate is used to determine the specific growth rate (μ) (Wood et al., 2005):

μ = F / V ……… Eq. 1

Where: F is the flow rate of the medium through the culture vessel (L/hour or L/day)

V is the volume of the culture vessel (L)

2.7.3 Counting the cells

Estimating the number of cells in the cultured population is the most common method of determining growth (Guillard and Sieracki, 2005). Cell counts should be expressed as the total number of cells in the whole culture or as number per unit volume of culture (Guillard and Sieracki, 2005). Population size is also often estimated by the biomass (wet or dry weight), chlorophyll *a* content, or the organic content of nitrogen, phosphorus or iron (Guillard and Sieracki, 2005). However, there is a more fundamental aspect to cell numbers; they give an indication of survival (Guillard and Sieracki, 2005).

The estimation of the rate of cell growth is the second application of cell counting (Guillard and Sieracki, 2005). It is equivalent to the rate at which the population increases (Guillard and Sieracki, 2005). This is expressed as the rate of cell division as the population increases when individuals divide to form two offspring cells (Guillard and Sieracki, 2005).

If counting chambers lack impressed rulings cells may be counted using transmitted light once they have settled into one plane within the depth of the focus of the objective-ocular system of a microscope (Guillard and Sieracki, 2005). The cells may have to be stained or immobilized to facilitate observation or to preserve them for counting at a later stage (Guillard and Sieracki, 2005). The size of the observation area must be known (Guillard and Sieracki, 2005). This is the technique that is used for all the counting chambers that lack impressed rulings on the surface of observation (Guillard and Sieracki, 2005). Several counting devices have been commercialised: the Sedgwick-Rafter Counting Slide (a rectangular chamber without rulings), the Palmer-Maloney Slide (a circular chamber without rulings), hemacytometer slides that are 0.2 or 0.1 mm deep with two or four separate chambers and a graticule on the base (Spears-Levy eosinophyll counter or those that are the Petroff-Hausser Bacteria Counting Slide (with an improved Neubauer ruling) (Guillard and Sieracki, 2005).

2.8 The potential of biodiesel from microalgae

In recent years biofuels have become an attractive source as an alternative fuel (Scott et al., 2010). Liquid biofuels, such as those derived from corn starch, sugar cane, palm and oilseed rape, allow energy to be stored as opposed to the other forms of renewable energy such as wind, tidal and solar energy.

Biodiesel is a fuel that is derived from either plant or animal oils (Scott et al., 2010). The source of the oils determines the detailed chemical composition, most importantly the fatty acid chain length (Scott et al., 2010). In order to produce biodiesel the oils should be

transesterfied, a process that involves replacing the glycerol attached to the fatty acid chain with another alcohol, usually methanol (Scott et al., 2010).

Microalgae rapidly convert solar energy into chemical energy because of their unicellular structure (Harun et al., 2010). As a result the biotechnology for obtaining biofuels from microalgae has developed and may be used in a variety of applications (Harun et al., 2010). Microalgae are suitable to use in the food and cosmetic industry, while some microalgal species produce bioactive compounds that can be used in the pharmaceutical industry (Harun et al., 2010). Many microalgae species contain lipids that can be extracted and converted into biofuels (Harun et al., 2010). Lipids can be extracted from microalgae in various ways; the most common methods include pressure, solvent extraction, superficial fluid extraction and ultrasound (Andrich et al., 2005, Demirbas et al., 2009). After lipid extraction the remaining algal waste may be converted into other types of biofuel (e.g. biomethane, bioethanol and biohydrogen; Harun et al., 2010). Not only can microalgae be used as an alternative fuel source, but they have also shown potential in reducing environmental problems such as the remediation of industrial water pollution and the in combatting the greenhouse effect (Harun et al., 2010).

Microalgae have a number of advantages over conventional oil crops such as soybeans (Wu et al., 2012): 1) Microalgae are photosythetically efficient, simple structured organisms that can be produced all year round; 2) There is a wide variety of species that can produce lipids over a broad spectrum of climates and geographic regions; 3) Microalgae are efficient at removing nutrients from wastewaters; and 4) Microalgae play an important role in sequestering large amounts of carbon.

When contemplating microalgae for biofuel, a main consideration must be the algae strain chosen for cultivation (Scott et al., 2010). Recent research focuses on a small number of species that are fast growing and produce large quantities of lipid under specific growth conditions (Scott et al., 2010). *Chlamydomonas reinhardtii, Dunaliella salina*, and various *Chlorella* spp. are the most commonly cultivated Chlorophyta species, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* the most important diatoms and other heterokonts including *Nannochloropsis* and *Isochrysis* spp. are the used for biofuel (Scott et al., 2010). There are three basic requirements that should be fulfilled in order for a biofuel to replace conventional fuel (Singh and Gu, 2010): firstly to produce commercial quantities of the biofuel a large feedstock is required, second it should be cheaper than conventional fossil fuel and lastly the standard and quality of the fuel must be appropriate (Singh and Gu, 2010). Microalgae provide a very promising alternative to fossil fuel, as they not only meet all three requirements but also have a high growth rate and the lipids they produce are mostly

neutral with a low of unsaturation (Singh and Gu, 2010). Some marine microalgal species that have been identified as strains that could be used for biofuel extraction are listed in Table 1.

Table 1: Lipid content and productivity of selected marine cultivated microalgae (from Rodolfi et al., 2009).

Algal Group	Microalgae strain	Habitat	Lipid Content (% biomass)	Lipid productivity (mg/l/d)
Bacillariophyta	*Chaetoceros muelleri* FandM-M43	Marine	33.6	21.8
	Chaetoceros calcitrans CS 178	Marine	39.8	17.6
	Phaeodactylum tricornutum FandM-M 40	Marine	18.7	44.8
	Skeletonoma costatum CS 181	Marine	21.0	17.4
	Skeletonoma species CS 252	Marine	31.8	27.3
	Thalassioria pseudonana CS 173	Marine	20.6	17.4
Chlorophyta	*Tetraselmis suecica* FandM-M33	Marine	8.5	27
	Tetraselmis species FandM-M34	Marine	14.7	43.4
	Tetraselmis suecica FandM-M35	Marine	12.9	36.4
	Ellipsoidion species FandM-M31	Marine	27.4	47.3
Heterokontophyta	*Nannochloropsis species* FandM-M26	Marine	29.6	61.0
	Nannochloropsis species FandM-M27	Marine	24.4	48.2
	Nannochloropsis species FandM-M24	Marine	30.9	54.8
	Nannochloropsis species FandM-M29	Marine	21.6	37.6
	Nannochloropsis species FandM-M28	Marine	35.7	60.9
	Isochrysis species (T-ISO) CS 177	Marine	22.4	37.7
	Isochrysis species FandM-M37	Marine	27.4	37.8
Haptophyta	*Pavlova salina* CS 49	Marine	30.9	49.4
	Pavlova lutheri CS 182	Marine	35.5	50.2
Rhodophyta	*Porphyridium cruentum*	Marine	9.5	34.8

3 Methodology

3.1 Study site

The Swartkops and Missionvale solar saltworks are situated approximately 8 km north-west of the Nelson Mandela Metropolitan city centre, in the Swartkops river valley. Industrial and residential areas of the city lie on both banks of the Swartkops estuary. Both Missionvale and Swartkops salinas are surrounded by formal and informal settlements (Plate 1). To the south of the Swartkops ponds, the informal township of KwaZakele occurs and in the west Veeplaas lies adjacent to the ponds (Plate 1). A 3.5 km water transfer pipe connects the Missionvale system to the Swartkops system (Du Toit, 2001). Bethalsdorp, Algoa Park and Zwide and KwaZakele completely surround the Missionvale salina in the west, south, north and east respectively (Plate 1). The informal settlements that surround the Missionvale salina are as close as 20 m from the edge of the ponds in places (Plate 1).

The sampling points at which abiotic and biotic variables were measure in the Swartkops (Figure 1) and Missionvale (Figure 2) salinas are given.

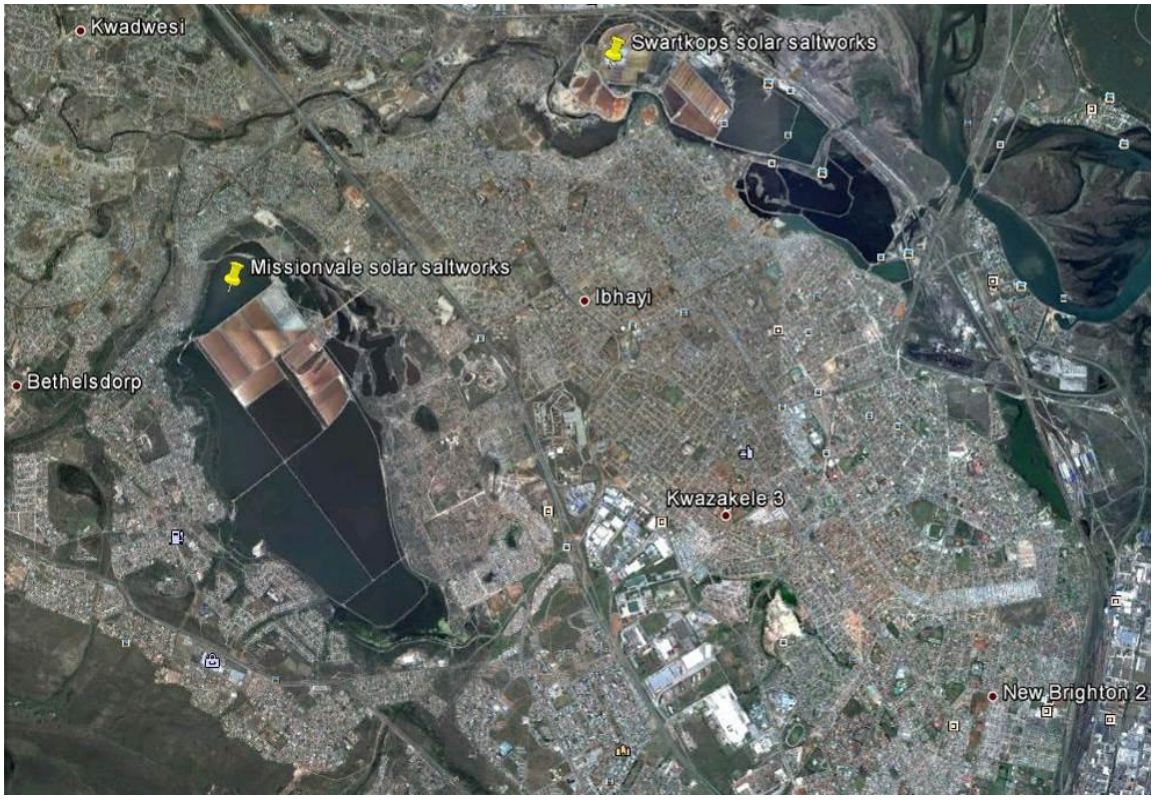


Plate 1: The locality of the Swartkops and Missionvale solar saltworks and the surrounding urban areas (Google Earth, 2011).

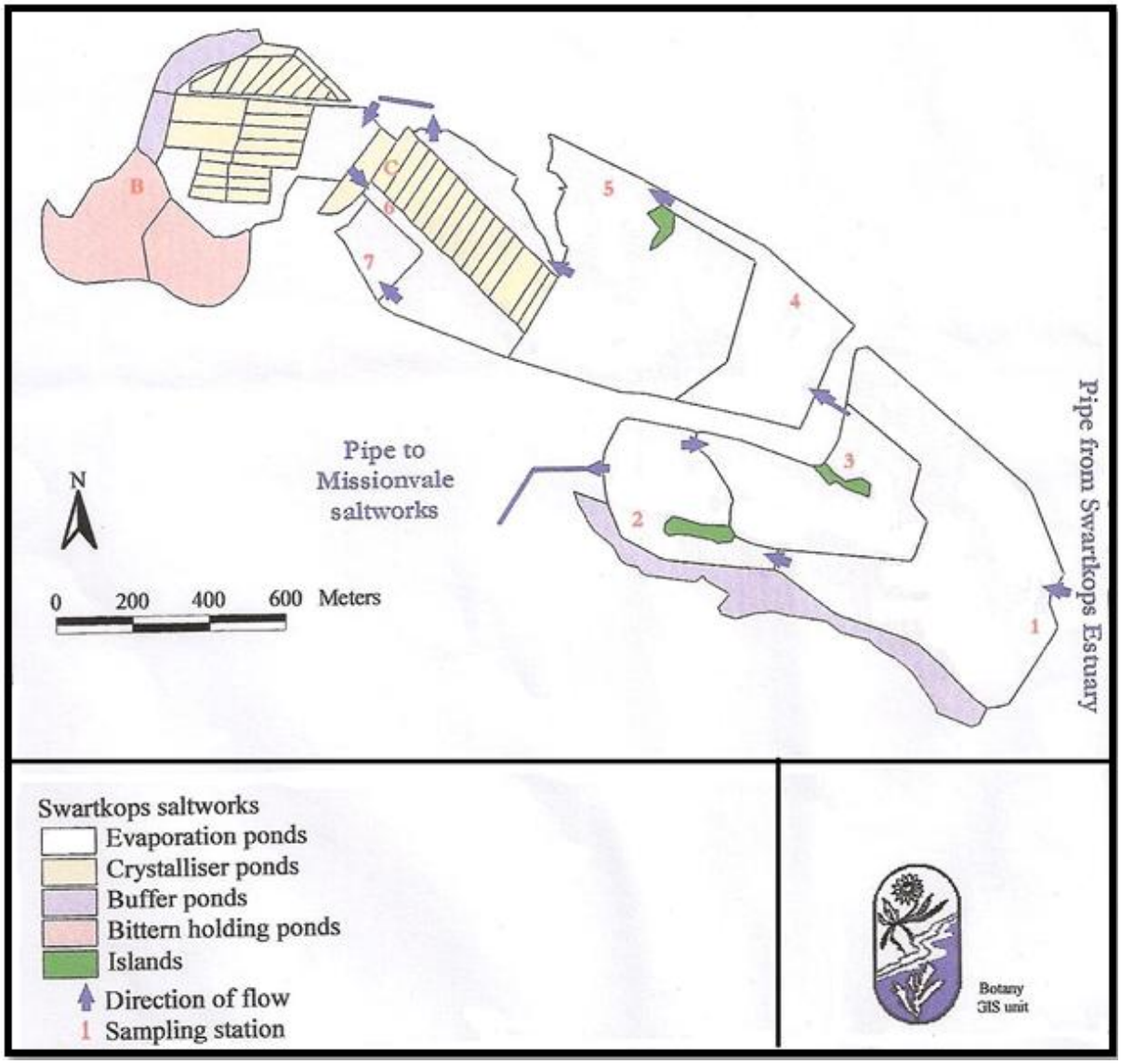


Figure 1: The Swartkops salina with sampling points 1-7 and C.

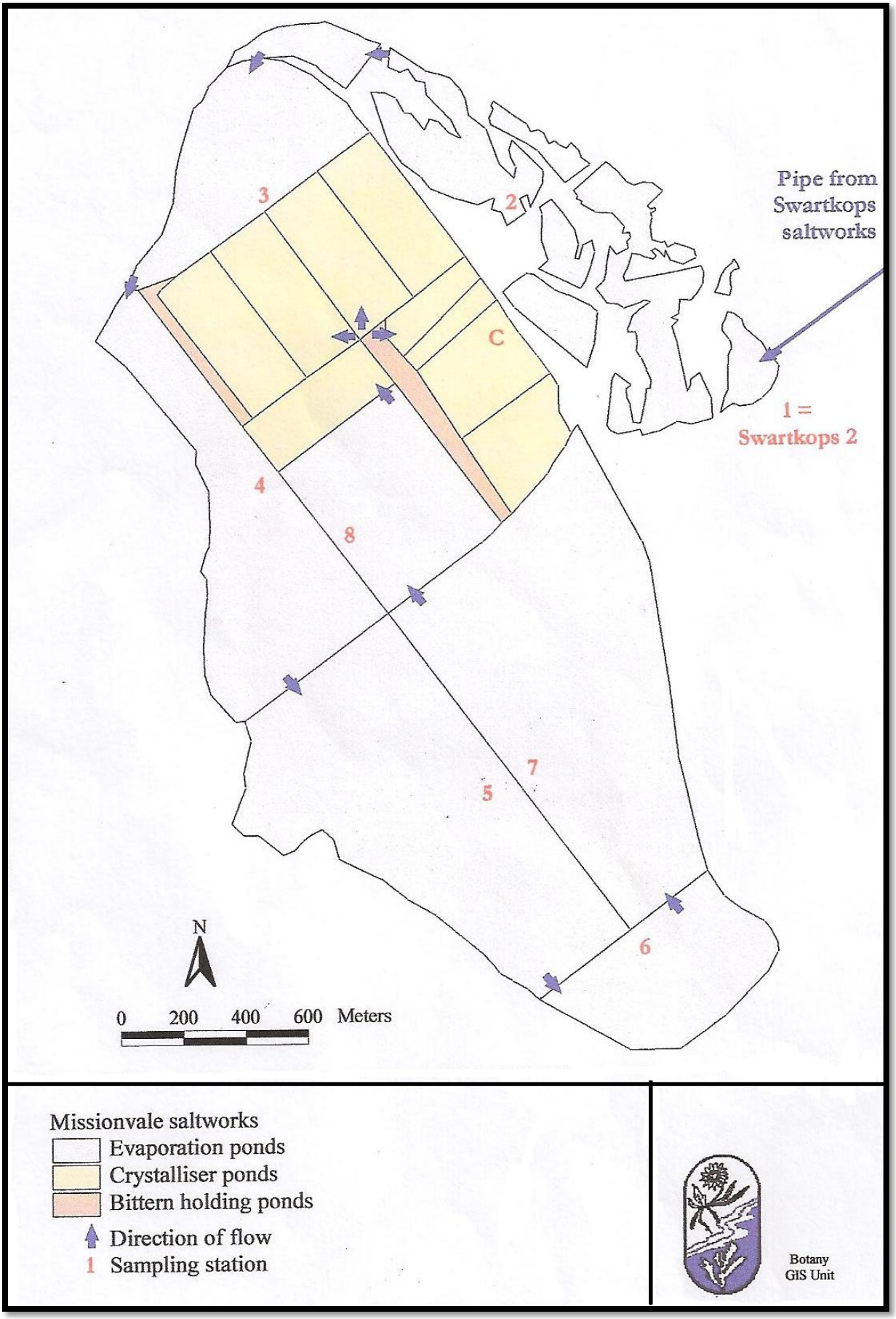


Figure 2: The Missionvale salina with sampling points 2-8 and C.

3.2 Sampling procedure

3.2.1 Physical Factors

The following physical factors were measured at each station:

Temperature

Temperature readings at each station were taken 20 m from the edge of each pond and 15 cm below the surface. The water temperature for both Swartkops and Missionvale salinas were taken in September 1999, 2011 and October 2012. Measurements were taken between 08h00 and 14h00.

Salinity

The salinity of the water was measured using an Atago S/Mill-E hand-held refractometer. Where the brine salinity was excess of 70 psu, samples were diluted with distilled water and the refractometer reading adjusted accordingly.

Pond depth

Pond depth was recorded at 100 points at each station using a meter stick graduated in centimetres (cm). The readings were taken from the edge to the centre of each evaporation pond. Only 40 readings were taken in the smaller crystallizer ponds.

3.2.2 Inorganic Nutrients

Water samples were collected in 2.5 L bottles at the edge, 1 m from the edge and in the middle of the pond at each station. All samples were filtered through Schleicher and Schuell GF/C filter paper. All colourimetric analyses (ammonium, nitrate, phosphate) were read on a spectrophotometer.

Ammonium

The ammonium was measured using the method of Solórzano (1969). The ammonium stock solution (10 mM NH_4^+) was prepared in advance of the field work. One mL of chloroform was added to the solution to keep it stable. The stock was diluted to give a standard series consisting of 0, 10, 20, 30 and 40 µM NH_4^+ solutions.

Nitrate

Nitrate was measured using the copper cadmium method of reduction modified from the Greiss (1879) and Ilosvay (1889) methods. In this method NO^{2-} is reduced by using copper sulphate treated granules (Bate and Heelas (1975). The nitrate stock solution (5 mM NO_3^-) was diluted to provide a standard series consisting of 0, 50, 100, 150 and 200 µM NO_3^-.

Soluble reactive phosphorus

The Strickland and Parsons (1972) method was used to determine the soluble reactive phosphorus in water. A phosphate stock solution (6 mM PO_4^{3-}) was diluted to provide a standard series consisting of 0, 0.3, 0.6, 0.9, and 1.2 µM PO_4^{3-}.

3.2.3 Chlorophyll *a*

Water column

The method recommended by Nusch (1980) was used to determine the chlorophyll *a* content. Between 50 and 500 ml of water was filtered through GF/C filter paper (depending on the concentration of microalgae in the water). The filter paper was placed in 10 ml 95% ethanol and left to extract overnight. The samples were filtered again and the filtrate was read at 665 nm using ethanol as a blank.

The absorption coefficient of 82 (in ethanol) and an acid ratio of 1.7 have been calculated and are used in the calculation as follows (Nusch, 1980):

Chlorophyll *a* (µg l^{-1}) = [R/(R-l)] * 1000/S * [A-(A/1.7)] * (v/V) Eq.2

Where R = the acid ratio of chlorophyll *a* (1.7)

l = path-length of the cuvette (1 cm)

1000 = convert mg to µg

S = specific absorption coefficient of chlorophyll *a* (82 g l^{-1} cm^{-1})

A = absorbance at 665 nm

v = volume of solvent used (ml)

V = volume of sample filtered

2.2.4 Elemental composition of particulate matter

These methods are the same methods that were carried out by Du Toit (2001) in order to make direct comparisons.

Particulate organic carbon

The Strickland and Parsons (1972) method was used to determine the particulate organic carbon (POC) in the water. The formula used to convert the absorbance readings to a final value in mg l^{-1} is as follows (Strickland and Parsons, 1972):

mg C l^{-1} = 275 A * V *E/v……Eq. 3

Where: A = the sample absorbance reading

275 is derived from $F = 120/E_c$ (F is a constant, Strickland and Parsons (1972))

V = volume in millilitres of oxidant used

E = the corrected resulting extinction (1.1)

V = volume filtered

Particulate organic nitrogen

Particulate organic nitrogen (PON) was determined using Kjeldahl digests. The volumes of water filtered through GF/C filter paper varied from pond to pond and the volumes were recorded. The filter paper was dried and placed into a digestion flask. The filter paper was digested using ammonia, followed by a back titration with sulphuric acid.

The following equation was used to convert the volume of sulphuric acid required to reach the end point to mg l^{-1} of nitrogen (Bremner, 1965):

mg N l^{-1} = ml H_2SO_4 * 28 * 50/V/v……..Eq. 4

Where: ml H_2SO_4 = volume titrated for sample-volume titrated for control blank,

28 is derived from the molecular mass,

50 = volume to which the sample is made up (ml),

V = volume distilled (ml), and

V = volume of sample filtered (l).

Particulate organic phosphorus

The Black (1965) method was used to analyse the particulate organic phosphorus (POP) concentrations in the water column. A stock solution of 2.2 g l^{-1} potassium dihydrogen

phosphate was made up. 0, 1, 2, 3 and 4 ml stock were made up to 100 ml, this being equivalent of 0, 0.5, 1, 1.5 and 2 mg P in the sample.

3.2.5 Macroalgae

Macroalgae were found in the early concentration ponds of both Swartkops and Missionvale salinas along the edges and in the shallower areas. The estimated percentage cover for macroalgae was determined by visual assessment (dividing the ponds in four and then determining the cover in each section). Even though this method is subjective its gives a good guideline as to the quantity of macroalgae present in a salina.

3.2.6 Zooplankton

Mesozooplankton were collected using 200 µm mesh conical plankton net with a 40 cm diameter. The net was trawled for approximately 25 m from the edge of the pond to the centre of each pond at each station.

3.2.7 Avifauna

Bird guano contributes to the nutrients in the ponds so it is useful to know the bird density and species richness in each pond. Birds associated with the ponds were counted between 08h00 and 14h00. Only the birds found in the ponds or on the immediate edge of the ponds were included.

3.3 Cultures

Water samples and scrapings from rocks and macroalgae were collected from each station. Serial dilutions, enrichment cultures and micropipetting was used to isolate monospecific cultures in either Provosoli or f/2 enriched, salinity adjusted seawater with or without silicon (0.03g) addition. Once monospecific, cultures were grown under standard conditions in a Conviron growth cabinet at a temperature of 18.8ºC and irradiance of 11.3 µmol l^{-1} and growth was determined by measuring the chlorophyll *a* content. Chlorophyll *a* was measured every day for six days using a PhytoPAM to determine the growth rate. Cultures were grown at 52, 70, 100 and 150 psu to compare growth and quantity of lipid produced. Microalgal cells were photographed at 400 times magnification using an Olympus BX51 light microscope.

3.3.1 Nile red stain

Nile Red is a fluorescent dye that is used for lipid analysis (Cooksey et al., 1987) with the dye-treated lipids fluorescing yellow under UV light (X-cite ® series 120 Q). The Cooksey et al. (1987) method was used to stain the culture. 250 µg Nile red was added to 1 ml acetone to form the Nile red solution. 4 µl Nile red solutions was added to 1 ml culture and mixed vigorously on a vortex mixer. The samples were used immediately or after 30 seconds for fluorescence microscopy. Lipid area was measured using ImageJ software. The images from which the lipids were measured were two dimensional. Calculating the amount of lipid per cell was done as follows: the area of the lipids in a cell was measured, then the area of the entire cell was measured and the amount of lipid was expressed as a percentage of the cell area. Ten cells per species were measured and the average percentage of lipid was determined per species.

3.4 Statistical Analysis

Statistical analyses were done using *Statistica* version 10 (2011). The Shapiro Wilks test indicated that some data was parametric and some nonparametric. The Spearman Rank test was used to determine the significant correlations. Significance levels were taken to be 0.05. A Student's t-test was done to test for significant differences between variables. Data for the biological survey from a study by Du Toit (2001) at the same sites ten years ago were used for comparison with the results from 2011 and 2012.

4. Results

4.1 Physical Factors

4.1.1 Temperature

Temperature was negatively correlated to pond depth in 2011 (Figure 3, Table 2) but this was not the case in 1999 and 2012. There was no significant difference between the water temperatures measured in 1999 and in 2011 and 2012 (Table 2).

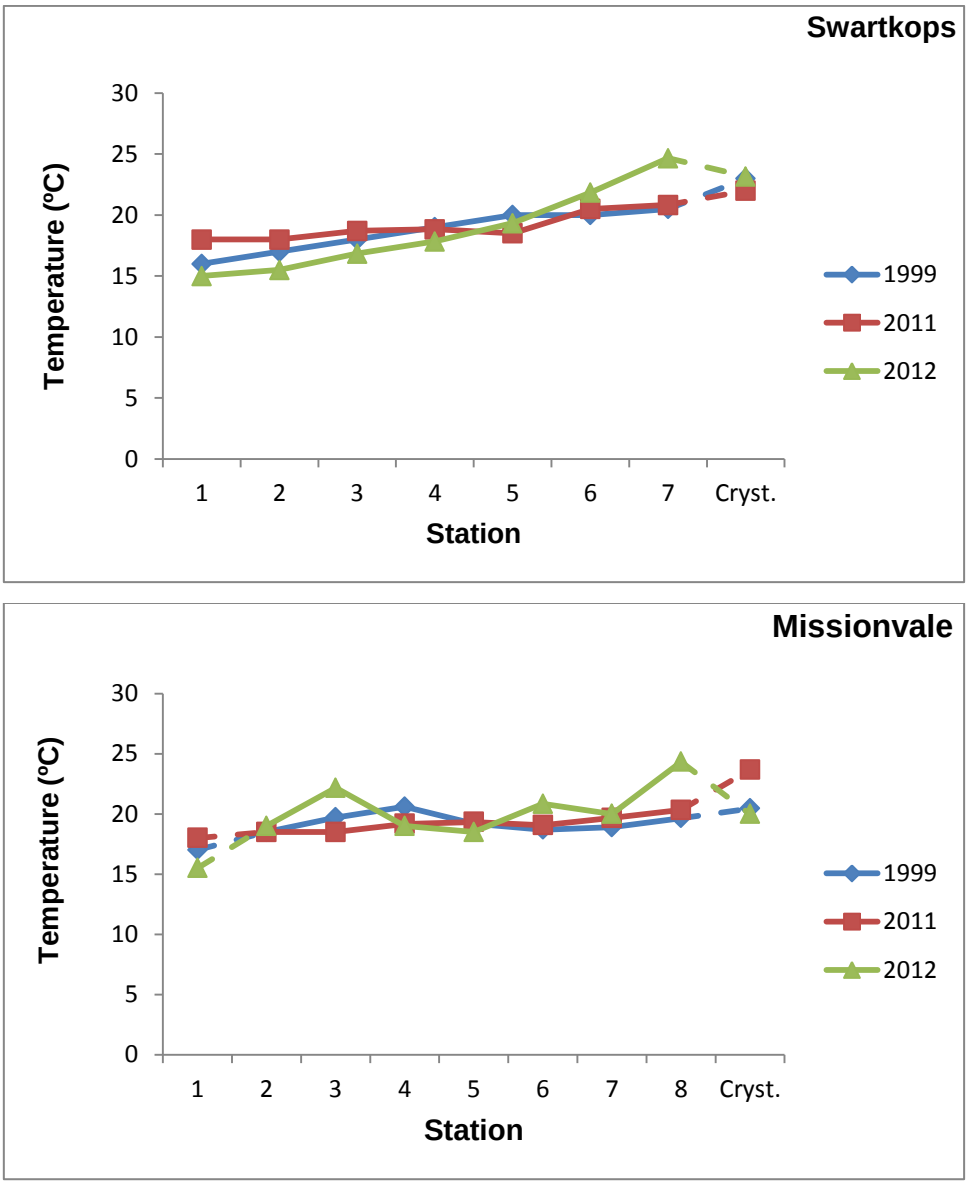


Figure 3: Water temperature of the Swartkops and Missionvale salinas

Table 2: Spearman's rank correlations (r) between pond depth and brine temperature in Swartkops and Missionvale salinas. Values in bold are significant at $p < 0.05$.

Salina	r	p	d.f.
Swartkops 1999	-0.674	0.066	14
Missionvale 1999	-0.048	0.91	14
Swartkops 2011	**-0.85**	< 0.01	14
Missionvale 2011	**-0.85**	< 0.01	14
Swartkops 2012	-0.595	0.119	14
Missionvale 2012	-0.301	0.468	14

4.1.2 Salinity

In 1999 the salinity of the water in the initial three stations of the Swartkops salina decreased, after which it increased until station 7 (Figure 4). The Swartkops salina crystallizer measured in 1999 had received substantial freshwater inflow as it had a salinity of 180 psu (Figure 4). However, in 2011 and 2012, the salinity of the Swartkops crystallizer pond was high at over 300 psu (Figure 4).

In 2011 the salinity profile of the Missionvale salina differed from the Swartkops salinity profile as it did not increase at each station throughout the system. In 2012 the salinity was similar to 2011 in that the salinity is not increase in each of the concentrating stations (Figure 4). The Missionvale crystallizer pond was harvested for salt in 2012, increasing the pond depth, decreasing the rate of evaporation, hence the lower salinity (Figure 4). The salinity profiles in 1999, 2011 and 2012 for both Swartkops and Missionvale salinas were not significantly different (Swartkops: 1999&2011 — $t = 0.108$, d.f. = 7, $p > 0.05$; 1999&2012 — $t = -0.568$, d.f. = 7, $p > 0.05$; 2011&2012 — $t = -1.036$, d.f. = 7, $p > 0.05$) (Missionvale: 1999&2011 — $t = 0.616$, d.f. = 7, $p > 0.05$; 1999&2012 — $t = -1.180$, d.f. = 7, $p > 0.05$; 2011&2012 — $t = -1.480$, d.f. = 7, $p > 0.05$).

4.1.3 Pond depth

The depth of the ponds plays an important role in a salina, particularly for salinity, thus the shallower the pond the faster the rate of evaporation and vice versa. Therefore the faster the rate of evaporation the quicker salt is produced. Due to the heavy rains, the depth of water in

the ponds increased. The rainwater had already been pumped off the concentrating ponds in 2012 for the Swartkops salina, however not for the Missionvale salina (Figure 5). The Missionvale crystallizer was deeper in 2012 compared to 2011 (Figure 5) because the salt was harvested from this crystallizer. The pond depths for the Swartkops and Missionvale salinas in 2011 and 2012 were not significantly different ($t = -0.333$, d.f. = 7, $p > 0.05$; $t = -1.021$, d.f. = 7, $p > 0.05$ respectively). In 2011, the salinity at the Missionvale salina was negatively correlated with pond depth, whereas in 2012 there were no significant correlations (Table 3).

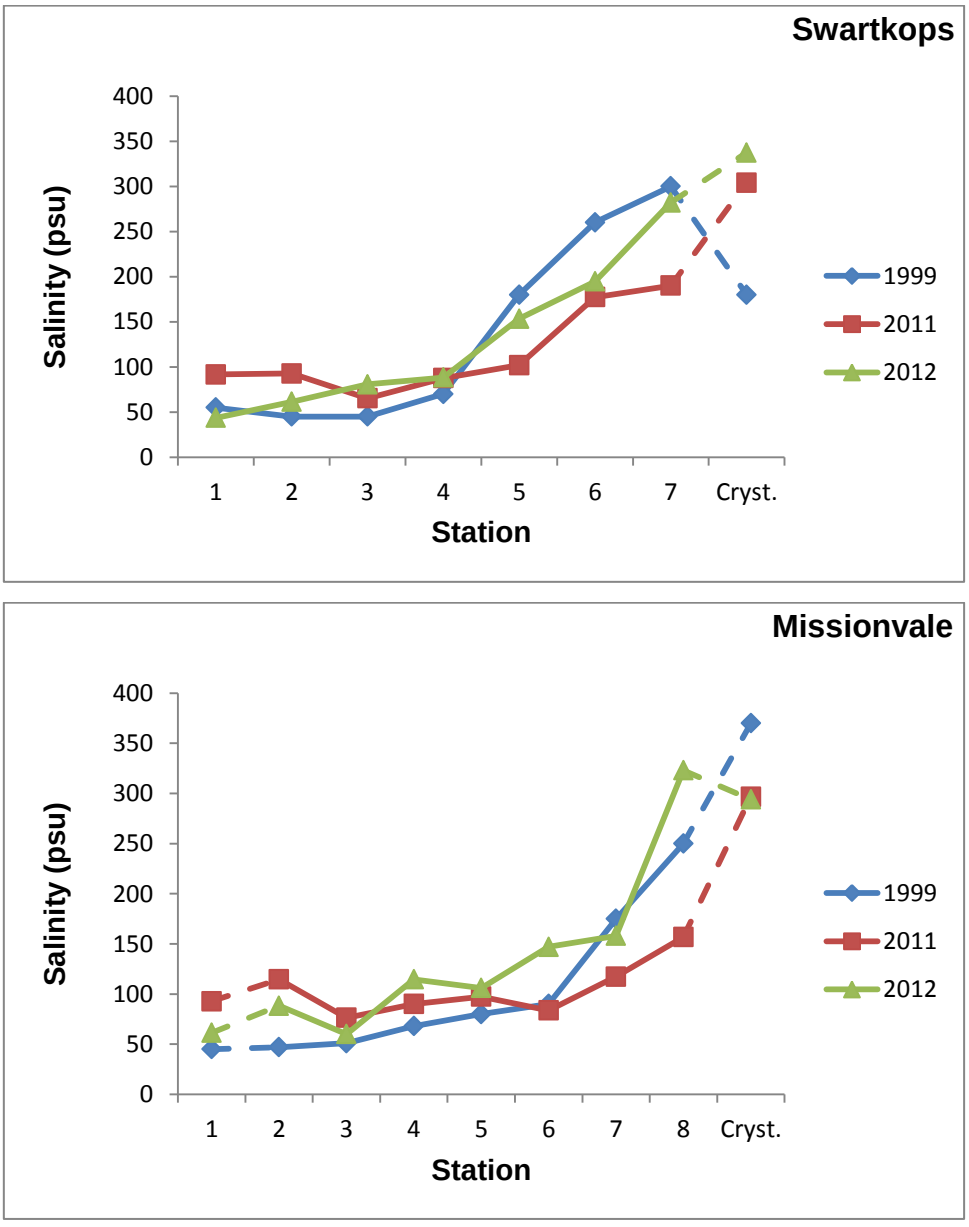
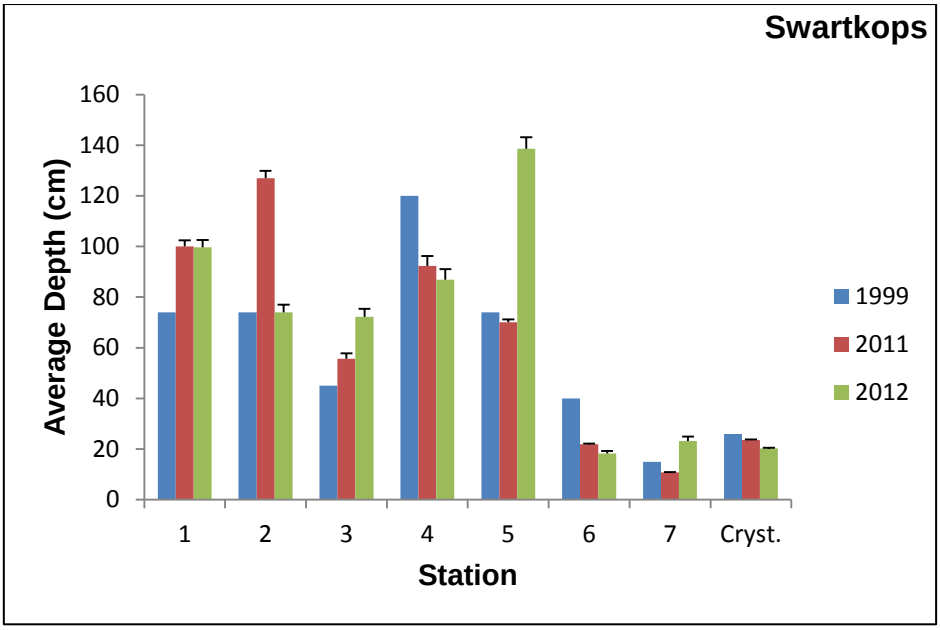


Figure 4: Water salinity of the Swartkops and Missionvale salinas



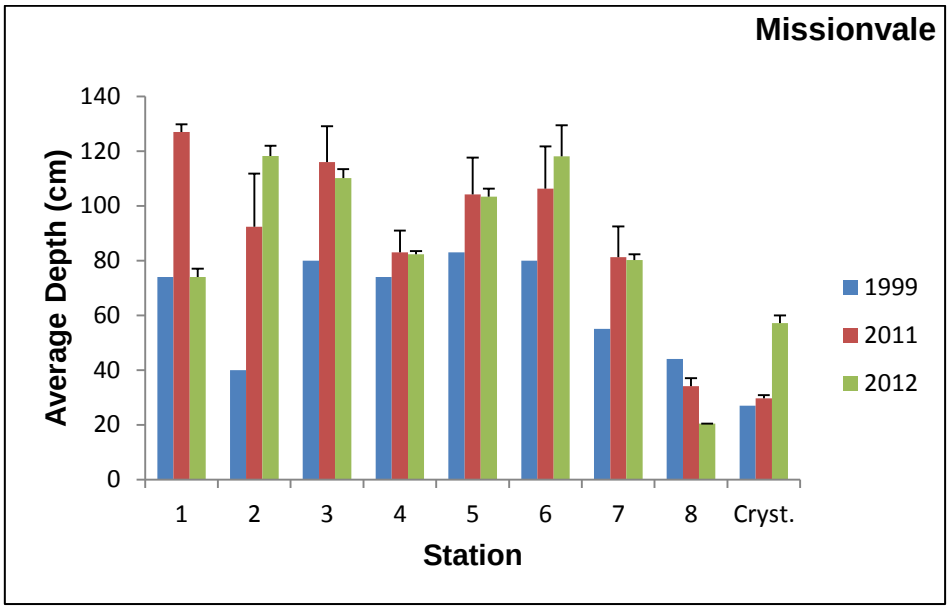


Figure 5: Pond depths of the Swartkops and Missionvale salinas at each station (n= 100 for evaporation ponds and 40 for crystallizers, vertical bars represent ± 1 S.E.)

Table 3: Spearman's rank correlations (r) between mean pond depths and salinity in Swartkops and Missionvale salinas. Values in bold are significant at $p < 0.05$.

Salina	r	p	d.f.
Swartkops 1999	-0.593	0.122	14
Missionvale 1999	-0.347	0.399	14
Swartkops 2011	-0.59	0.12	14
Missionvale 2011	**-0.93**	< 0.01	14
Swartkops 2012	-0.619	0.102	14
Missionvale 2012	-0.809	0.015	14

4.1.4 Rainfall

The Swartkops and Missionvale salinas lie in an area that receives rainfall all year round (Stone et al. 1998). Port Elizabeth had particularly high rainfall in 2011 and 2012 with July 2011 receiving 215 mm of rain (Figure 6). The winter of 2012 was the second wettest winter in the city's history.

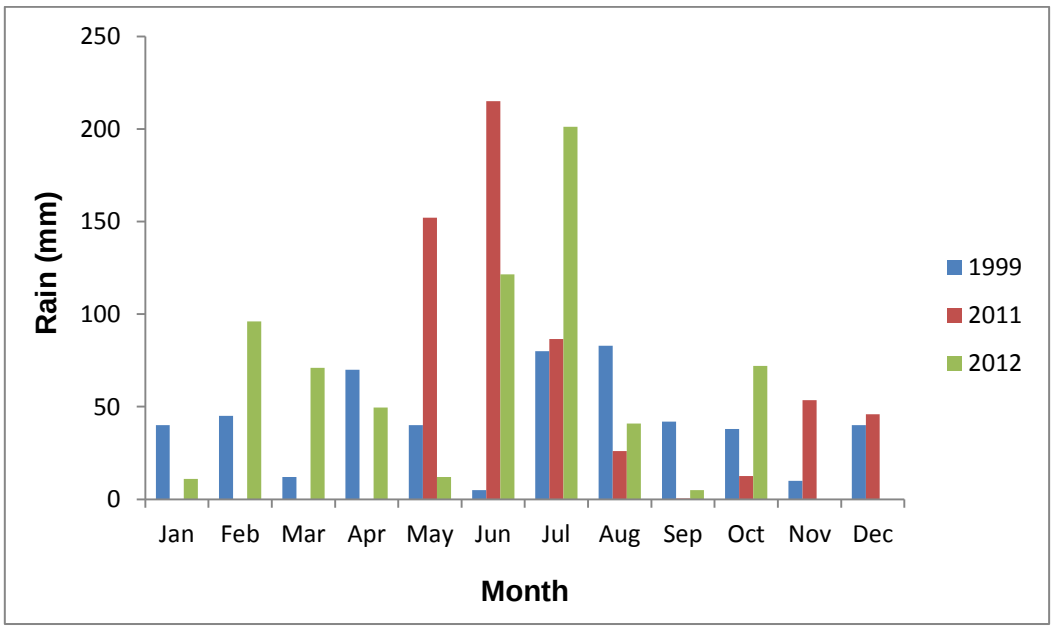


Figure 6: Monthly rainfall for the Port Elizabeth area (data from Marina Seesout and South African Weather Bureau)

4.2 Inorganic Nutrients

4.2.1 Nitrogen

In the Swartkops salina ammonium concentrations found in 2012 were significantly higher than those found in 1999 ($t = 0$, $z = 2.52$, $p = 0.0117$) and 2011 ($t = 0$, $z = 2.10$, $p = 0.036$) (Figure 7). Likewise in the Missionvale salina the ammonium concentrations found in 2012 were significantly higher than concentrations in 1999 ($t = 2$, $z = 2.24$, $p = 0.025$) (Figure 7). The nitrogenous nutrients were greatest for the Missionvale salina. In the Missionvale salina ammonium contributed largely to the inorganic nitrogen concentrations and in the Swartkops salina nitrate and nitrite contributed the most (Figure 7).

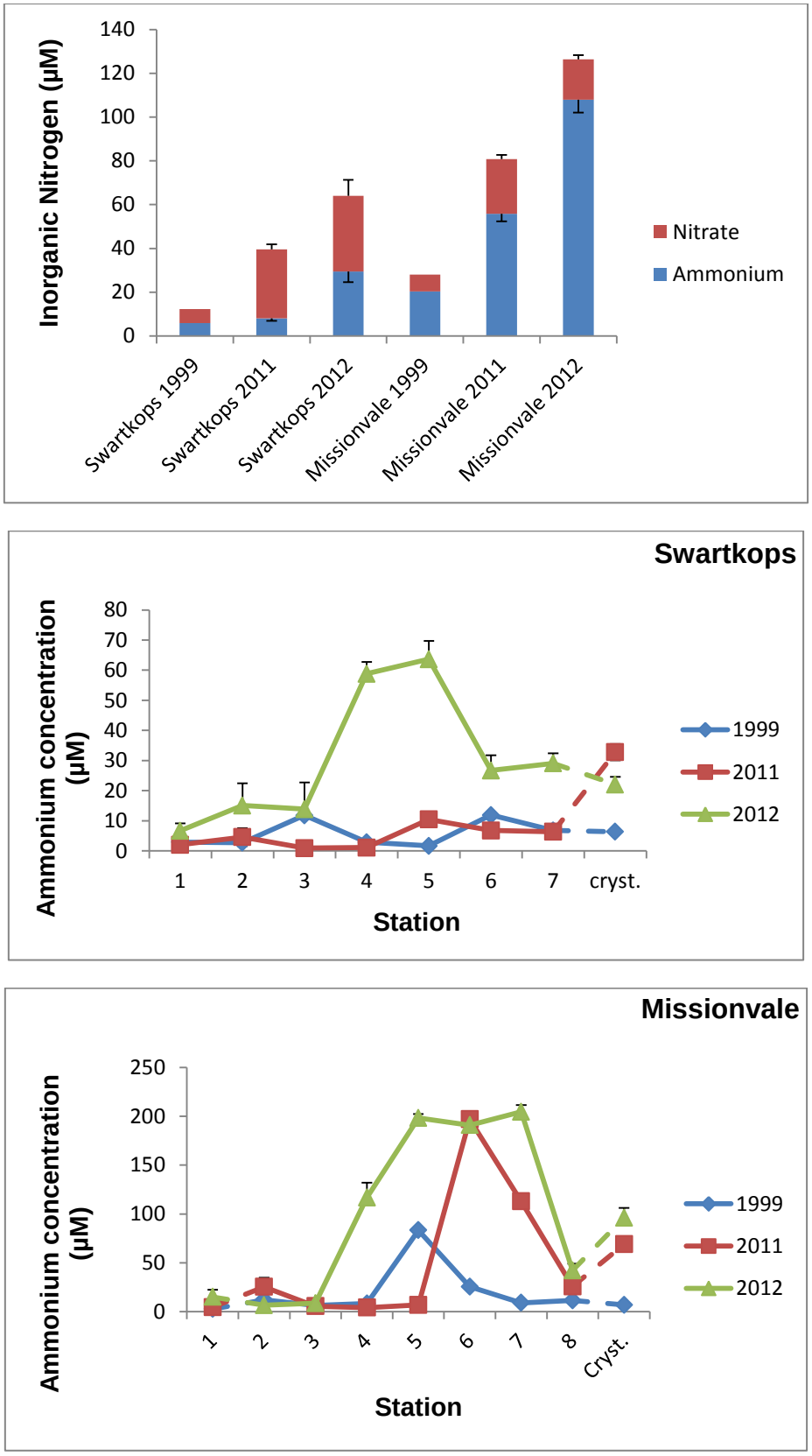


Figure 7: The mean inorganic nitrogen concentrations and the water column ammonium concentrations of the ponds in the Swartkops and Missionvale salinas (n = 3, vertical bars represent ± 1 S.E.)

4.2.2 Nitrate

In 1999 the water column nitrate concentrations were low for both Swartkops and Missionvale salinas. In 2011 and 2012 the Swartkops salina had inorganic nitrogen concentrations above the 25 µM target (Davis and Giordano, 1996) (Figure 8). In the Missionvale salina in 2011 and 2012 the inorganic nitrogen concentrations ranged between 25 and 30 µM (Figure 8). The nitrate concentrations in the Swartkops and Missionvale salina in 2011 and 2012 were significantly higher than the concentrations in 1999 ($t = 0$, $z = 2.52$, $p = 0.012$). The nitrate concentrations in 2011 in the Missionvale salina were significantly higher than in 2012 ($t = 3$, $z = 2.10$, $p = 0.036$).

4.2.3 Soluble Reactive Phosphorus

In 1999, 2011 and 2012 the Swartkops salina had high initial soluble reactive phosphorus concentrations (Figure 9). The soluble reactive phosphorus concentration in the Swartkops salina in 1999 and 2011 were significantly higher than in 2012 ($t = 01$, $z = 2.10$, $p = 0.036$) and ($t = 0$, $z = 2.24$, $p = 0.025$) respectively. In the Missionvale salina the 1999 and 2011 soluble reactive phosphorus concentrations were similar and fell within the recommended concentration (1 µM) (Figure 9); however the 2012 Missionvale soluble reactive phosphorus concentrations were significantly higher than 1999 ($t = 2$, $z = 2.24$, $p = 0.025$) and 2011 ($t = 0$, $z = 2.52$, $p = 0.012$) soluble reactive phosphorus concentrations.

4.3 Chlorophyll *a*

The water chlorophyll *a* concentration was taken as a measure of the phytoplankton biomass in each pond. 2011 chlorophyll *a* concentration was higher the Swartkops higher and Missionvale salina than in 2012 ($t = 3.203$, d.f. = 7, $p < 0.05$) and in 1999 and 2011 in the Missionvale salina had high water column phytoplankton biomass. Both Swartkops and Missionvale salinas in 2012 and Swartkops salina in 1999 had lower concentrations of chlorophyll *a,* anywhere between 10 and 50 µg l^{-1} lower than in 2011 (Figure 10). The phytoplankton biomass (measured as chlorophyll *a* concentration) for 1999 was significantly higher than 2012 in both salinas (Swartkops: $t = 2.946$, d.f. = 7, $p < 0.05$; Missionvale: $t = 3.511$, d.f. = 7, $p < 0.05$).

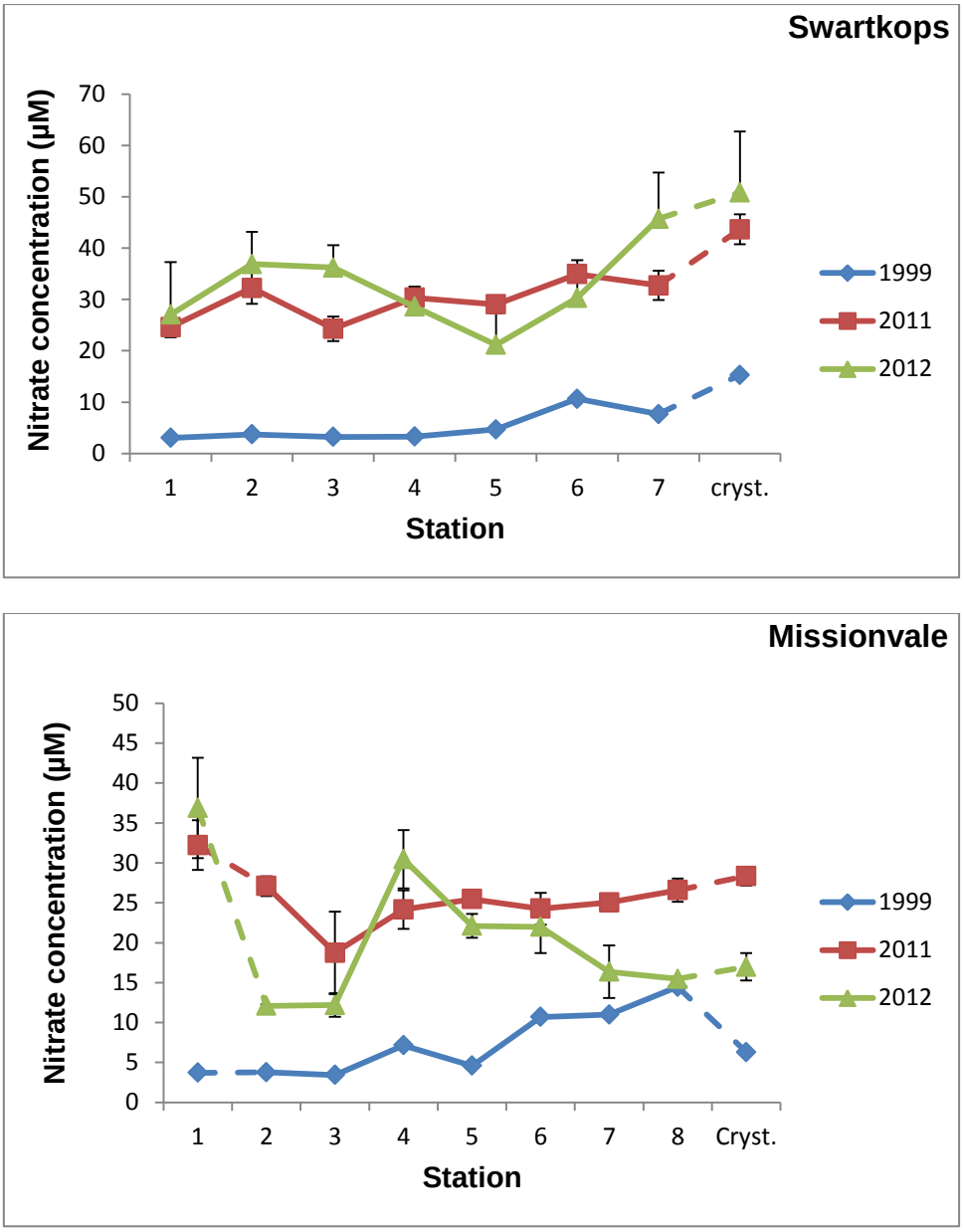


Figure 8: Water column nitrate concentrations at each station in the Swartkops and Missionvale salinas (n = 3, vertical bars represent ± 1 S.E.)

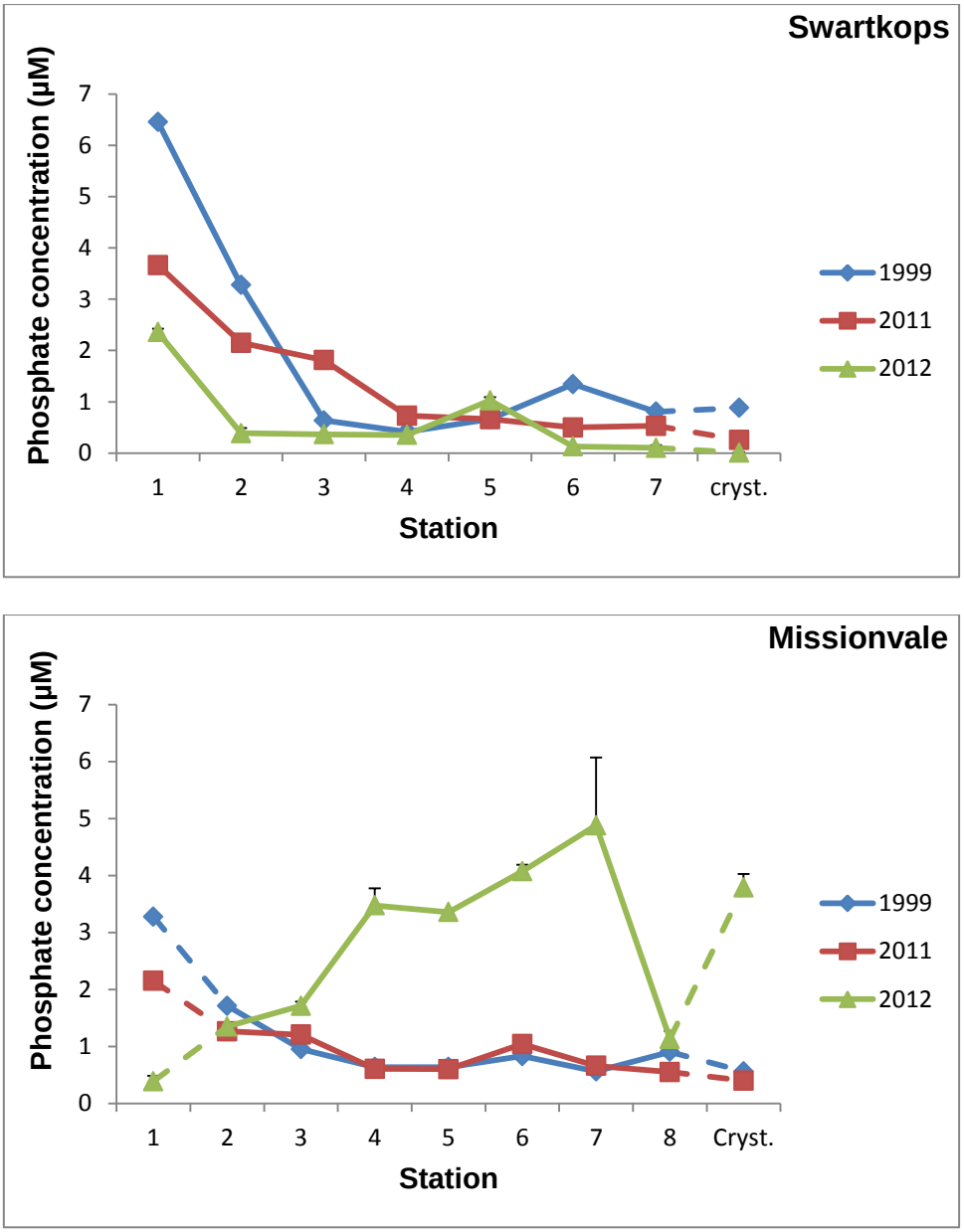


Figure 9: The water column soluble reactive phosphorus concentrations at each station of the Swartkops and Missionvale salinas (n = 3, vertical bars represent ± 1 S.E.)

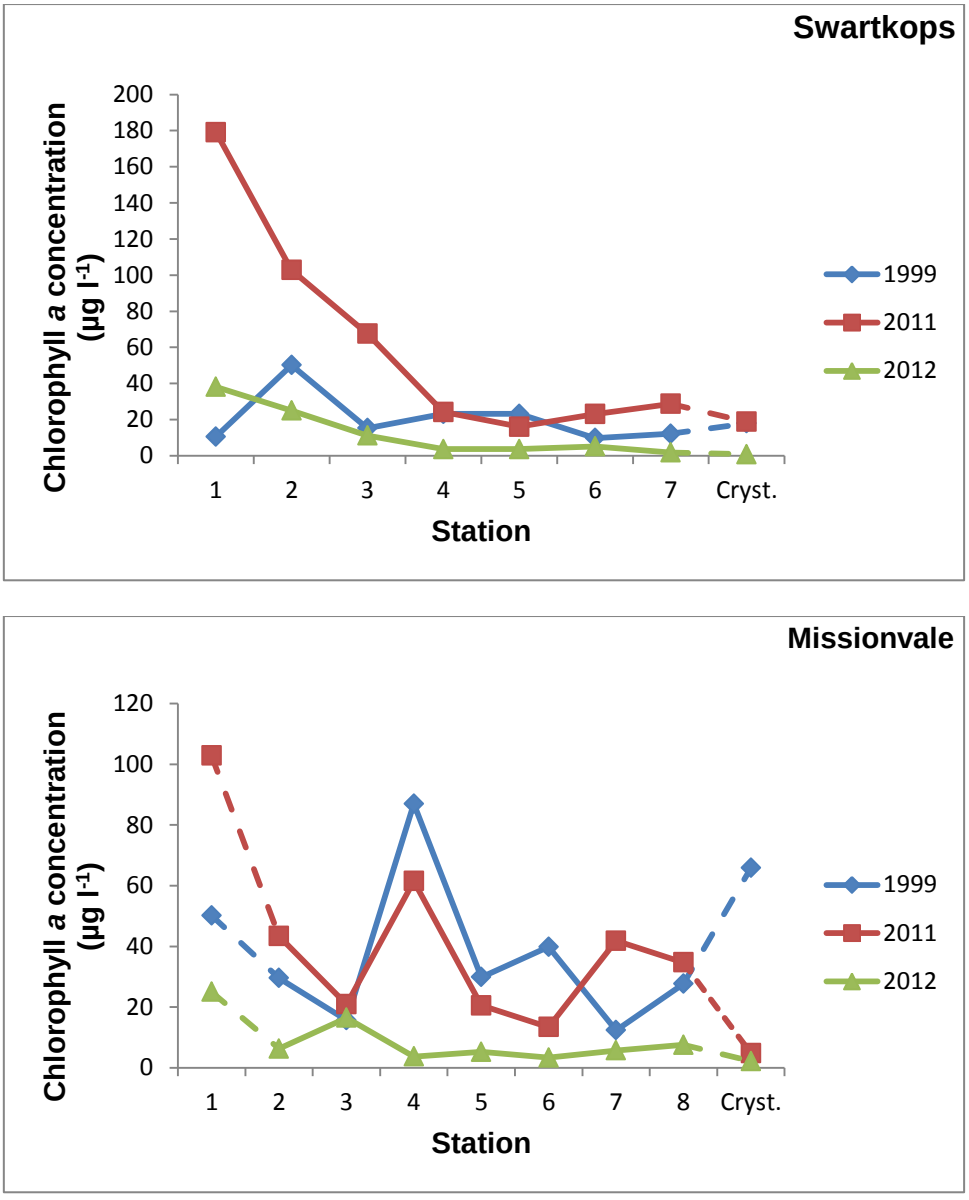


Figure 10: The water chlorophyll *a* concentration at each station of the Swartkops and Missionvale salinas, (n = 3, vertical bars represent ± 1 S.E.)

4.4 Elemental composition of particulate matter

4.4.1 Particulate organic carbon

The particular organic carbon (POC) in the Swartkops salina in 1999 remained constant at the evaporation stations (1 to 5) and tripled at the pre-crystallizer station (Figure 11). In 2011 the POC decreased as the brine moved from station 1 to the crystallizer, and 2012 POC concentrations fluctuated substantially (Figure 11). Overall, the 2012 POC concentrations were significantly higher than in 1999 ($t = - 3.183$, $d.f. = 7$, $p = 0.015$) and 2011 ($t = - 3.847$, $d.f. = 7$, $p = 0.006$).

The POC of the Missionvale salina in 1999 increased from station 4 to station 6 and again in the crystallizer (Figure 11). In 2011 and 2012 the POC concentration remained within a narrow range (give range) throughout the system (Figure 11). The POC concentrations between the years were not significantly different.

4.4.2 Particulate organic nitrogen

The water column particulate organic nitrogen (PON) for both salinas in 2011 was under 2 mg l^{-1} (Figure 12). As the salinity of the brine increased, the PON decreased in both Swartkops and Missionvale salinas (Figure 12). The largest increase was from station 5 to 6 in the Swartkops salina were the PON increased by 1 mg l^{-1}. The 1999 PON concentrations were under 3 mg l^{-1} for both Swartkops and Missionvale salinas.

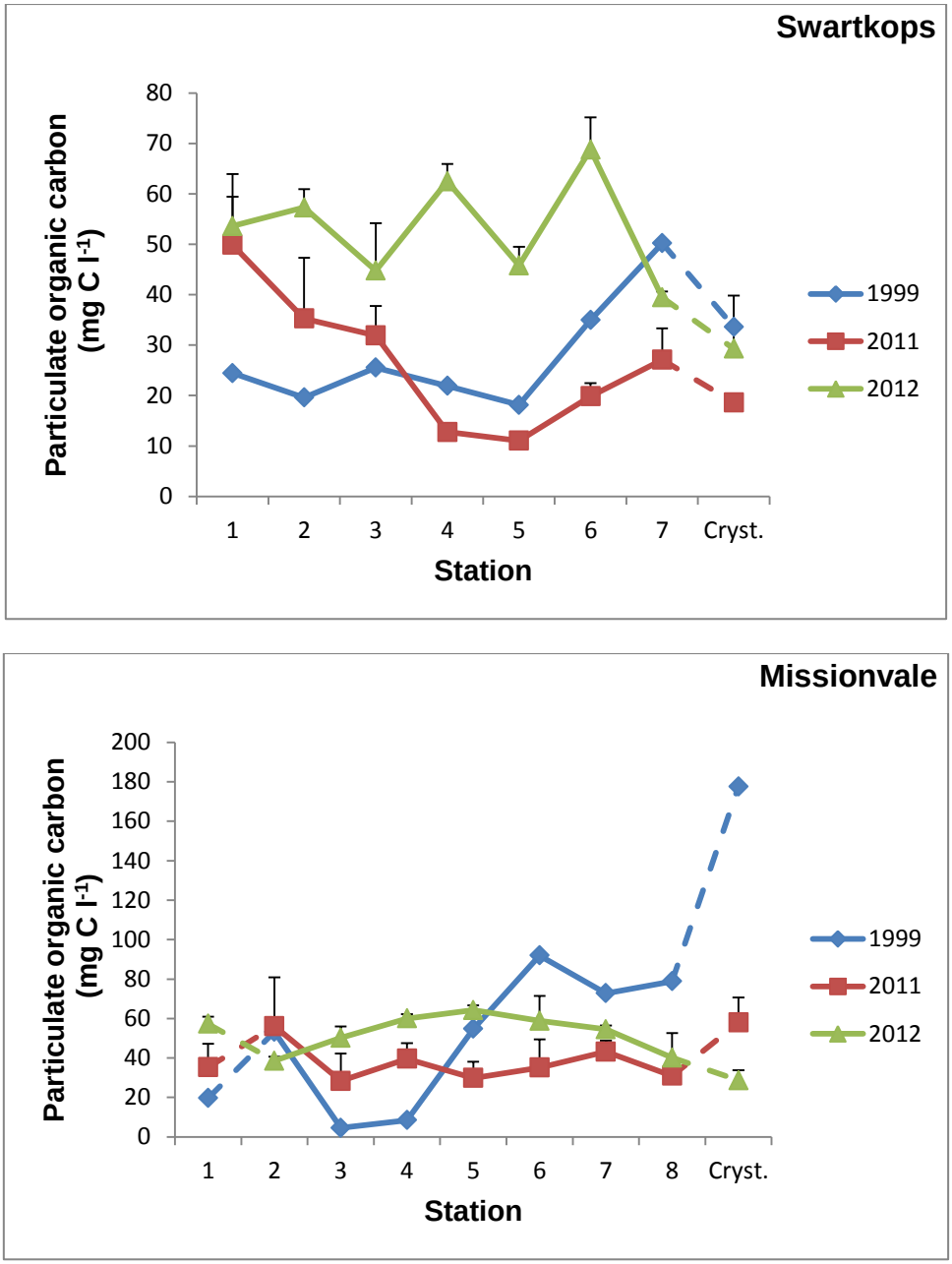


Figure 11: Particulate organic carbon concentrations of the Swartkops and Missionvale salinas (n = 2, vertical bars represent ± 1 S.E.)

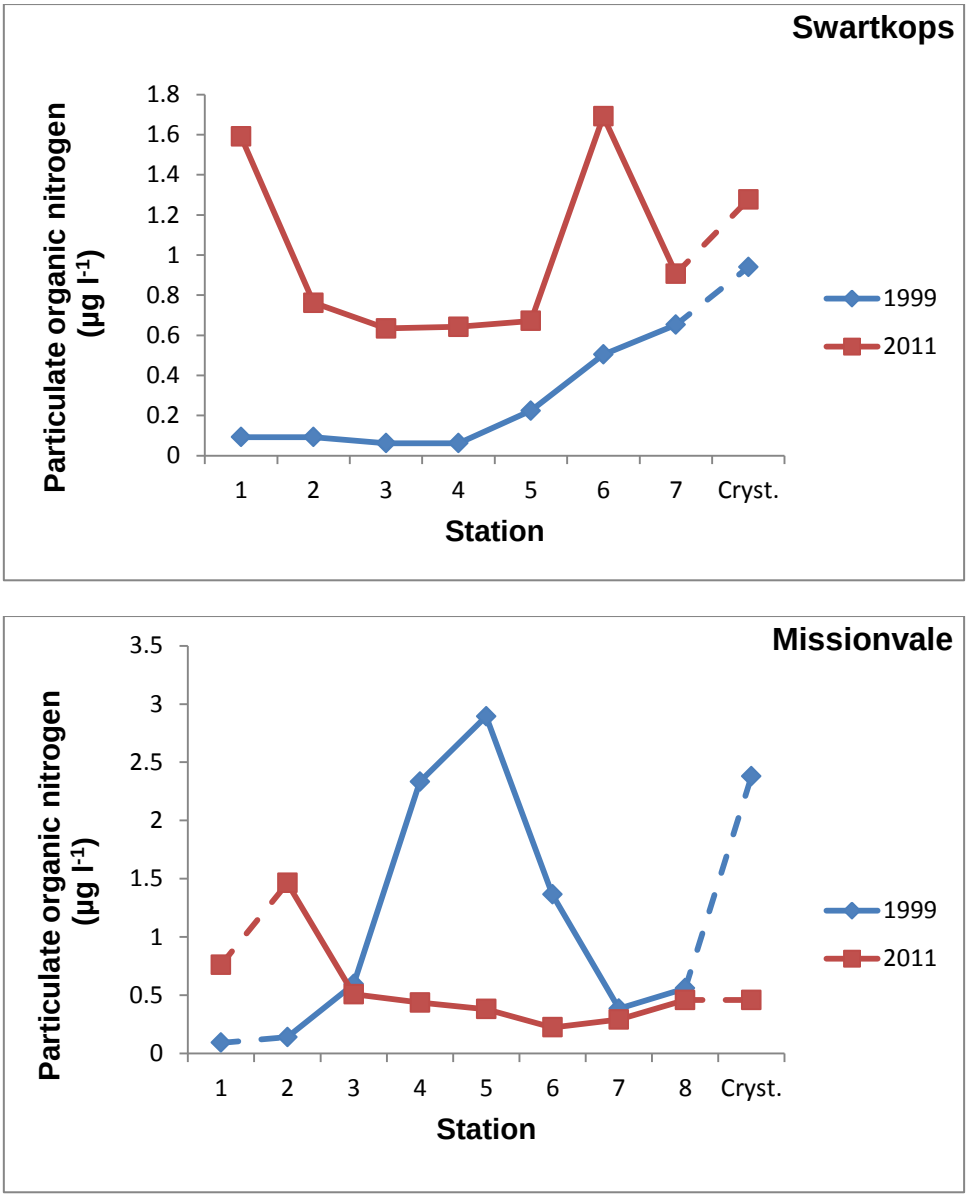
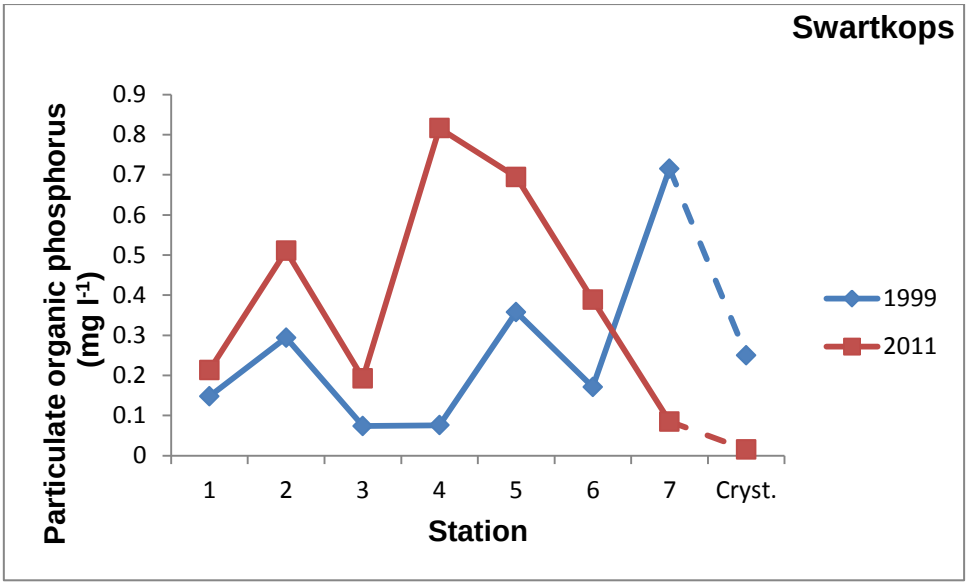


Figure 12: Particulate organic nitrogen concentrations of the Swartkops and Missionvale salinas.

4.4.3 Particulate organic phosphorus

The water column particulate organic phosphorus (POP) concentrations in 2011 in the Swartkops salina were generally lower than the POP concentrations for the Missionvale salina (Figure 13), with Swartkops and Missionvale having very similar average concentrations (0.36 mg l^{-1} ± 0.1 mg l^{-1} and 0.48 mg l^{-1} ± 0.09 mg l^{-1} respectively). In 1999 the mean POP concentrations for Missionvale (0.84 mg l^{-1} ± 0.08 mg l^{-1}) was higher than for Swartkops (0.26 mg l^{-1} ± 0.07 mg l^{-1}) (Figure 13).

Particulate organic phosphorus concentrations in 2011 in the Swartkops system started to decrease from station 4 as the brine progressed through the system (Figure 13), while the POP in the Missionvale system decreased from station 3 to station 7 and then increases in the crystallizer (Figure 13). This increase of POP in the crystallizer in the Missionvale system is consistent with an increase of POC and PON in the crystallizer pond. In 1999 there were no trends evident.



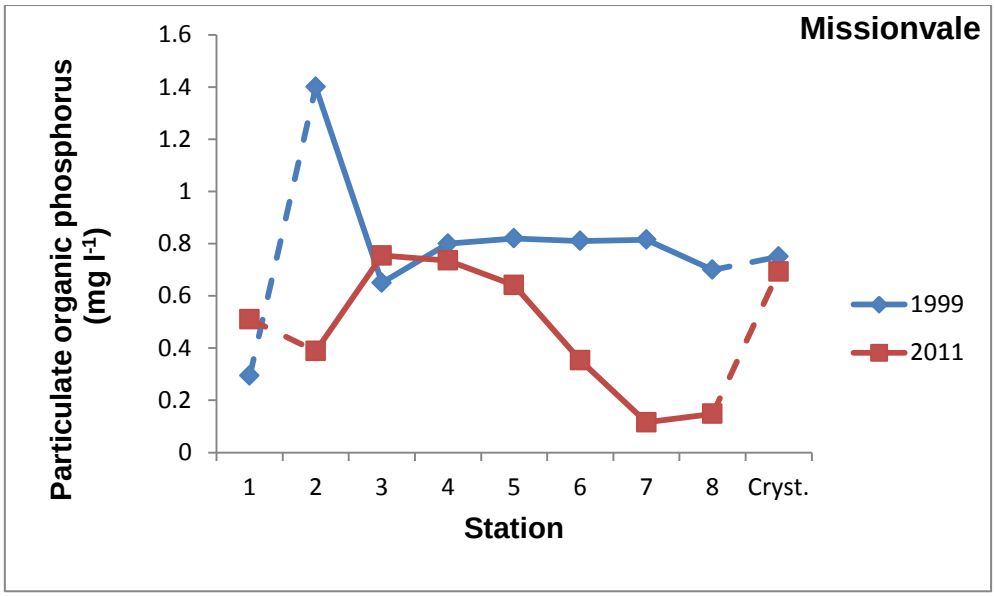


Figure 13: Particulate organic phosphorus concentrations of the Swartkops and Missionvale salinas.

4.5 Comparative analyses

The particulate organic carbon in the Swartkops ponds comprised mostly of phytoplankton biomass (significant correlations between POC and water chlorophyll *a* in Table 4). The particulate organic nitrogen and the particulate organic phosphorus had no effect on the phytoplankton biomass in the Swartkops ponds (Table 4). The phytoplankton biomass in the Missionvale ponds was neither affected by the particulate organic carbon, nor the particulate organic nitrogen, nor the particular organic phosphorus (Table 4).

Table 4: Spearman rank correlations (r) between water column chlorophyll a and organic concentrations of the Swartkops and Missionvale salina. Values in bold are significant at $p < 0.05$.

Salina	POC		PON		POP	
	r	p	r	p	r	p
Swartkops 1999	**-0.743**	**0.035**	-0.285	0.091	-0.179	0.243
Missionvale 1999	0.214	0.61	0.667	0.071	0.048	0.911
Swartkops 2011	**0.928**	**< 0.01**	0	1	-0.92	0.823
Missionvale 2011	0.095	0.823	0.239	0.567	-0.071	0.867
Swartkops 2012	0.548	0.16				
Missionvale 2012	0.071	0.867				

A useful guide to indicating any disturbances in the solar saltworks systems is the ratios between the organic fractions in the brine. Redfield (1934) calculated the ideal ratio for carbon: nitrogen: phosphorus which is 106 C: 16N: 1P. A high carbon load was found by Du Toit (2001) in the Swartkops and Missionvale salinas where the C:N ratio was up to four times higher than 250:1 whereas in 2011 Swartkops particulate organic carbon to particulate organic nitrogen (C:N) ratios were comparatively stable at below 50:1, while Missionvale ratios were up to 3 times higher (Figure 14).

The particulate organic carbon to particulate organic phosphorus (C: P) ratios for both Swartkops and Missionvale in 1999 and 2011 fluctuated throughout the system. The C: P ratio in 2011 of the Swartkops (260.3 ± 141) salina was higher than Missionvale (130.9 ± 40)

(Figure 15), whereas the ratios by Du Toit (2001) for Swartkops (203.7 ±36.5) and Missionvale (209.6 ± 50.4) were similar.

In the Swartkops salina 2011 the particulate organic nitrogen to particulate organic phosphorus (N: P) ratio remained below 7:1 for all station except station 7 and 8, whereas the Missionvale salina 2011 N: P ratio remained below 4:1 (Figure 16). This indicates that there were low levels of nitrogen in the salinas, lower than the recommended 16:1 (Redfield, 1934). The low-salinity stations of Swartkops had a mean N: P ratio of 4, while the mid-salinity stations had a ratio of 2, indicating that nitrogen became a limiting factor. Du Toit (2001) found that N:P ratio for both Swartkops and Missionvale salinas were below 7:1 with the low salinity stations of Missionvale having a mean N : P of 2 while the mid-salinity stations ratio was 8; thus indicating that either inorganic nitrogen was increasing or soluble reactive phosphorus was depleted.

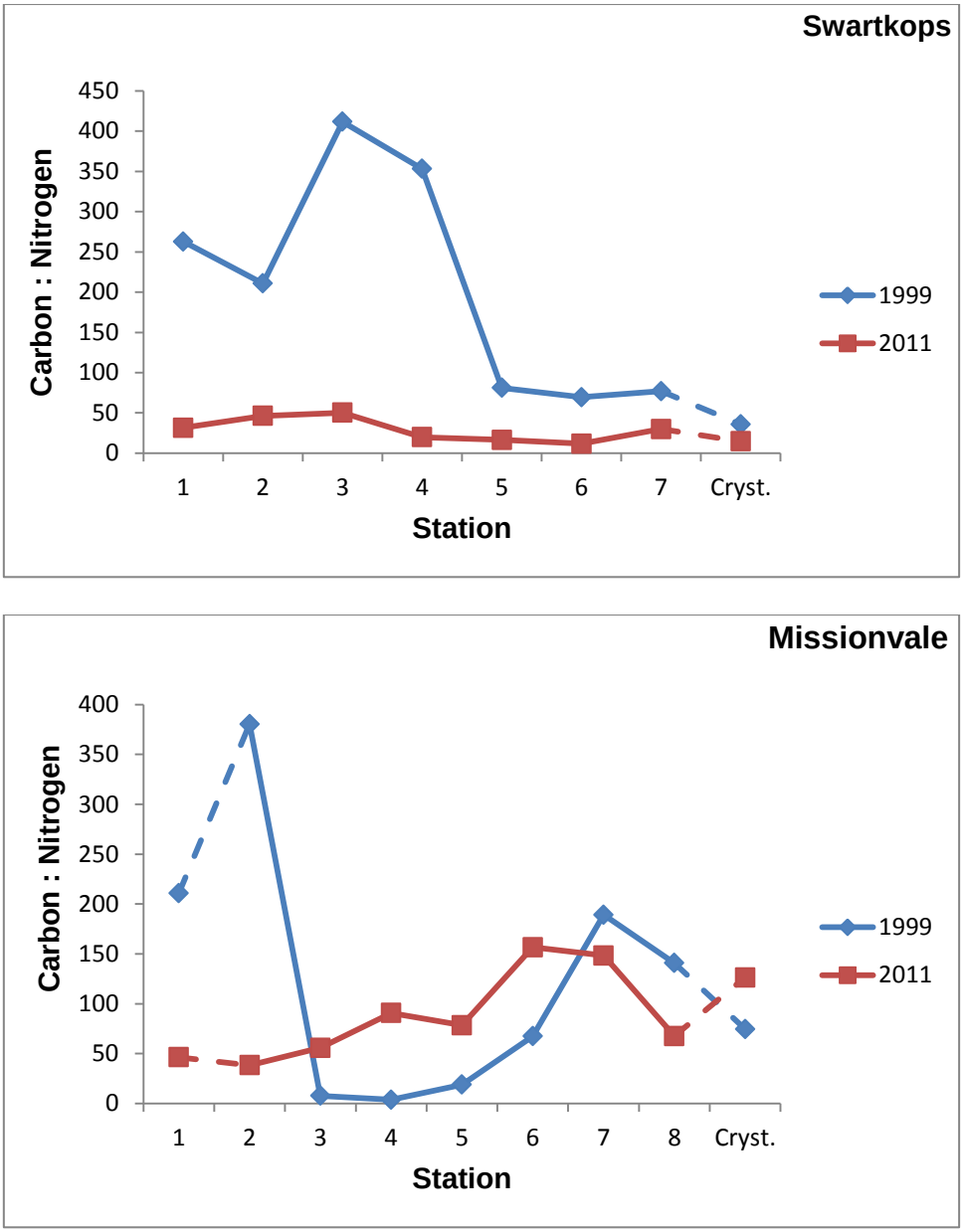


Figure 14: Particulate C: N ratios of the Swartkops and Missionvale salinas.

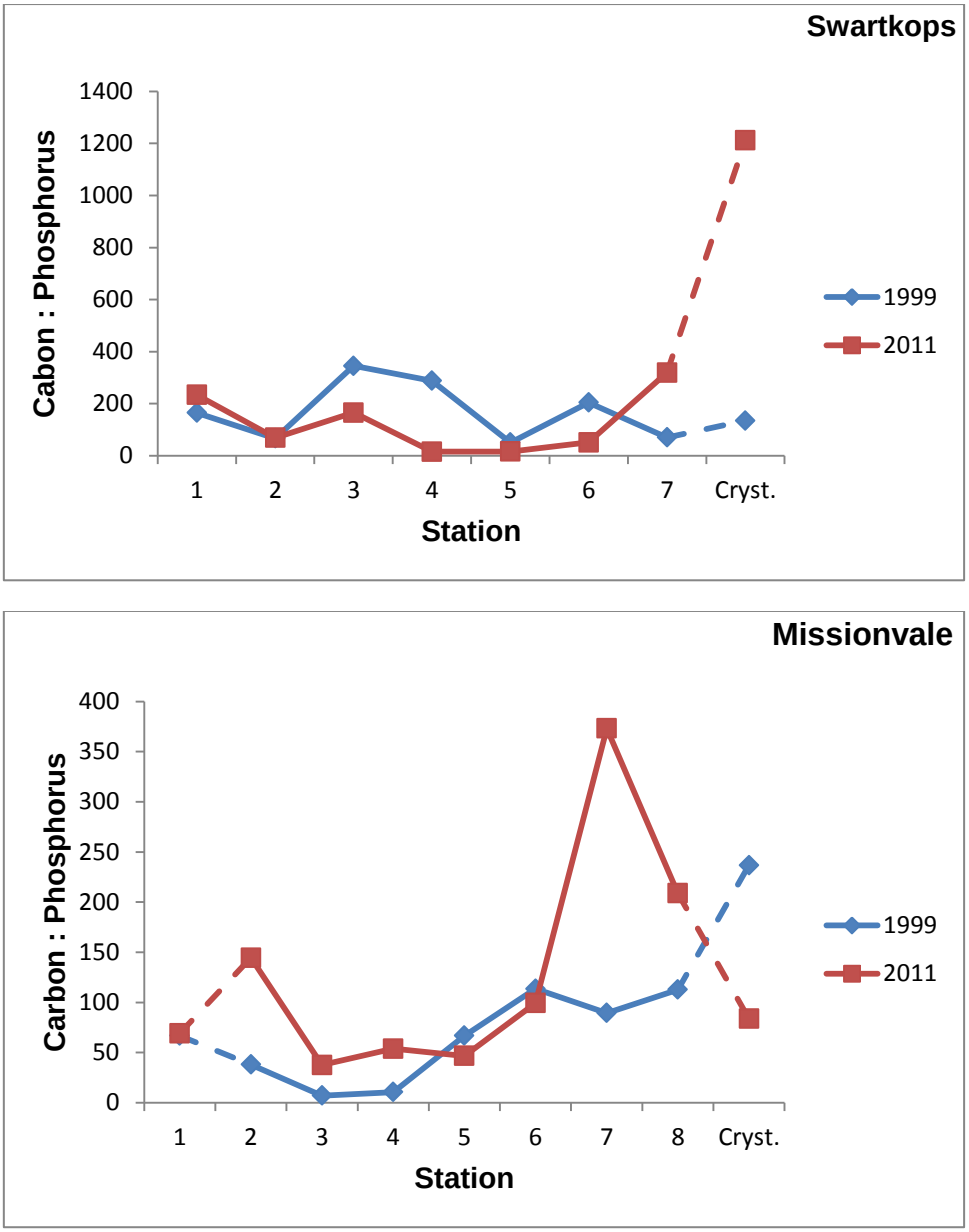


Figure 15: Particulate C: P ratios of the Swartkops and Missionvale salinas.

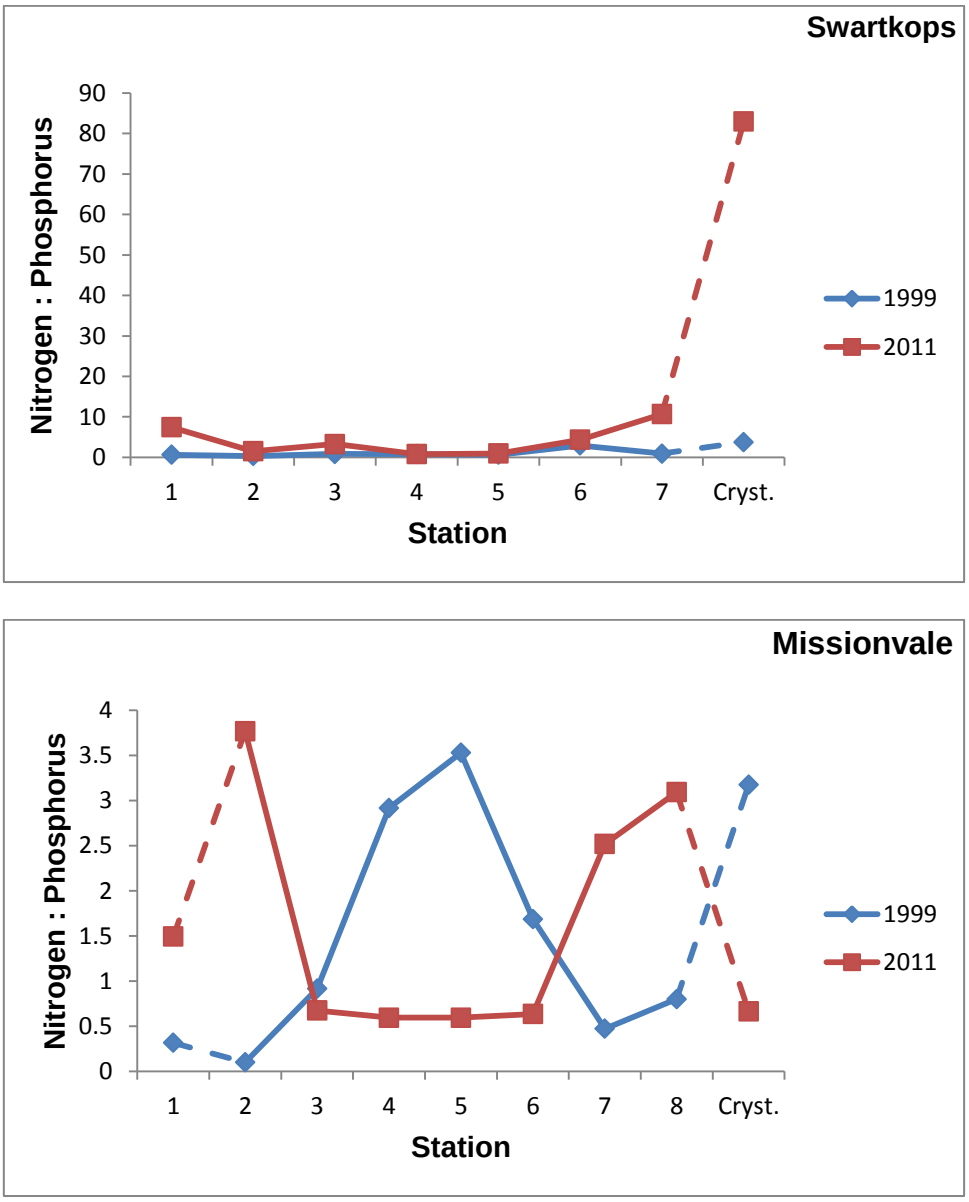


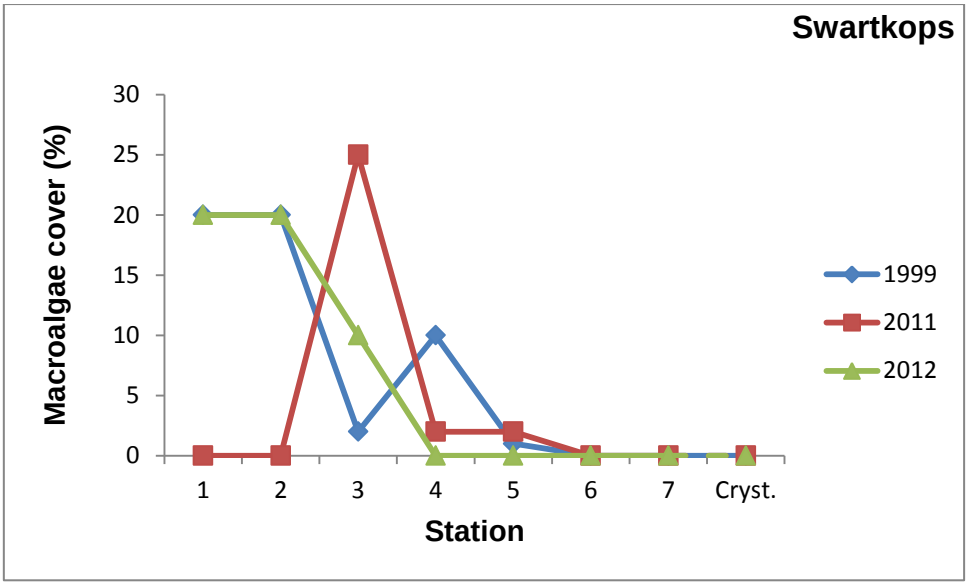
Figure 16: Particulate N: P ratios of the Swartkops and Missionvale salinas.

4.6 Macroalgae

Macroalgae were found in ponds below 150 psu (Figure 17) with the biomass (estimated as percentage cover) being similar in all three years (Figure 16; Swartkops: 1999&2011 – t = 0.108, d.f. = 7, p >0.05; 1999&2012 – t = -0.568, d.f. = 7, p >0.05; 2011&2012 – t = -1.036, d.f. = 7, p >0.05; Missionvale: 1999&2011 – t = 0.561, d.f. = 7, p >0.05; 1999&2012 – t = 0.801, d.f. = 7, p >0.05; 2011&2012 – t = -0.361, d.f. = 7, p >0.05)

4.7 Zooplankton

Artemia salina numbers were negligible in both salinas in 1999 as the maximum number found was 10 *Artemia* per litre (Figure 18). A high abundance of brine shrimp was found at both Swartkops and Missionvale in 2011 and 2012 in ponds were the salinity ranged from 70 psu to 180 psu (Figure 18). The abundance of brine shrimps did not change in 2012 compared to 2011 (Swartkops: t = -0.398, d.f. = 7, p > 0.05; Missionvale: t = -1.058, d.f. = 7, p > 0.05).



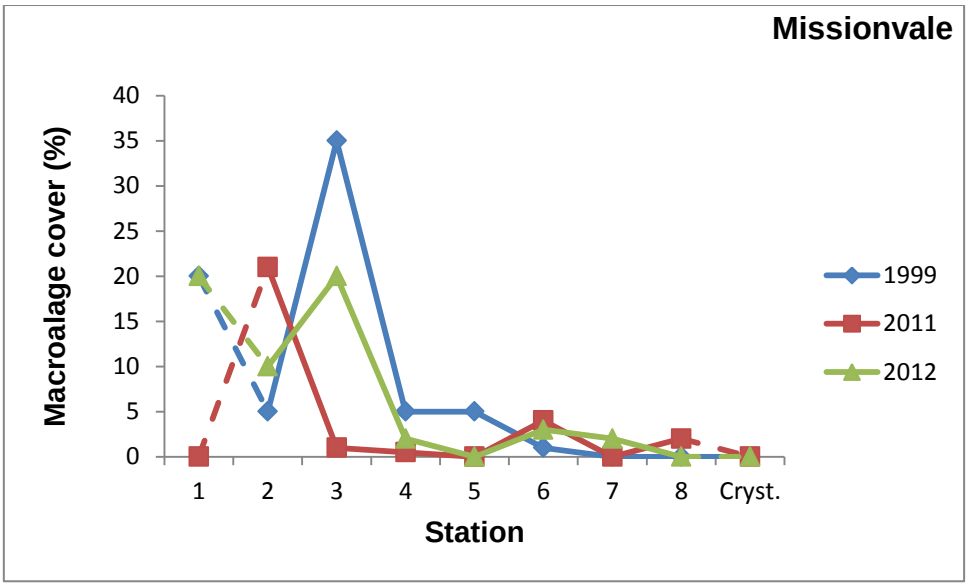


Figure 17: Percentage macroalgal cover of the Swartkops and Missionvale salinas.

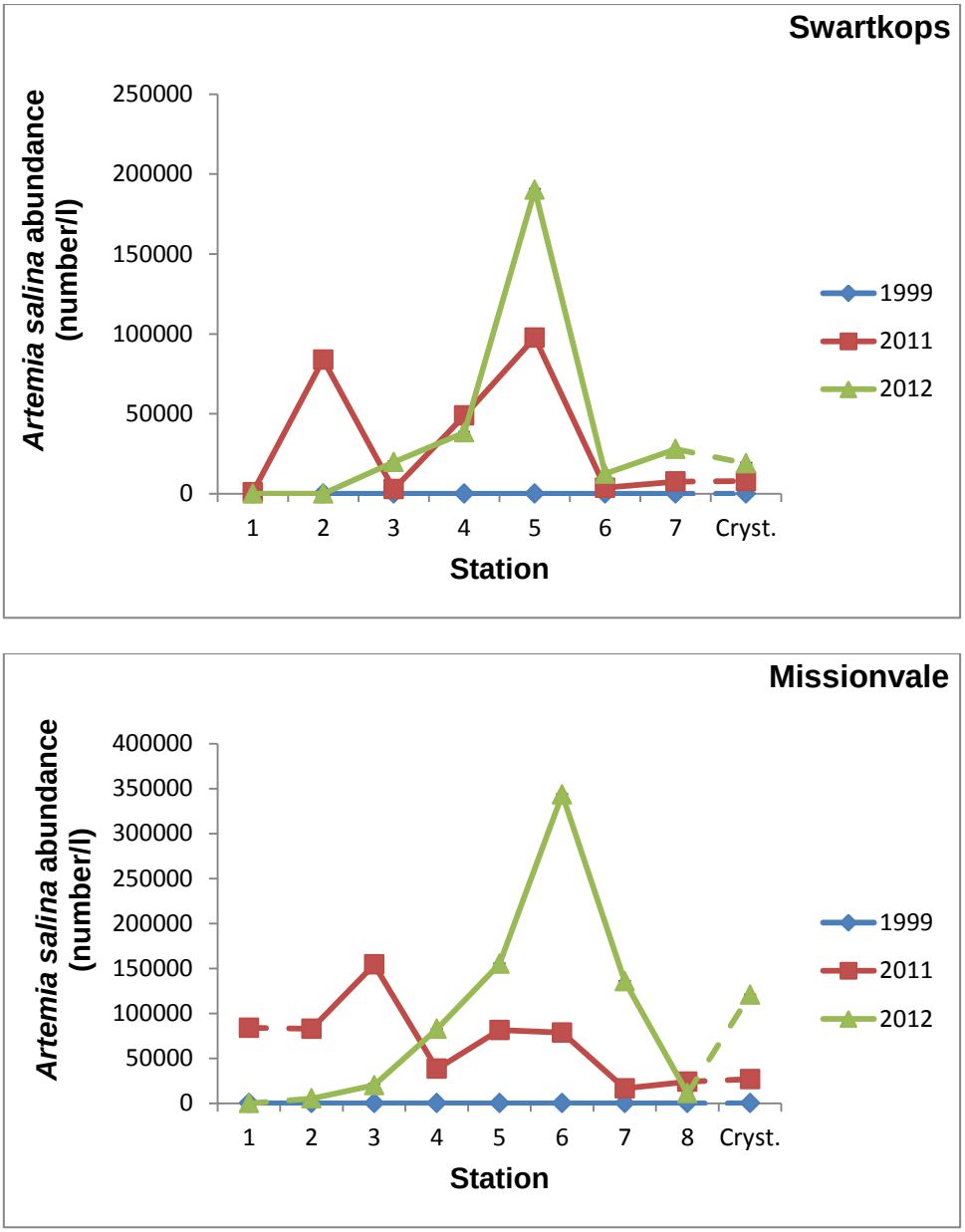


Figure 18: The abundance of *Artemia salina* in the Swartkops and Missionvale salinas (n = 3, vertical bars represent ± 1 S.E.)

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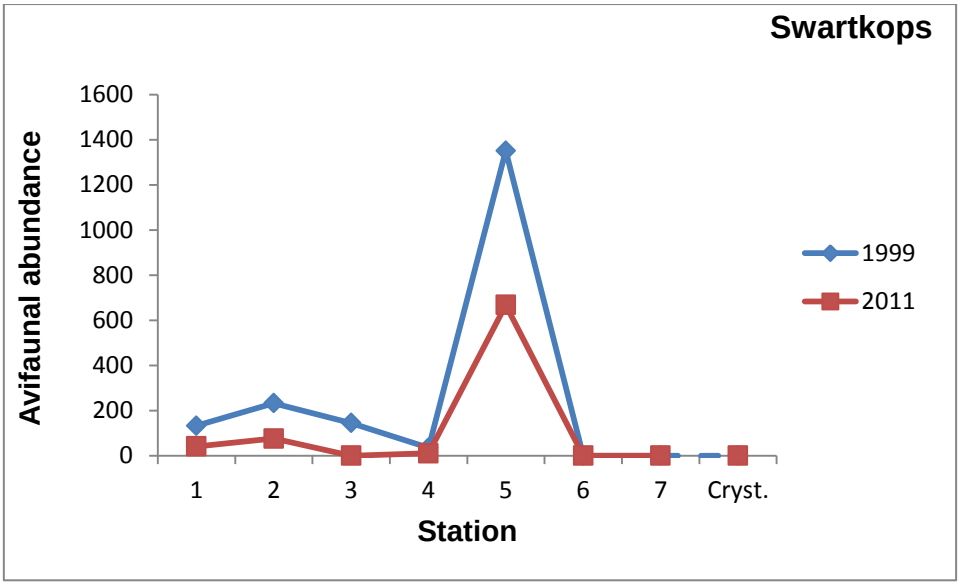
4.8 Avifauna

Avifauna in 1999 and 2011 in the Swartkops salina was limited to the initial evaporation ponds, but birds were found throughout the Missionvale salina (Figure 19). In the Swartkops salina in 1999 the avifaunal abundance (Figure 19) was greatest at station 5 and the species richness was greatest at station 1, while station 6 in the Missionvale salina had the highest abundance (Figure 19) and station 2 had the highest species richness (Figure 20). In the Swartkops salina in 2011 the avifaunal abundance (Figure 19) and species richness (Figure 19) were greatest at station 5, while station 4 in the Missionvale salina had the highest abundance (Figure 19) and species richness (Figure 20).

More birds were found on the lower salinity ponds in the Swartkops and Missionvale salinas, with the exception of station 5 of the Swartkops salina in 1999 and 2011, however there were no significant correlations between bird numbers and salinity at the Swartkops and Missionvale salinas ($r = -0.372$, $t = -0.982$, $p > 0.05$; $r = -0.167$, $t = -0.414$, $p > 0.05$ respectively) . At this station there was substantially more birds compared to the other stations of the Swartkops salina (e.g. Pond 4; $z = 2.31$, $n = 8$, $p < 0.05$). The bird species found at this station were predominantly from the families Phoenicopteridae, the lesser flamingo – most commonly associated with salinas and Podicipedidae, the black-necked Grebe (Appendix 1). There was no difference in the number of birds found on the Swartkops salina compared to the Missionvale salina.



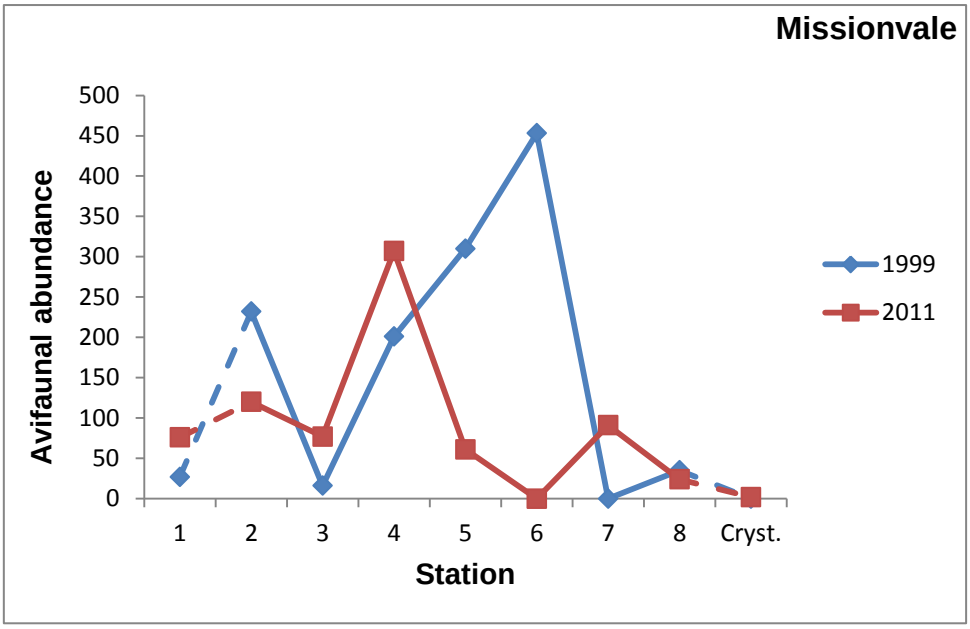


Figure 19: Avifaunal abundance (birds per pond) in the Swartkops and Missionvale salinas in 1999 and 2011.

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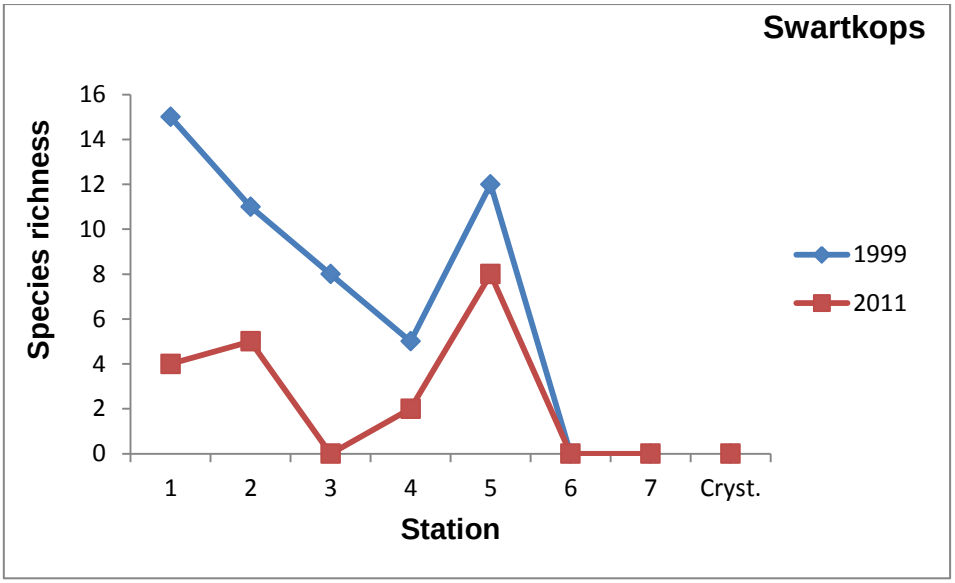

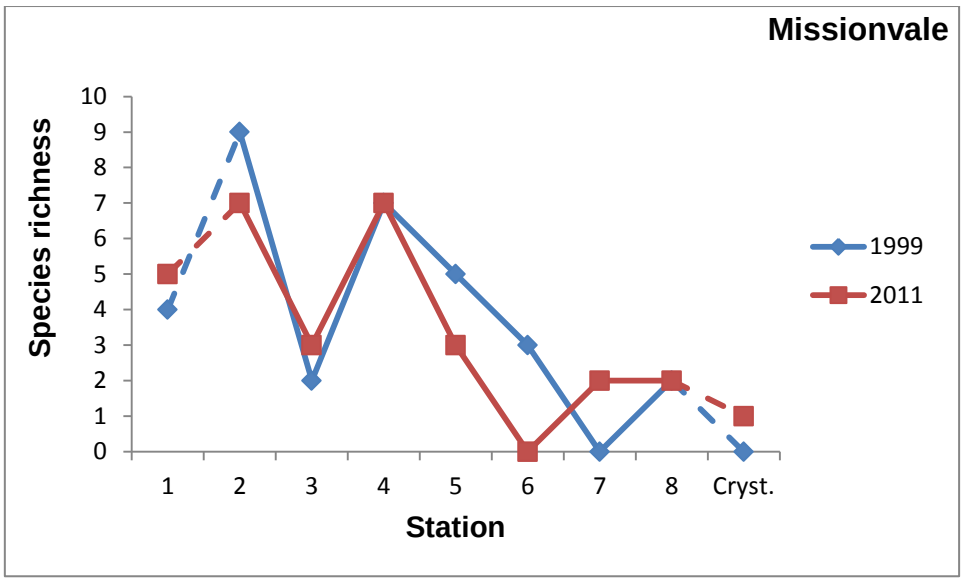

Figure 20: The avifaunal species richness (species per pond) in the Swartkops and Missionvale salinas in 1999 and 2011.

4.9 Impact of the environment on the biota

Phytoplankton biomass was negatively affected by the ammonium concentration in the Swartkops salina in 1999 and 2011 (significant correlations between NH_4^+ and chlorophyll *a* concentrations Tables 5 and 6). The availability of soluble reactive phosphorus to the phytoplankton in the Swartkops salina in 2011 and 2012 positively influenced biomass production (significant correlations between PO_4^{2-} and chlorophyll *a* concentrations Tables 6 and 7). Salinity negatively affects the growth of algae (phytoplankton and macroalgae). (Significant correlation between salinity and chlorophyll *a* concentrations Table7 and significant correlations between salinity and macroalgae Tables 5, 7, 8 and 10). Ammonium concentrations in the Swartkops salina in 2012 negatively affected macroalgal growth (significant correlation between NH_4^+ and macroalgae Table 7). Macroalgae prohibits phytoplankton from flourishing as they compete for the same resources (significant correlation between chlorophyll *a* and macroalgae Table 7). The ammonium concentration in 2012 is significantly correlated to brine shrimp indicating that their feeding and excretion controls the concentration (Table 7). There were no significant correlation found in 2012 at Missionvale between the environment and the biota (Table 9). Salinity had an overall negative effect on the phytoplankton biomass and the macroalgae (significant negative correlations between salinity and phytoplankton and salinity and macroalgae Table 11) indicating that the higher the salinity the less productive the algae are. Ammonium concentration had an overall negative effect on phytoplankton and a positive effect on the brine shrimp indicating that brine shrimp control the amount of ammonium present in the brine and controls phytoplankton biomass (significant negative correlation between ammonium and phytoplankton and significant positive correlation between ammonium and brine shrimp Table 11). Macroalgae were significantly positively correlated to soluble reactive phosphorus (Table 11). Birds added phosphorus to the water and since macroalgae are found in the lower salinity pond and so are the birds (Du Toit, 2001) this allows macroalgae to bloom. Phytoplankton biomass is negatively affected by brine shrimp (significant negative correlation, Table 11). Phytoplankton is the main food source for brine shrimp, therefore it is expected that there would be a negative relationship.

Table 5: Spearman's rank correlations between water quality and biota of Swartkops salina in **1999.** Values in bold are significant at p < 0.05, for these the p-value is given in parentheses.

	Phytoplankton biomass	**Macroalgal biomass (% cover)**	***Artemia salina* (numbers/l)**
Salinity	-0.43	**-0.839 (>0.01)**	-
NH_4^+	**-0.79 (0.02)**	-0.54	-
NO_3^{2-}	-0.072	**-0.847 (>0.01)**	-
PO_4^{2-}	0.311	0.209	-
Phytoplankton biomass	-	0.395	-
Macroalgae	0.395	-	-
Brine shrimp	-	-	-

Table 6: Spearman's rank correlations between water quality and biota of Swartkops salina in **2011**. Values in bold are significant at p < 0.05, for these the p-value is given in parentheses.

	Phytoplankton biomass (µg chl *a* l^{-1})	**Macroalgal biomass (% cover)**	***Artemia salina* (numbers/l)**
Salinity	-0.524	-0.674	-0.143
NH_4^+	**-0.714 (0.046)**	-0.481	0
NO_3^{2-}	-0.452	-0.674	-0.143
PO_4^{2-}	**0.809 (0.015)**	0.206	0.167
Phytoplankton biomass	-	-0.179	-0.19
Macroalgae	-0.179	-	0.275
Brine shrimp	-0.19	0.275	-

Table 7: Spearman's rank correlations between water quality and biota of Swartkops salina in **2012**. Values in bold are significant at $p < 0.05$, for these the p-value is given in parentheses.

	Phytoplankton biomass	**Macroalgal biomass (% cover)**	***Artemia salina* (numbers/l)**
Salinity	**-0.929 (>0.01)**	**-0.866 (>0.01)**	0.419
NH_4^+	-0.667	**-0.825 (0.012)**	**0.814 (0.014)**
NO_3^{2-}	-0.405	-0.082	-0.239
PO_4^{2-}	**0.738 (0.037)**	0.659	-0.168
Phytoplankton biomass	-	**0.866 (>0.01)**	-0.635
Macroalgae	**0.866 (>0.01)**	-	-0.705
Brine shrimp	-0.635	-0.705	-

Table 8: Spearman's rank correlations between water quality and biota of Missionvale salina in **1999**. Values in bold are significant at $p < 0.05$, for these the p-value is given in parentheses.

	Phytoplankton biomass	**Macroalgal biomass (% cover)**	***Artemia salina* (numbers/l)**
Salinity	0.0714	**-0.901 (>0.01)**	-
NH_4^+	0.0714	-0.05	-
NO_3^{2-}	-0.048	**-0.801 (0.017)**	-
PO_4^{2-}	-0.253	0.557	-
Phytoplankton biomass	-	0.05	-
Macroalgae	0.05	-	-
Brine shrimp	-	-	-

Table 9: Spearman's rank correlations between water quality and biota of Missionvale salina in **2011**. Values in bold are significant at $p < 0.05$, for these the p-value is given in parentheses.

	Phytoplankton biomass	**Macroalgal biomass (% cover)**	***Artemia salina* (numbers/l)**
Salinity	-0.095	-0.317	-0.69
NH_4^+	-0.476	-0.048	-0.5
NO_3^{2-}	-0.238	-0.048	-0.285
PO_4^{2-}	0.381	0.609	0.619
Phytoplankton biomass	-	0.244	-0.042
Macroalgae	0.244	-	0.415
Brine shrimp	-0.048	0.415	-

Table 10: Spearman's rank correlations between water quality and biota of Missionvale salina in **2012**. Values in bold are significant at $p < 0.05$, for these the p-value is given in parentheses.

	Phytoplankton biomass	**Macroalgal biomass (% cover)**	***Artemia salina* (numbers/l)**
Salinity	-0.262	**-0.712 (0.048)**	0.167
NH_4^+	-0.309	-0.405	**0.857 (>0.01)**
NO_3^{2-}	-0.643	-0.467	0.691
PO_4^{2-}	-0.524	-0.037	**0.786 (0.021)**
Phytoplankton biomass	-	0.331	-0.524
Macroalgae	0.331	-	-0.246
Brine shrimp	-0.524	-0.246	-

Table 11: Spearman's rank correlations between water quality and biota of both the Swartkops and Missionvale salinas over twelve years. Values in bold are significant at $p < 0.05$, for these the p-value is given in parentheses.

	Phytoplankton biomass	**Macroalgal biomass (% cover)**	***Artemia salina* (numbers/l)**
Salinity	**-0.279 (0.055)**	**-0.779 (>0.001)**	0.227
NH_4^+	**-0.534 (>0.001)**	-0.232	**0.459 (0.001)**
NO_3^{2-}	-0.296	**-0.285 (0.049)**	**0.501 (>0.001)**
PO_4^{2-}	-0.026	**0.306 (0.034)**	0.122
Phytoplankton biomass		0.199	**-0.384 (0.007)**
Macroalgae	0.199		-0.231
Brine shrimp	**-0.384 (0.007)**	-0.231	

4.10 Cultures

Of the genera isolated from the Swartkops salina six (Plates 2 to 9) produced lipids and grew rapidly. All six species were found in the mid- to high salinity (Ca. 60 – 300 psu) stations. The species found were (Table):

Table 12: Species isolated from the Swartkops salina

Genus	**specific epithet**	**Authority**	**Isolation station**	**Culture media**
Nitzschia	*acicularis*	(Kützing) W.Smith	S2, 3, 5	PES & PES +si
Trachyneis	*aspera*	(Erhenberg) Cleve	S5	PES + si
Navicula	*lanceolata*	Erhenberg	S7	PES
Halamphora	*coffeaeformis*	(Agardh) Levkov	S Crystallizer	PES
Navicula	sp.	Bory de Saint-Vincent	S7	PES

Plate 2: *Nitzschia acicularis* photographed using brightfield light microscopy (top) and under UV florescence (bottom) (lipid droplets stained yellow).

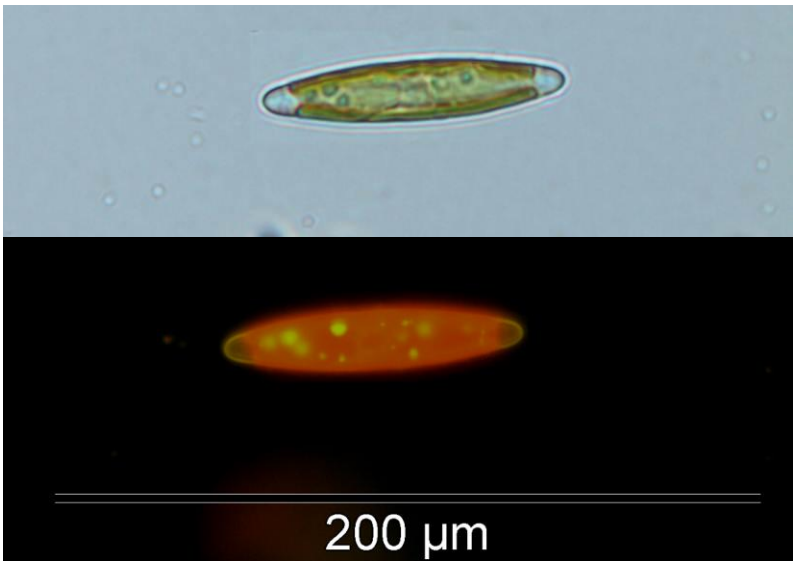

Plate 3: *Trachyneis aspera* photographed using brightfield light microscopy and under UV florescence (lipid droplets stained yellow).

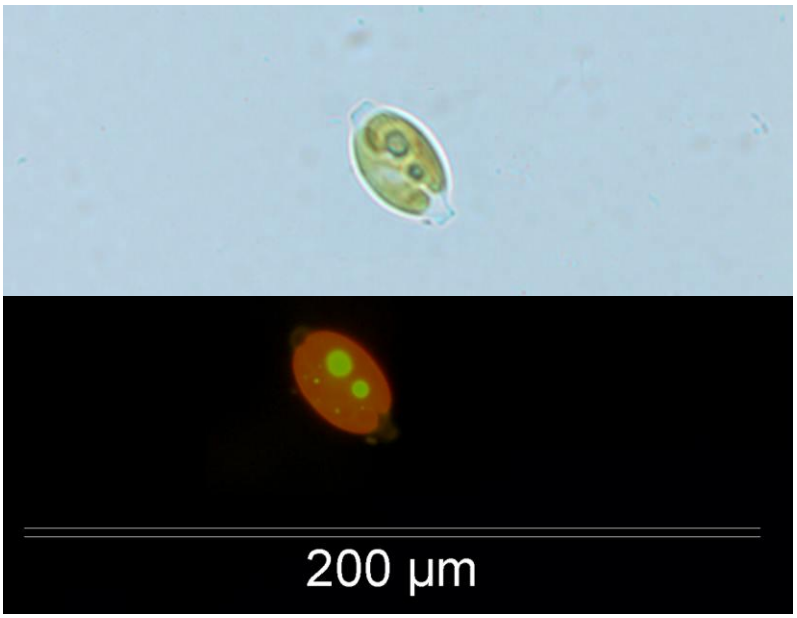

Plate 4: *Halamphora coffeaeformis* photographed using brightfield light microscopy and under UV florescence (lipid droplets stained yellow).

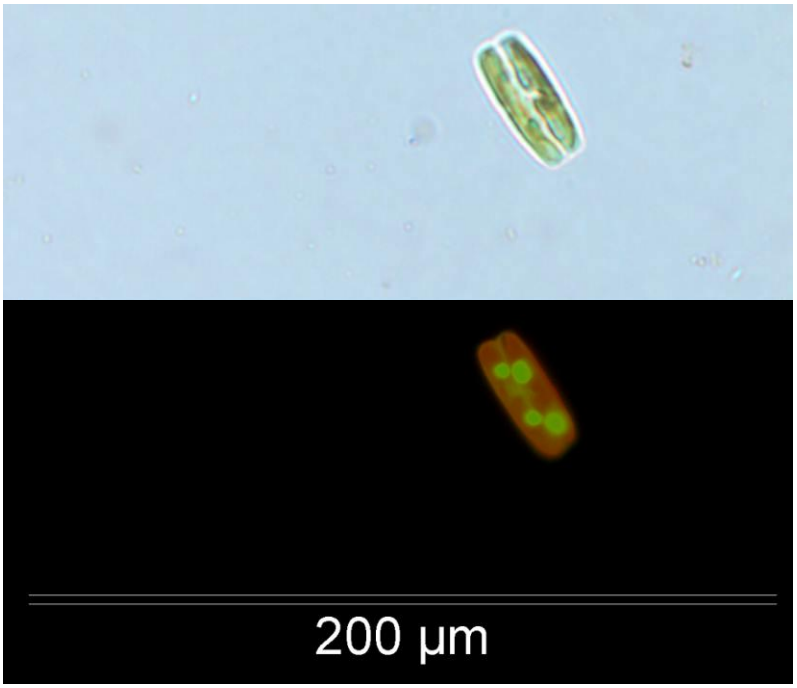

Plate 5: *Navicula* sp. photographed using brightfield light microscopy and under UV florescence (lipid droplets stained yellow).

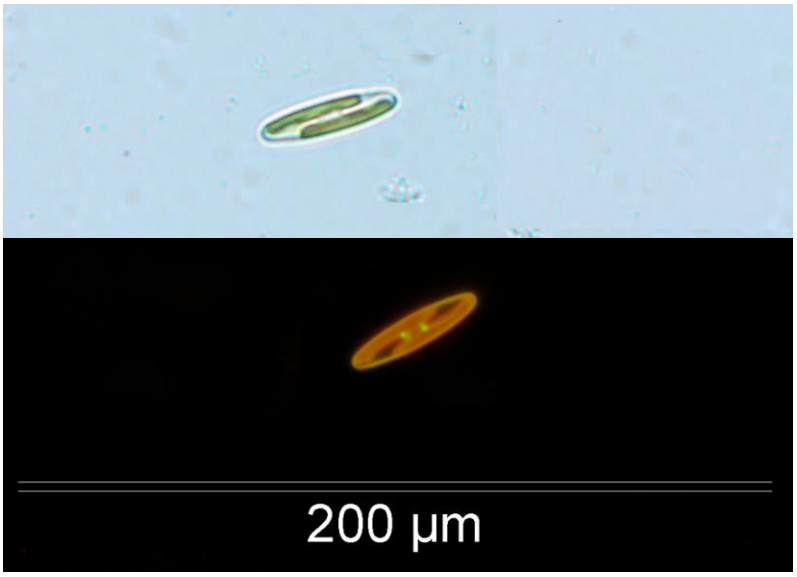

Plate 6: *Navicula lanceolata* photographed using brightfield light microscopy and under UV florescence (lipid droplets stained yellow).

Nitzschia acicularis (Plate 2) was isolated from the phytoplankton of stations 2 and 5 and from the benthos at station 3. *Trachyneis aspera* (Plate 3) was isolated from the phytoplankton of station 5. These two species grew fastest at a salinity of 70 psu (Figures 21 and 22). The growth rate of *Nitzschia acicularis* isolated from the benthos at station 3 at 70 psu was significantly higher than growth at 100 psu ($t = 3.17$, d.f. = 5, $p<0.05$) and 150 psu ($t = 2.9$, d.f. = 5, $p<0.05$).

Nitzschia acicularis from station 2 (a low salinity pond) grew fastest at a salinity of 52 psu (Figure 21). However it was only significantly higher than growth at 70 psu ($t = 2.92$, d.f. = 5, $p<0.05$). The *Nitzschia acicularis* found in station 5 (a mid-salinity pond) grew best at 70 psu (Figure 21), which is significantly higher than the growth at 150 psu ($t = 2.7$, d.f. = 5, $p<0.05$). There were two species growing equally in station 7 (*Navicula* sp. and *Navicula lanceolata*, with both growing fastest at 100 psu (Figure 22). This growth was significantly higher than the species' growth at 35 psu ($t = 3.23$, d.f. = 5, $p<0.05$), 52 psu ($t = 4.59$, d.f. = 5, $p<0.01$), 70 psu ($t = 4.41$, d.f. = 5, $p<0.01$), and 150 psu ($t = 3.38$, d.f. = 5, $p<0.01$). The *Halamphora coffeaeformis* found in the crystallizer station (highest salinity) grew the fastest at 52 psu (Figure 22), which is significantly higher than growth at 35 psu ($t = 3.08$, d.f. = 5, $p <0.01$), and 150 psu ($t = 2.72$, d.f. = 5, $p < 0.01$).

Nitzschia acicularis cultivated in Provosoli Enriched Seawater (PES) + si from station 3 grew better than the *Trachyneis aspera* at salinity of 52 psu ($t = 4.22$, d.f. = 5, $p < 0.01$), 70 psu ($t = 2.67$, d.f. = 5, $p < 0.05$) and 150 psu ($t = 3.5$, d.f. = 5, $p < 0.05$). *Halamphora coffeaeformis* cultivated in PES from the crystallizer station grew better than the *Nitzschia acicularis* cultivated in PES from station 5 at salinity 52 psu ($t = 3$, d.f. = 5, $p < 0.05$), 100 psu ($t = 4.14$, d.f. = 5, $p < 0.01$) and 150 psu ($t = 3.2$,d.f.=5,$p<0.05$).

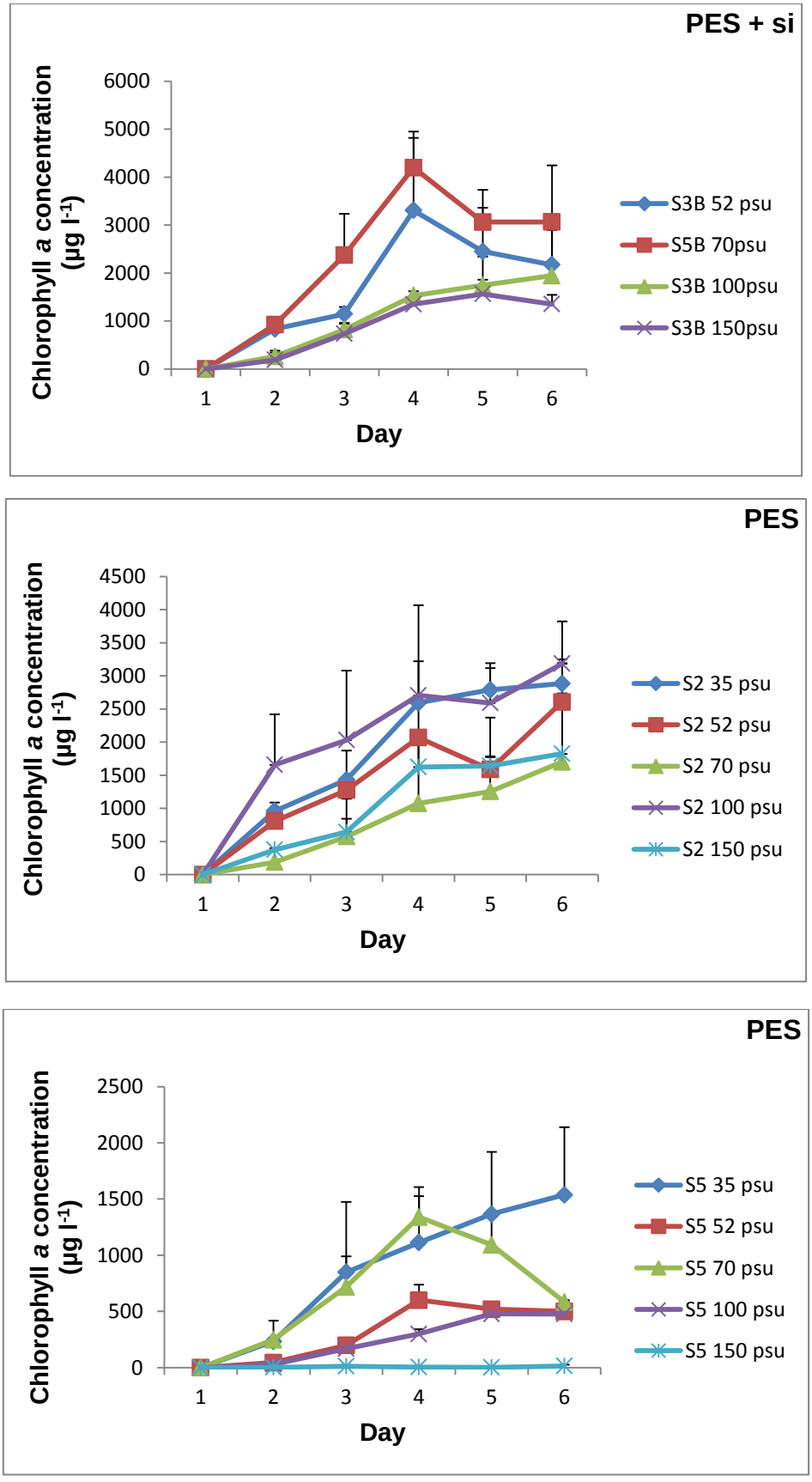

Figure 21: Chlorophyll *a* concentration of cultures of *Nitzschia acicularis* isolated from stations 2, 3B (benthic), and 5 grown in Provosoli Enriched Seawater with silicon (PES + si) and Provosoli Enriched Seawater (PES) over six days.

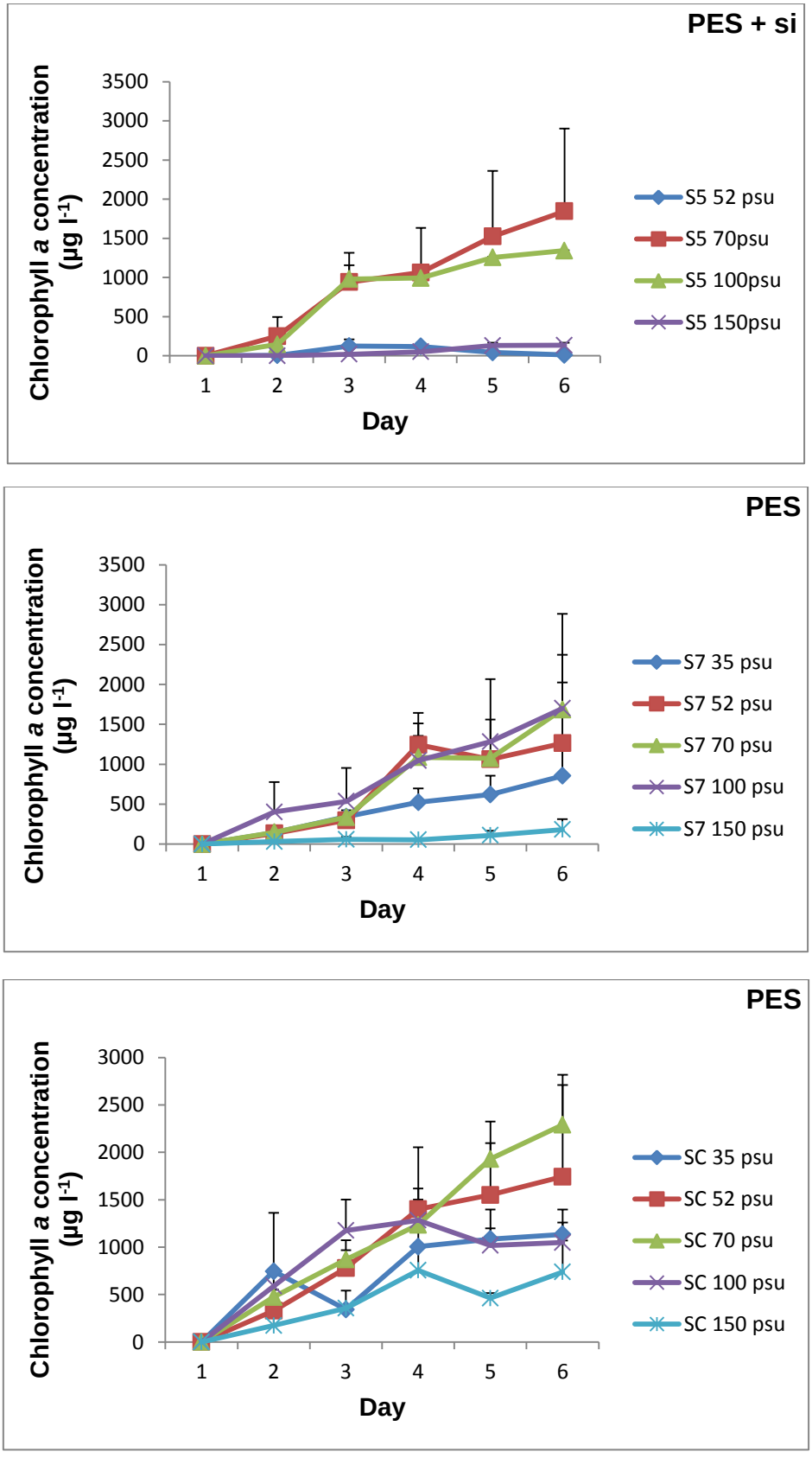

Figure 22: Chlorophyll *a* concentration of cultures of *Trachyneis aspera* isolated from station 5 grown in PES + si, *Navicula lanceolata* and *Navicula* sp. isolated from station 7 and *Halamphora coffeaeformis* isolated from the crystallizer station grown in PES over six days.

The *Nitzschia acicularis* found in the benthos of station 3 produced the most lipid at a salinity of 150 psu (Figure 23); however it was only significantly higher than the amount of lipid produced at 100 psu ($t = 2.79$, d.f. = 9, $p < 0.05$). *Trachyneis aspera* produced the highest amount of lipid at 100 psu, and this amount of lipid was significantly more than that was produced at 150 psu ($t = 2.38$, d.f. = 9, $p < 0.05$). *Nitzschia acicularis*, *Navicula* sp., and *Navicula lanceolata* grew best in the PES medium producing the most lipid at a salinity of 70 psu (Figure 23). The lipid produced by *Navicula* sp. at 70 psu was significantly more than the lipid produced at 35 psu ($t = 3.58$, d.f. = 9, $p < 0.01$), 52 psu ($t = 4.95$, d.f. = 9, $p <0.01$) and 100 psu ($t = 4.48$, d.f. = 9, $p < 0.01$). *Navicula lanceolata* produced significantly more lipid at 70 psu compared to 52 psu ($t = 4.54$, d.f. = 9, $p < 0.01$), 100 psu ($t = 3.67$, d.f. = 9, $p < 0.01$), and 150 psu ($t = 4.28$, d.f. = 9, $p < 0.01$). *Halamphora coffeaeformis* produced the most lipid at a salinity of 100 psu; however this was not significantly higher than the amount of lipid produced at any other salinity.

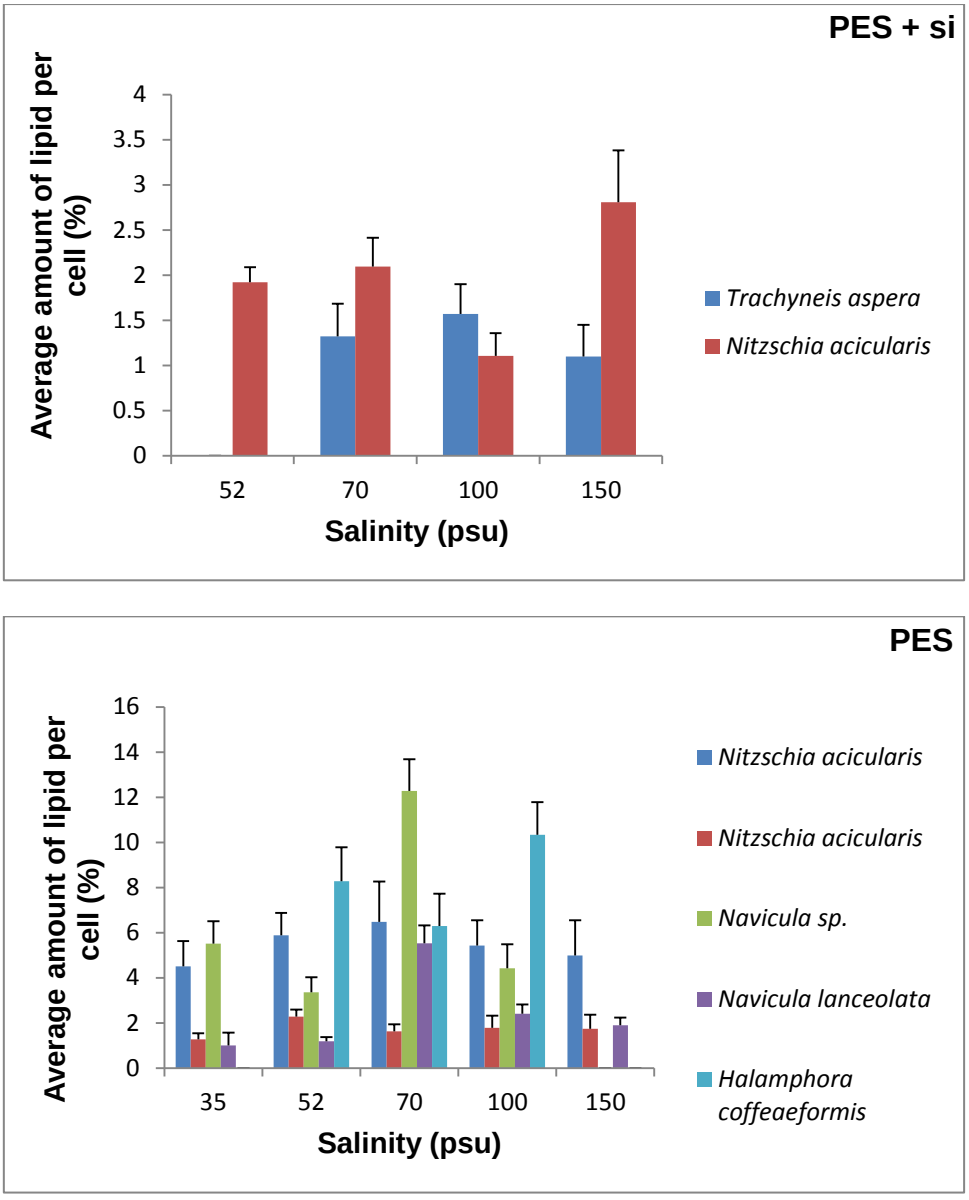

Figure 23: Average percentage of lipid per cell for species grown in PES with silicon and without silicon.

5. Discussion

South African salinas are highly productive ecosystems. The Swartkops River Estuary is the main source of water to both the Swartkops and Missionvale salinas. This river and estuary is surrounded by industries and large informal settlements with little or no sewage and refuse disposal facilities. The result is a steadily increasing level of pollution in the estuary (Binning, 1999, Mackay, 1994) with a consequent increase in nutrient loading via the inlet water. Nutrient loading into the salt pans is often due to rainfall runoff as well as the occasional sewage spillage depositing high-nutrient water into the ponds (Mackay, 1994).

The salinity profile of a salina is the most important abiotic factor, as this not only controls the biotic components in each pond but also determines the quality and quantity of salt produced. The ponds at Swartkops and Missionvale are built so that excess freshwater flows out of the ponds and if the quantity of freshwater entering the ponds is too great the excess is pumped out. When there is high rainfall (as experienced in 2011 and 2012) no seawater is pumped into the system, in order to first stabilise the ponds. To maintain biological stability in any salina the salinity in each pond must be kept within a narrow range (Davis and Giordano, 1996). By regulating the flow rates and allowing any rainwater to flow out of the crystallizer ponds large salinity fluctuations can be avoided (Davis and Giordano, 1996).

A study by Mackay (1994) on the Swartkops Estuary found that the most significant threat the estuary was facing was urban runoff. The inorganic nutrient concentrations for both the Swartkops and Missionvale salinas were particularly high in 2011 and 2012. Inorganic nitrogen concentration in the Swartkops salina in 2012 and in the Missionvale salina in 2011 and 2012 was more than double the recommended 25 µM (Davis and Giordano, 1996). This is possibly due to nutrients being washed into the ponds or into the source water which feeds the ponds, as prior to the sampling there were heavy rains increasing urban runoff. Nutrient concentrations vary greatly in solar saltworks: Ammonium concentrations of up to 700 µM (Jones et al., 1981) have been reported, while nitrate in excess of 40 µM have been found (Du Toit, 2001), indicating that salinas can function at high nutrient inputs.

The ammonium concentrations of the Swartkops salina in 1999 and 2011 fell within the recommended 15 µM (Davis and Giordano, 1996), with the exception of the crystallizer pond in 2011. This could be partially due to the high number of brine shrimp found in the Swartkops crystallizer. The 2012 ammonium concentrations were much higher than the recommendation possibly due to both salinas having exceptionally high brine shrimp numbers. Ammonium is excreted in the faeces of the brine shrimp (Colvin and Brand, 1977), therefore if there is a high number of brine shrimp there will be a higher concentration of ammonium and thus a significant negative effect on the phytoplankton. Phytoplankton is able

to adapt to its surrounding environments in order to survive; for example Thakur and Kumar (1999) removed ammonium from the surrounding medium using immobilised *Dunaliella salina* cells.

Nitrate concentrations were, however, correlated to salinity. Camargo et al. (2005) found that nitrate was released during the decomposition of the numerous organisms that can no longer tolerate the increasing salinity. Increased runoff during the rainy season often provides substantial nitrogen loading to lagoons, estuaries and coastal waters (Abreu et al., 1995). The nitrate concentrations in both salinas in 2011 and 2012 were more than double the recommended 10 µM (Davis and Giordano, 1996). These inorganic nitrogen concentrations for Swartkops and Missionvale salinas are still not as high as the 700 µM that Jones et al. (1981) recorded for a South Australia saltworks.

Soluble reactive phosphorus is often the growth-limiting nutrient for benthic organisms, as the microphytobenthos remove soluble reactive phosphorus from the water (Davis, 1999). The recommended phosphate concentration for salinas is 1 µM (Davis and Giordano, 1996). The phosphates were most probably deposited in abundance by birds (Du Toit, 2001); however avifaunal abundance was not significantly correlated with the soluble reactive phosphorus concentrations in the water in 2011. Jones et al. (1981) recorded excessively high phosphate concentrations (approximately 16 µM) and this can be detrimental to the biological functioning of a salina and salt production (Du Toit, 2001).

Water column phytoplankton are affected by salinity, light intensity and nutrient concentrations (Rijstenbil, 1987), with salinity being one of the most variable abiotic factors (Remane, 1955, 1958, Gasiunaite, 2000). Salinas however increase in salinity throughout the system creating an environment where the biota has to adapt to an increase in salinity but not a decrease. The specific growth rates of the phytoplankters differ depending on the salinity fluctuations (Rijstenbil, 1987). The Swartkops and Missionvale salinas showed the required replacement of dominant biota as the salinity increased as found by Davis (1990). The early ponds were dominated by algae (macroalgae and phytoplankton; Figures 10 and 17). A common phenomenon in estuarine (Fong et al., 1996) and hypersaline (Difford, 2008) habitats is for the macroalgae in the earlier ponds to out-compete the phytoplankton. A “bloom-and-bust” cycle in solar saltworks was postulated by Difford (2008). It occurs when the phytoplankton population in a salina collapses due to macroalgal blooms in the ponds. The macroalgae float on the surface of the water shading the phytoplankton below, and resulting phytoplankton death and decomposition (Difford, 2008). This decay of the phytoplankton biomass leads to an increase of nutrients into the water column causing macroalgal blooms again (Difford, 2008).

Studies by Britten and Johnson (1987), Campbell et al. (2001) and Du Toit (2001) found almost linear increases in the concentrations of particulate organic matter with salinity. It is characteristic for salinas to have an increase in organic matter levels with an increase in salinity (Du Toit, 2001); however this was not the case in this study. Most organic matter levels fluctuated greatly from station to station, some with a high initial concentration and others with a high final concentration. A high organic concentration in the crystallizer ponds is not good for salt production as it affects the size and quality of the salt crystals.

Phosphorous limitation and nitrogen limitation can be expressed in marine ecosystems that have been heavily loaded with nutrients for urban areas, with these limitations being altered with seasonality (Conley et al., 2009). Algal blooms are a key indication of eutrophication in marine ecosystems (Petrovic, 1998, Conley et al., 2009). Nixon (1995) stated eutrophication must be linked to the organic nutrient input in marine ecosystems rather than the inorganic nutrients. However, in the Swartkops and Missionvale salinas both the organic and inorganic nutrients are high affecting the biota of the salinas. The carbon in a marine ecosystem either comes for the primary producers or organic input from outside the system (Nixon, 1995). In the current study organic carbon comes from within the salina, from the decomposing algae. The trophic status of a system can be determined by the amount of organic carbon found in the system (Nixon, 1995). If primary production in a system is continually increasing, then it can be said that eutrophication is occurring in that system (Nixon, 1995). In this case the organic carbon concentration is comprised of phytoplankton (significant correlations Table 4), however the phytoplankton biomass was higher in 2011, which would indicate that eutrophication was occurring, but in 2012 the phytoplankton biomass was less. Therefore according to Nixon (1995) eutrophication should be decreasing, but the organic carbon in 2012 is still high. This supports hypothesis 1 (Eutrophication remains a problem at both Swartkops and Missionvale salt pans). The inorganic nutrients in the system also remained high.

Irrespective of the nutrient concentrations, the macroalgae are the first to be affected by the increasing salinity. Macroalgae cannot survive salinities above 70 psu in the long term as shown by the low percentages of macroalgae present throughout the system. As found in decline above 70 psu (Difford, 2008). Although the macroalgae dominated the primary production in the low salinity ponds, it was not carried through to the higher salinity ponds.

Brine shrimp populations naturally occur in salt lakes, with their salinity tolerance ranges generally between 100 psu to 250 psu. The mid-salinity stations of both Swartkops and Missionvale salinas show high abundance of *Artemia salina* in 2011 and 2012, with a maximum of ten *Artemia salina* per litre being present in 1999. The *Artemia salina* population

dominated the zooplankton population in both systems with the mean abundance at Swartkops being 28 144 l^{-1}, and 34 178 l^{-1} and at Missionvale 62 838 l^{-1}, and 109 188 l^{-1} for 2011 and 2012 respectively. These values are substantially higher than the 1 l^{-1} Campbell et al. (2001) found in the study of Tankatara salina on the South African east coast. A good *Artemia* population in a salina helps in reducing the organic matter in the system and improves the production of salt (Vanhaecke and Sorgeloos, 1989). This was demonstrated in a salina in China that had excessive accumulation of organic matter (Vanhaecke and Sorgeloos, 1989). When Du Toit (2001) conducted his study the brine shrimp numbers were very low and his recommendation was to introduce a more productive strain into the system. This recommendation was carried out and the system now has a flourishing brine shrimp community throughout the system. The food is sufficient for the brine shrimp to grow and reproduce and the physical variables are satisfactory for their development. Difford (2008) found that brine shrimps occur at salinities greater than 70 psu, but do best at salinities of 120 to 140 psu where they have protection from less halotolerant predators. At around 250 psu *Artemia salina* food sources generally become limiting (Sorgeloos et al., 1991) causing their decline. However, in 2011 and 2012 brine shrimp were found in the crystallizer station, this is not good for salt production, not only as they feed on the halophilic bacteria and microalgae, but they also add nutrients into the salt (Sorgeloos et al., 1991).

Birds favour ponds that are relatively shallow and have a high abundance of food, which then results in greater number of birds and high species diversity (Du Toit, 2001). The brine shrimp are a food source for a variety of birds (Du Toit, 2001). Swartkops and Missionvale salinas provide refuge for the birds from the densely populated surroundings (Du Toit, 2001). A study by Warnock et al. (2002) on the salt pans of San Fransico Bay found that the most dominant species occurring in the salinas were American avocet and Black-bellied plover. The great flamingo, pied avocet and black-headed gull were found to be the most dominant species on a Spanish salina in an undated report by Castro-Nogueira et al. During the summer months the shell ducks, flamingos and avocet species are the only birds that venture into the high salinity pons (Britten and Johnson, 1987). The pre-crystallizer ponds of the Swartkops salina hosted similar filter feeders as well as numerous other species (Du Toit, 2001). These included black-necked grebe, lesser flamingo, Cape teal, and Curlew sandpiper (Appendix 1). Warnock et al. (2002) found that the largest number of birds occurred at a salinity of 140 psu, whereas in this study, in 2011, station 5 at Swartkops had the largest number of birds at a salinity of 120 psu. These hypersaline habitats have favoured the colonisation of avifauna, and provide a resting place for migratory birds; however too many birds in a salina affect the nutrient concentrations, as in the study by Du Toit (2001) where there was significant correlations between the number of birds and

ammonium concentrations. Therefore bird number should be controlled and where birds are unwanted scarecrows should be erected. However, as it would be beneficial to the production of salt to chase the birds away, it would be a great loss to the ecotourism in this area. People travel to Swartkops to see the magnificent bird life and to enjoy them in their natural habitat.

This study showed that marine diatoms have wide salinity tolerances which are consistent with literature (Williams, 1964). Species that grew best at differing salinity were *Nitzschia acicularis* and *Halamphora coffeaeformis*, indicating that these species adapt well to changing environments (nutrient depletion each day and different salinity).
Halamphora coffeaeformis produced the most lipid at 100 psu, thus supporting the second hypothesis which stated that "algae cultured from the high salinity ponds will have a higher lipid yield than those cultured from lower salinity ponds". However this species is best grown at a lower salinity than where it was found before growing it in a higher salinity to produce more lipid. These findings are contrary to others in the literature, for instance Cucchiari et al. (2008) showed that species growing in different geographical positions each have a different salinity preference; meaning that a species found in a high salinity pond will grow better in a higher salinity which was clearly not the case for the *Halamphora coffeaeformis* in this study. Williams (1964) found the *Nitzschia closterium* strain used preferred a salinity of 17 psu, whereas in the present study the *Nitzschia acicularis* grew best at salinity 35 psu, 52 psu and 70 psu.

With their production of lipids and growth doubling every day, the cultivated microalgae have the potential to be a source of biofuel. However, small quantities of nutrients need to be added every week to ensure that the microalgae continue to grow. At this stage it is not economically feasible to start commercial production until further large scale experiments are carried out and the lipids are harvested to determine the yield, therefore leaving hypothesis 3 "Some phytoplankton species will have sufficient lipid content and sufficiently high growth rates to be used for harvesting" unanswered.

5.1 Management Recommendations

There are various alternatives available to improve the functioning of these solar saltworks. These include:

1. Removal of macroalgae

Macroalgae is present in these Salinas because of the high nutrient load providing perfect environmental conditions for it to flourish. It is also a major contributor to the nutrients in the brine. A short term solution would be to remove the dead and dying algae to ensure that the excess nutrients are not carried throughout the system. A long term solution would be to physically remove as much algae as possible periodically, however this could be time consuming and costly. This will benefit the phytoplankton as macroalgae will not provide a shading effect and the temperature of the ponds should increase as the macroalgae will not shade the brine below.

2. Correction of pond depths

Due to the heavy rains over the past two years the pond depths have increased indicating that the evaporation process takes longer. The salinity increases in small increments and takes longer to produce salt crystals. The shallower the ponds the higher the brine temperature therefore the faster the brine is evaporated. In order to accomplish the correct pond depth the fresh water should be pumped out of the ponds. This will also help with removing some of the excess nutrients and ensure that the brine is well mixed.

3. Aquaculture options

External ponds could be built for the culturing of microalgae that can be used as biofuel. However this process is still very experimental. The quantity and quality of oil produced from the microalgae must meet certain standards. It will take time and be fairly costly, but will provide an extra source of income eventually. For *Halamphora coffeaeformis* it is recommend that larger scale experiments should be conducted to grow this species at 52 psu to acquire the maximum biomass and then shock it at 100 psu for a couple of days for maximum lipid production. *Navicula lanceolata* and *Navicula* sp. species from station 7 produced the most lipids at 70 psu and growth increased each day at this salinity. It is recommended that further research should be done on these species to see how they cope on a large scale. It is difficult to compare the amount of lipid produced to other studies as the lipid was not harvested, and therefore the wet weight was not calculated. The third hypothesis which stated that “some phytoplankton species will have sufficient lipid content and sufficiently high growth rates to be used for harvesting” is

therefore not supported by the results presented herein. In order to test this hypothesis further, larger aquaculture experiments need to be carried out so that lipids can be harvested. *Halamphora coffeaeformis* species and *Navicula* sp. species would be the best two to start with as they grow well at different salinities and can produce lipids of up to 10 % of the cell.

References

Abid, O., Sellami-Kammoun, A., Ayadi, H., Drira, Z., Bouain, A., and Aleya, L. 2008. Biochemical adaptation of phytoplankton to salinity and nutrient gradients in a coastal solar saltern, Tunisia. *Estuarine, Coastal and Shelf Science* **80:** 391–400.

Abreu, P.C., Hartmann, C., and Odebrecht, C. 1995. Nutrient-rich Saltwater and its influence on the phytoplankton of the Patos Lagoon Estuary, Southern Brazil. *Estuarine, Coastal and Shelf Science* **40:** 219-229.

Allen, E.J., and Nelson, E.W. 1910. On the artificial culture of marine plankton organisms. *Journal of Marine Biological Assessment* **8:** 421 – 474.

Andersen, R.A., and Kawachi, M. 2005. Traditional Microalgae Isolation Techniques. *In*: Andersen, R. A. [Ed.] *Algal Culturing Techniques.* Academic Press, San Diego, 578 pg.

Andrich, G., Nesti, U., Venturi, F., Zinnai, A., and Fiorentini, R. 2005. Supercritical fluid extraction of bioactive lipids from the microalgae *Nannochloropsis* species. *European Journal of Lipid Science and Technology* **6:** 107:381.

Bate, G.C., and Heelas, B.V. 1975. Studies on the nitrate nutrition of two indigenous Rhodesian grasses. *Journal of Applied Ecology* **12:** 941-952.

Becker, E.W. 1994. Microalgae: Biotechnology and microbiology. Cambridge University Press, 293 pg.

Berges, J.A., Franklin, D.J., and Harrison, P.J. 2001. Evolution of an artificial seawater medium: Improvements in enriched seawater, artificial water over the last two decades. *Journal of Phycology* **37**: 1138-1145.

Binning, K.A. 1999. *An investigation into the relationship between water quality, sediment, and meiofaunal communities of the Swartkops River System*. University of Port Elizabeth, South Africa, MSc dissertation, 147 pg.

Black, C.A. 1965. Methods of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy, Inc. Publisher, Madison.

Bolhuis, H., Poele, E.M., and Rodriguez-Valera, F. 2004. Isolation and cultivation of Walsby's square archaeon. *Environmental Microbiology* **6:** 1287-1291.

Borowitzka, M.A. 2005. Culturing Microalgae in Outdoor Ponds. *In*: Andersen, R. A. [Ed.] *Algal Culturing Techniques.* Academic Press, San Diego, 578 pg.

Bremmer, J.M. 1965. Total nitrogen. *In:* Black, C.A. et al. [Eds.] *Methods of soil Analysis*. American Society of Agronomy Inc., Madison, Wisconsin, USA. Part 2, 1149-1174 pg.

Britten, R.H., and Johnson, R. 1987. An ecological account of a Mediterranean Salina: the Salin de Giraud, Camargue (S. France). *Biological Conservation* ***42*****:** 185-230.

Brzezinski, M.A. 1985. The Si-C-N ratio of marine diatoms: Interspecific variability and the effect of some environmental variables. *Journal of Phycology* **21**: 347-357.

Camargo, J.A., Alonso, A., and Salamanca, A. 2005. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere* **58**: 1255-1267.

Campbell, E.E., and Wooldridge, T. 1994. *Biological Assessment of the Missionvale and Swartkops Solar Saltworks*. Institute for Coastal Research, University of Port Elizabeth, 42 pg.

Campbell, E.E., Davis, J.S. and Wooldridge, Y. 2001. *The biota of newly installed solar saltworks at Tankatara, east of Port Elizabeth*, South Africa. Institute for coastal research, University of Port Elizabeth, 146 pg.

Carpelan, L.H., 1957. Hydrobiology of the Alviso salt ponds. *Ecology* **38:** 376-390.

Castro-Nogueira, H., Lopez-Carrique, A. and Aguilera, P.A. Undated. Salt production in salt pans: a model of sustainable development. *Ecosystems and Sustainable Development,* 73-81pg.

Chen, C.Y., and Durbin, E.G. 1994. Effects of pH on the growth and carbon uptake of marine phytoplankton. *Marine Ecology Progress Series* **109**: 83-94.

Coffaro, G., and Bocci, M. 1997. Resources competition between *Ulva rigida* and *Zostera marina:* a quantitative approach applied to the Lagoon of Venice. *Ecological Modelling* **102**: 81-95.

Coffaro, G., Bocci, M., and Bendoricchio, G. 1997. Application of structural dynamic approach to estimate space variability of primary producers in shallow marine water. *Ecological Modelling* **102**: 97-114.

Coleman, M.U. 2009. Oxygen production and use in benthic mats of solar salt ponds. *Natural Resources and Environmental Issues* **15**: 235-239.

Colvin, L.B., and Brand, C.W. 1977. The Protein Requirement of penaeid shrimp at various life-cycle stages in controlled environment systems. Proceedings of the annual meeting - *World Mariculture Society* **8:** 821-840.

Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., Lancelot, and Likens, G.E. 2009. Controlling Eutrophication: Nitrogen and Phosphorus. *Science* **323:** 1014-1015.

Cooksey, K.E., Guckert, J.B., Williams, S.A., and Callis, P.R. 1987. Fluorometric determination of the neutral lipid content of microalgal cells using Nile Red. *Journal of Microbiological Methods* **6:** 333-345.

Copeland, B.J. 1967. Environmental characteristics of hypersaline lagoons. University of Texas. *Contributions in Marine Science* **12:** 207-218.

Cucchiari, E., Guerrini, F., Penna, A., Totti, C., and Pistocchi, R. 2008. Effect of salinity, temperature, organic and inorganic nutrients on growth of cultured *Fibrocapsa japonica* (Raphidophyceae) from the northern Adriatic Sea. *Harmful Algae* **7:** 405-14.

Davis, J.S. 1978. Biological communities of a nutrient enriched Salina. *Aquatic Botany* **4:** 23-42.

Davis, J.S. 1990. Biological Management for the Production of Salt from Sea water. In: Akutsuka, I. (Ed.). *Introduction to Applied Phycology*, 479-488 pg.

Davis, J.S. 1999. *Solar Saltwork- An environmentally friendly industry*. Department of Botany, University of Florida, Gainesville, Florida.

Davis, J.S. 2001. Structure, function and management of the biological system for seasonal solar saltworks. *Global Nest: The International Journal* **2:** 217-226.

Davis, J.S. and Giordano, M. 1996. Biological and physical events involved in the origin, effects, and control of organic matter in solar saltworks. *International Journal of Salt Lake Research* **4:** 335-347.

Demirbas, A. 2009. Production of biodiesel from algae oils. Energy Sources Part A. *Recovery Utilization and Environmental Effects* **8:** 31:163.

Difford, M. 2008. *Towards the Bioremediation of the Hypertrophic Swartkops Solar Salt-works. PhD Thesis*. Nelson Mandela Metropolitan University, 296 pg.

Dortch, Q., Ahmed, S.I., and Packard, T.T. 1979. *Nitrate reductase and glutamate dehydrogenase activities in Skeletonerna costaturn as measures of nitrogen assimilation rates*. Journal of Plankton Resources **1:** 169-186

Dortch, Q. 1990. The interaction between ammonium and nitrate uptake in phytoplankton. *Marine Ecology Progress Series* **61:** 183-201.

Droop, M.R. 1954. A note on the isolation of small marine algae and flagellates for pure cultures. *Journal of Marine Biological Assessment* **33:** 511-514.

Du Toit, S.R. 1998. *Artificial wetlands in the management of solar saltworks. Unpublished M.Sc. dissertation*, Department of Botany, University of Port Elizabeth, 117 pg.

Du Toit, S.R. 2001. *Biological management of South African solar saltworks. PhD thesis*, University of Port Elizabeth, 260 pg.

Elevi Bardavid, R., and Oren, A. 2008. Dihydroxyacetone metabolism in *Salinibacter ruber* and in *Haloquadratum walsbyi*. *Extremophiles* **12:** 125-131.

Emmerson, W.D. 1985. The nutrient status of the Swartkops river estuary, Eastern Cape. Water SA 11 **4:** 189-198.

EU-China. 2009. *Salt production: A reference book for the industry*. Beijing. (*www.chinaeci.com/admin/upload/20090817022608.pdf)*

Fong, P., Boyer, K.E., and Zedler, J.B. 1993. Competition with macroalgae and benthic cyanobacterial mats limits phytoplankton abundance in experimental microcosms. *Marine Ecological Progress Series* **100:** 97-102.

Fryxell, G.A., Garza, S.A., and Roelke, D.L. 1991. Auxospore formation in an Antarctic clone of *Nitzschia subcurvata* Hasle. *Diatom Research* **6:** 235-245.

Gasiunaite, Z.R. 2000. Coupling of the limnetic and brackishwater plankton crustaceans in the Curonian lagoon (Baltic Sea). *International Review of Hydrobiology* **85:** 649-657.

Griess, P. 1879. Bemerkungen zu der abhandlung der H.H. Weselsky und Benedikt Ueber einige azoverbindungen. *Berichte der Deutschen chemischen Gesellschaft* **12:** 426-428.

Grobbelaar, J.U. 2004. Algal Nutrition. *In:* Richmond, A. [Ed.] *Handbook of Microalgal Culture Biotechnology and Applied Phycology.* Blackwell Publishing Ltd., UK, 97-115 pg.

Guillard, R.L., and Sieracki, M.S. 2005. Counting cells in cultures with the light microscope. *In*: Andersen, R. A. [Ed.] *Algal Culturing Techniques.* Academic Press, San Diego, 578 pg.

Harrison, P.J., and Berges, J.A. 2005. Marine Culture Media. *In*: Andersen, R. A. [Ed.] *Algal Culturing Techniques.* Academic Press, San Diego, 578 pg.

Harun, R., Singh, M., Forde, G.M., and Danquah, M.K. 2010. Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and Sustainable Energy Reviews* **14:** 1037-1047.

Hinga, K.R. 1992. CO-occurrence of dinoflagellate blooms and high pH in marine enclosures. *Marine Ecological Progress Series* **86:** 181-187.

Ilosvay, M.L. 1889. L'acide azoteux dans la salive et dans l'aire exhale. *Bulletin de la Société Chimique de France* **2:** 388-391.

Javor, B.J. 1983. Planktonic standing crop and nutrients in a saltern ecosystem. *Limnology and Oceanography* **28:** 153-159.

Javor, B.J., 1989. *Hypersaline Environments : Microbiology and Biogeochemistry*. Springer Series in Contemporary Bioscience. Springer-Verlag, Berlin, 195 pg.

Jones, A.G., Ewing, C.M., and Melvin, M.V. 1981. Biotechnology of solar saltfields. *Hydrobiologia* **82**: 391-406.

Kawachi, M., and Noël, M. 2005. Sterilization and Sterile Technique. *In*: Andersen, R. A. [Ed.] *Algal Culturing Techniques.* Academic Press, San Diego, 65 pg.

Keller, M.D., Selvin, R.C., Claus, W., and Guillard, R.R.L. 1987. Media for the culture of oceanic ultraphytoplankton. *Journal of Phycology* **23:** 176-179.

Lee, Y., and Shen, H. 2004. Basic Culturing Techniques. *In:* Richmond, A. [Ed.] *Handbook of Microalgal Culture Biotechnology and Applied Phycology.* Blackwell Publishing Ltd., UK, 40-56 pg.

Lippemeier, S., Klaus, R.H., Vanselow, K.H., Hartig, P. and Colijn, F. 2001. In-line recording of PAM fluorescence of phytoplankton cultures as a new tool for studying effects of fluctuating nutrient supply on photosynthesis. *European Journal of Phycology* **36:** 89-100.

Litchfield, C.D. 1991. Red - the magic color for solar salt production. *In*: Hocquet JC, Palme R. Schwaz: Berenkamp [Ed.] *Das Salz in der Rechts - und Handelsgeschichte*, 403-412 pg.

Litchfield, C.D., Oren, A., Irby, A., Sikaroodi, M., and Gillevet, P.M. 2008. Temporal and salinity impacts on the microbial diversity at the Eilat, Israel Solar Salt Plant. *Global NEST Journal* **11:** 86-90.

López, E., Aguilera, P.A., Schmitz, M.F., Castro, H., and Pineda, F.D. 2010. Selection of ecological indicators for the conservation, management and monitoring of Mediterranean coastal Salinas*. Environmental Monitoring Assessment* **166:** 241-256.

Losada, M., and Guerrero, M.G. 1979. The photosynthetic reduction of nitrate and its regulation. *In:* Barber, J (Ed.) *Photosynthesis in relation to model systems*. Elsevier/North- Holland Biomedical Press, Amsterdam, 363-408 pg.

MacKay, H.M. 1994. *Management of water quality in an urban estuary. PhD thesis*. University of Port Elizabeth, Port Elizabeth, South Africa, 242 pg.

Masero, J.A. 2003. Assessing alternative anthropogenic habitats for conserving waterbirds: Salinas as buffer areas against the impact of natural habitat loss for shorebirds. *Biodiversity and Conservation* **12:** 1157-1173.

Miquel, P. 1890-1893. De la culture artificielle des diatomées. *Le Diatomiste* 1: Introduction, 73-75 pg; Cultures ordinaires des diatomées, 93-99 pg; Culture artificielle des diatomées marines, 121-28; Cultures pures des diatomées, 149-56 pg; Culture des diatomées sous le microscope. De l'avenir de cultures de diatomées, 165-72 pg.

Mohebbi, F. 2010. The Brine Shrimp *Artemia* and hypersaline environments microalgal composition: a mutual interaction*. International Journal of Aquatic Science* **1:** 19-27.

Moraiti-Ioannidou, M., Castritsi-Catharios, J., Miliou, H., and Kotzamanis, Y.P. 2007. Fatty acid composition and biometry of five Greek Artemia populations suitable for aquaculture purposes. *Aquaculture Research* **38:** 1664-1672.

Nixon, S.W. 1995. Coastal marine eutrophication: A definition, social causes and future concerns. *Ophelia* **41:** 199-219.

Nusch, E.A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* **14:** 14-36.

Oren, A. 2002. Halophilic Microorganisms and their Environments. Kluwer, Dordrecht.

Oren, A. 2009. Saltern evaporation ponds as model systems for the study of primary production processes under hypersaline conditions. *Aquatic Microbial Ecology* **56:** 193-204.

Oren, A. and Mana, L. 2002. Amino acid composition of bulk protein and salt relationships of selected enzymes of Salinibacter ruber, an extremely halophilic Bacterium. *Extremophiles* **6:** 217–223.

Petrovic, A. 1998. Diatoms from the saltern of Ston (Croatia). *Rapp. Comm. Int. Mer. Medit* **35:** 574-575.

Ranga Rao, A., Dayananda, C., Sarada, R., Shamala, T.R., and Ravishankar, G.A. 2007. Effect of Salinity on growth on green alga *Botryococcus braunii* and its constituents. *Bioresource Technology* **98:** 560-564.

Rahaman, A.A., Ambikadevi, M., and Esso, S. 1993. Biological management of Indian solar saltworks. *Seventh Symposium on Salt.* Elsevier Science Publishes B.V., Amsterdam **1:** 633-643.

Redfield, A.C. 1934. On the proportions of organic derivatives in seawater and their relation to the composition of plankton. *In: James Johnstone Memorial Volume*. University Press of Liverpool, 176-192 pg.

Remane, A. 1955. Die Brackwasser e Submergenz und die Unkomposition der Coenosen in Belt- und Ostsee. *Kieler Meeresforschung* **11:** 59-73.

Remane, A. 1958. Okologie des Brackwassers. In: Remane, A., Schlieper,C. (Eds.),Die Biologie desBrackwassers. Schweizerbartsche Verlagsbuchhandlung, Stuttgart, p. 216.

Richmond, A., and Becker, E.W. 1986. Technological aspects of mass cultivation – A general outline *In*: Richmond, A [Ed.] *Handbook of Microalgal Mass Culture*. CRC Press, Inc., Boca Raton, 245-263 pg.

Riebesell, U., Wolf-Gladrow, D.A., and Smetacek, V. 1993. Carbon dioxide limitation of marine phytoplankton growth rates. *Nature* **361:** 249-251.

Rijstenbil, J.W. 1987. Phytoplankton composition of stagnant and tidal ecosystems in relation to salinity, nutrients, light and turbulence. *Netherlands Journal of Sea Research* **21:** 113-123.

Rocha, R., Costa, D., Lucena-Filho, M., Bezerra, R., Medeiros, D., Azevedo-Silva, A., Araújo, C., and Xavier-Filho, L. 2012. Brazilian solar saltworks - ancient uses and future possibilities. *Aquatic Biosystems* **8:** 8.

Rodolfi, L., Zittelli, G.C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., and Tredici, M.R. 2009. Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor

mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering* **102:** 100-112.

Sammy, N. 1983. Biological Systems in north-western Australian solar salt fields. In: Schreiber, B.C. and Harner, H.L. [Eds.] *sixth Symposium on Salt*. Vol. 1. The Salt Institute, Alexandria, Virginia, 207-215 pg.

Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-smith, D.J., and Smith, A.G. 2010. Biodiesel from algae: challenges and prospects. *Current Opinion in Biotechnology* **21:** 277-286.

See, D.S. 1960. Solar Salt*. In:* D.W. Kaufmann (Ed.) *Sodium Chloride: The production and properties of brine.* Am. Chem. Soc. Monogr. Ser. Reinhold, New York, 96-108 pg.

Singh, J., and Gu, S. 2010. Commercialization potential of microalgae for biofuels production. *Renewable and Sustainable Energy Reviews* **14:** 2596-2610.

Solórzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography* **14:** 799-801.

Sørensen, K., Canfield, B.D.E., Teske, A.P., and Oren, A. 2005. Community composition of a hypersaline endoevaporitic microbial mat. *Applied and Environmental Microbiology* **71:** 7352-7365.

Sorgeloos, P., Lavens, P., Leger, P., Tackaert, W., and Versichele. 1986. *Manual for culture and use of brine shrimp Artemia in aquaculture*. State University of Ghent. Faculty of Agriculture, 319 pg.

Sorgeloos, P., and Tackaert, W. 1991. Roles and Potentials of *Artemia* in coastal Saltworks. *In:* Cheng, L. [Ed.] P*roceedings of International Symposium on Biotechnology of Saltponds, Tanggu, Tianjin, PR China*. Salt Research Institute, Tanggu, Tianji, PR China, 283 pg.

Strickland, J.D.H., and Parsons, T.R. 1972. *A practical handbook of seawater analysis. 2nd Edition*, Bulletin 167. Fisheries Research Board of Canada, Ottawa.

Stone, A.W., Weaver, A., and West, W.O. 1998. Climate and Weather. In: Lubke, R. and De Moor, I. (Eds.). *Field Guide to the Eastern and Southern Cape Coasts.* UCT Press, 557 pg.

Syrett, P.J. 1981. Nitrogen metabolism of microalgae. *In:* Platt, T. (Ed.) *Physiological bases of phytoplankton ecol*ogy. Bulletin No. 210. Canadian Government Publishing Center. Hull, Quebec, Canada, 182-210 pg.

Thakur, A., and Kumar, H.D. 1999. Nitrate, Ammonium, and Phosphate Uptake by the lmmobilized Cells of *Dunaliella salina. Bulletin Environmental Contamamination Toxicology* **62:** 70-78.

Torzillo, G., Bernardini, P., and Masojidek, J. 1998. On-line monitoring of chlorophyll fluorescence to assess the extent of photoinhibition of photosynthesis induced by

high oxygen concentration and low temperature and its effect on the productivity of outdoor cultures of *Spirulina platensis* (Cyanobacteria). *Journal of Phycology* **34:** 504-10.

Vanhaecke, P., and Sorgeloos, P. 1989. International study on *Artemia.* XLVII. The effect of temperature on cyst hatching, larval survival and biomass production of diferent geographical strains of *Artemia spp. Annual Society R. Zoology Belgium* **119:** 7-23.

Warnock, N., Page, G.W., Ruhlen, T.D., Nur, N., Takekawa, J.Y., and Hanson, J.T. 2002. Management and Conservation of San Francisco Bay Salt Ponds: Effects of Pond Salinity, Area, Tide, and Season on Pacific Flyway Waterbirds. *The International Journal of Waterbird Biology* **25:** 79-92.

Williams, R.B. 1964. Division Rates of Salt Marsh Diatoms in Relation to Salinity and Cell Size. *Ecology* **45**: 877-80.

Winter, P.E.D. 1990. *THe estuarine-marine exchange of dissolved and particulate material at the Swartkops River estuary, Algoa Bay, South Africa.* PhD thesis, University of Port Elizabeth, Southt Arica, 106 pg.

Wood, A.M., Everroad, R.C., and Wingard, L.M. 2005. Measuring Growth Rates in Microalgal Cultures. *In*: Andersen, R. A. [Ed.] *Algal Culturing Techniques.* Academic Press, San Diego, 578 pg.

Wu, X., Ruan, R., Du, Z., and Liu, Y. 2012. Current Status and Prospects of Biodiesel Production from Microalgae. *Energies* ***5*****:** 2667-2682.

Wurtsbaugh, W.A., and Gliwicz, Z.M. 2001. Limnological control of brine shrimp population dynamics and cyst production in the Great Salt Lake, Utah. *Hydrobiologia* **466:** 119-132.

Yoo, K. 1991. Population dynamics of dinoflagellate community in Masan Bay with a note on the impact of environmental parameters. *Marine Pollution Bulletin* **23:** 185-188.

Appendix 1

Bird species recorded in the Swartkops and Missionvale Salinas for 1999 and 2011 and linked to the nutrients from 1999 and 2011.

Swartkops Marina

May 1999

No	Common Name	Scientific Name	Buffer	P1	2	3	4	5
7	Blacknecked grebe	*Podiceps nigricollis*	2	1		32	20	420
8	Dabchick	*Tachybaptus ruficollis*	65	6	10			
55	Whitebreasted cormorant	*Phalacrocorax carbo*		4				
56	Cape cormorant	*Phalacrocorax capensis*		6	38	10	2	30
58	Reed cormorant	*Phalacrocorax africanus*		6				
67	Little egret	*Egretta garzetta*			100			
95	African spoonbill	*Platalea alba*				2		
96	Greater flamingo	*Phoenicopterus ruber*				70		200
97	Lesser flamingo	*Phoenicopterus minor*						250
102	Egyptian goose	*Alopochen aegyptiacus*				2		
106	Cape teal	*Anas capensis*	30	12	20		6	20
112	Cape shoveller	*Anassmithii*	2	70	20			
228	Redknobbed coot	*Fulica cristata*		1		25		
237	Kittliz's Sandplover	*Charadrius pecuarius*						1
258	Blacksmith plover	*Vanellus armatus*		4	2			
270	Greenshank	*Tringa nebularia*		1				
294	Avocet	*Recurvirostra*		2				10
295	Blackwinged stilt	*Himantopus himantopus*	2	6	21			353
312	Kelp gull	*Larus dominicanus*	2	4	10	3	2	10
315	Greyheaded gull	*Larus cirrocephalus*		8	8		5	55
322	Caspian tern	*Hydroprogne caspia*		2				1

327	Common tern	*Sterna hirundo*			2			1
339	Whitewinged tern	*Chlidonias leucopterus*	2					
713	Cape wagtail	*Motacilla capensis*	1		2	1		
		Number of individuals	**106**	**133**	**233**	**145**	**35**	**1351**
		Number of species	**8**	**15**	**11**	**8**	**5**	**12**

Swartkops Marina

October 2011

No	Common Name	Scientific Name	Buffer	P1	2	3	4	5
7	Blacknecked grebe	*Podiceps nigricollis*		4			9	329
55	Whitebreasted cormorant	*Phalacrocorax carbo*						1
56	Cape cormorant	*Phalacrocorax capensis*						1
96	Greater flamingo	*Phoenicopterus ruber*						6
97	Lesser flamingo	*Phoenicopterus minor*						316
102	Egyptian goose	*Alopochen aegyptiacus*			7		2	
106	Cape teal	*Anas capensis*	136	16	21			
228	Redknobbed coot	*Fulica cristata*						6
254	Grey plover	*Pluvialis squatarola*		18				
258	Blacksmith plover	*Vanellus armatus*	3		2			
262	Turnstone	*Arenaria interpres*	3					
270	Greenshank	*Tringa nebularia*						6
272	Curlew sandpiper	*Calidris ferruginea*	39	3	16			
295	Blackwinged stilt	*Himantopus himantopus*	12					
312	Kelp gull	*Larus dominicanus*						3
315	Greyheaded gull	*Larus cirrocephalus*	5		30			
		Number of individuals	**198**	**41**	**76**	**0**	**11**	**668**
		Number of species	**6**	**4**	**5**	**0**	**2**	**8**

Missionvale Marina

May 1999

No	Common Name	Scientific Name	P1	2	3	4	5	6	7	8	Cryst.
7	Blacknecked grebe	*Podiceps nigricollis*		25		65	1	30		30	
8	Dabchick	*Tachybaptus ruficollis*								5	
55	Whitebreasted cormorant	*Phalacrocorax carbo*		1							
62	Grey heron	*Ardea cineria*				1	1				
67	Little egret	*Egretta garzetta*				1					
96	Greater flamingo	*Phoenicopterus ruber*		16	8	128	301	420			
106	Cape teal	*Anas capensis*		60	8		2				
228	Redknobbed coot	*Fulica cristata*				2					
237	Kittliz's Sandplover	*Charadrius pecuarius*		1							
249	Threebanded plover	*Charadrius tricollaris*	3								
254	Grey plover	*Pluvialis squatarola*									
258	Blacksmith plover	*Vanellus armatus*	2			2					
262	Turnstone	*Arenaria interpres*					5	3			
272	Curlew sandpiper	*Calidris ferruginea*	10	65							
274	Little stint	*Calidris minuta*		10		2					
294	Avocet	*Recurvirostra*		4							
295	Blackwinged stilt	*Himantopus himantopus*	12	50							
		Number of individuals	**27**	**232**	**16**	**201**	**310**	**453**	**0**	**35**	**0**
		Number of species	**4**	**9**	**2**	**7**	**5**	**3**	**0**	**2**	**0**

Missionvale Marina

October 2011

No	Common Name	Scientific Name	P1	2	3	4	5	6	7	8	Cryst.
7	Blacknecked grebe	*Podiceps nigricollis*		8	69	254	17		85		
96	Greater flamingo	*Phoenicopterus ruber*								18	
106	Cape teal	*Anas capensis*				3					
237	Kittliz's Sandplover	*Charadrius pecuarius*		12							
245	Ringed plover	*Charadrius hiaticula*			6						2
254	Grey plover	*Pluvialis squatarola*								6	
258	Blacksmith plover	*Vanellus armatus*		1	2	6	2				
270	Greenshank	*Tringa nebularia*		1							
272	Curlew sandpiper	*Calidris ferruginea*		5		10					
294	Avocet	*Recurvirostra avosetta*		5		16					
295	Blackwinged stilt	*Himantopus himantopus*				8					
315	Greyheaded gull	*Larus cirrocephalus*		88		10	42		6		
		Number of individuals		**120**	**77**	**307**	**61**	**0**	**91**	**24**	**2**
		Number of species		**7**	**3**	**7**	**3**	**0**	**2**	**2**	**1**