The hatching success of egg banks of selected endorheic wetland (Pan) Fauna and a suggested water quality classification of Pans

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The Hatching Success of Egg Banks of Selected Endorheic Wetland (Pan) Fauna and a Suggested Water Quality Classification of Pans

Report to the

WATER RESEARCH COMMISSION

by

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EXECUTIVE SUMMARY

BACKGROUND AND RATIONALE

Pans (endorheic wetlands) are more vulnerable to anthropogenic stress because of their isolated nature and hydrological regime. There are constant fluctuations in the duration and frequency of the inundation period. Small changes in the natural hydrology can have significant impacts on the ecology of these wetlands. It is necessary to assess the ecology of these unique and fragile ecosystems to fully understand the impacts anthropogenic activities will have. It is also a necessity to find new ways of monitoring for such impacts in the shortest time possible with minimal efforts, for the benefit of both the environment and researchers involved. Studies have shown there is large variability within the physico-chemical parameters of pans. This variability has a major influence on the presence and abundance of aquatic invertebrates and plants and the invertebrate fauna that inhabit these environments have various physiological, behavioural and structural adaptations enabling their survival in a constantly changing environment.

With many of the endorheic wetlands (pans) in South Africa occurring in areas where there has been an increase in mining activities, it has not been unexpected that the number of Environmental Impact Assessments (EIA) and monitoring programs has also increased. As is the case with most biotic indices and methods used in water resource management, studies on pans are also reliant on the selection of relevant reference conditions. The variability observed within these ecosystems also complicates the selection of appropriate reference conditions.

The South African climate and geology varies to a great extent from the west to the east of the country. This variability can have significant effects on the ecology of pan ecosystems and results in different biological communities and water quality conditions. As pans do occur over a wide range of climates, three areas with a high density of pans were selected as study areas. These areas are the Lake Chrissie area in Mpumalanga, Wesselsbron in the western Free State and Delareyville in the North West Province. These areas differ in climate and rainfall but still contain perennial and ephemeral pans. An initial survey was undertaken during May 2012 to select appropriate study sites and to collect sediment for the hatching experiments as well as water samples for physico-chemical analyses. Surveys were also undertaken between December 2012 and January 2013 and between March and April 2013. These surveys were undertaken to collect water samples for physico-chemical analyses.

AIMS

1. To develop methods to assess the hatching success of egg banks in pans.
2. To determine the impact of Acid Mine Drainage (AMD) on the hatching of egg banks.
3. To determine whether pans affected by AMD can still sustain egg banks ("ecological sustainability").
4. To study the applicability of a trophic state based classification system for pans.
5. To study the applicability of this classification system in selecting reference conditions.

The aquatic invertebrate communities were also collected during the various surveys. Although this section of the report was not part of the original aims of the study the results have been included to increase the limited literature available regarding the biological communities of pans.

RESULTS AND DISCUSSION

AIM 1

As indicated above, temporary wetlands are highly variable ecosystems and undergo changes in physical and chemical characteristics on a regular basis. The invertebrate fauna that inhabit these systems are highly adapted to survive these constant changing environments. A particularly important group of fauna that inhabit these ecosystems are the Branchiopoda. The class Branchiopoda, descriptively termed phyllopods, consist of the orders Anostraca (fairy and brine shrimp), Notostraca (tadpole shrimp), Conchostraca (clam shrimp) and Cladocera (water fleas). Branchiopods and a few other zooplankton taxa make use of the escape in time survival strategy, achieved through the production of an egg bank. A detailed methodology that was implemented during the current study is presented in chapter 3. A review of the important variables to consider during these hatching studies is also represented in this chapter.

Branchiopod diversity and successional patterns could be determined from small amounts of sediment. Abundances obtained here are comparable to other studies assessing the hatching success of zooplankton communities. The North West and Free State provinces in particularly had high cumulative abundances. The Mpumalanga pans had the lowest abundance of hatchlings emerging from the sediment. This was not an unusual result as pans sampled in the North West and Free State provinces were largely ephemeral in nature, while those sampled in the Mpumalanga Province were of a perennial nature. The patterns of hatching found during the study, throughout all three provinces, were similar to *in situ* patterns of pan succession observed in other studies. Anostracans were generally the first group of crustaceans to be identified after inundation followed by the Cladocera. The Ostracoda and Conchostraca were the last group to be identified. It is not unusual to find a single representative of Branchiopoda in a pan. The data will prove very useful in future studies and monitoring of these pans. Reference conditions of pan communities can effectively be obtained through egg bank analysis. The diversity of pan communities was different between pans and between regions for the larger part of the pans studied. From this it seems that pans have their own unique communities, and each one contributes towards the regional diversity.

AIM 2 and AIM 3

Recovery experiments were performed after the initial hatching experiments on the sediment exposed to AMD only. The AMD was allowed to completely evaporate from the containers. Once this had occurred, the sediment was again left to undergo desiccation for a period of two weeks which was substantial enough for the sediment to be completely dry given the quantity within the containers (25 g). After the desiccation period 1 L distilled water was added to the AMD containers.

The hatching of branchiopod crustaceans was inhibited by the presence of AMD. An explanation for eggs not hatching in the presence of AMD is that AMD has a high concentration of mineral salts (consisting of toxic metals) and a low pH. The high concentration of mineral salts increases the osmotic pressure of the water. Should enough water pass through the tertiary membrane into the egg, metabolic processes within the metanauplius will be activated. Should the metabolism be activated glycerol will start building up inside the egg, creating an osmotic gradient for more water to pass into the cyst. This water build up creates an osmotic pressure inside the egg which results in the bursting of the outer membrane enabling hatching.

It was also demonstrated that the recovery of these aquatic invertebrates after AMD exposure was low. When compared to the diversity of aquatic invertebrates obtained from the controls it could clearly be seen that AMD altered the community structure of the branchiopods which recovered. The diversity of individuals was much lower as a result of the AMD. This shows how poorly the community will respond to the removal of this stressor. A possible factor that could have played a role in the survival of eggs is the type of dormancy. Diapausing eggs may be more tolerant to stressors than quiescent eggs as they rely on internal conditions to hatch regardless of external conditions being favourable. The diapausing eggs of copepods have been found to be less sensitive to metal pollution due to the thick chorion membrane surrounding it. Quiescent eggs relying directly on external conditions could possibly be stimulated to hatch by favourable light and temperature conditions, but conditions such as pH and conductivity which were likely unfavourable could counteract this and inhibit hatching. In this regard diapausing eggs can lie dormant for longer without external factors interfering in the hatching process. The effect that low pH has on hatching has to do with the optimal functioning of the hatching enzyme. The enzyme is secreted by the metanauplii allowing it to break free of the inner membrane, the final membrane that has to be broken to allow the release of the free-swimming nauplii. The low pH of the AMD may have denatured this enzyme which would prevent hatching even if the metanauplii were successful in bursting through the outer membrane.

Even though recovery did take place in a few pans, the number of individuals may be too low to replace the number of eggs affected by the AMD. The buffering capacity of the egg bank will be lost, and the egg bank will eventually deplete itself during future inundations. Species extinctions as a result are inevitable, which raises the concern that wetlands impacted to such an extent by such a stressor may be beyond rehabilitation.

AIM 4 and AIM 5

Pan ecosystems are some of the most variable systems in South Africa. Their endorheic nature and the resultant variability make comparison to other wetlands and even other pans problematic. To determine the spatial and temporal variation in the physico-chemical characteristics of the water from the various pans in the Mpumalanga, North West and Free State provinces, surface water samples were collected during each of the surveys. All water quality analyses were carried out by Chemtech Laboratories (a SANAS accredited laboratory). The water analysis included nutrients, salts and metals. The in situ physico-chemical variables that were sampled during the current survey included temperature, pH, dissolved oxygen concentration ([DO]) and saturation (DO%), total dissolved solids (TDS) and electrical conductivity (EC). The in situ analysis was undertaken using a pre-calibrated WTW 340i multi-parameter hand-held water quality meter.

Large variability was observed in the nutrient and salt concentrations of the selected study sites. This variability observed on a spatial scale was expected. The climate and rainfall varies between the different provinces. The pans from Mpumalanga are generally more perennial in nature when compared to pans in the North West and Free State. This was evident in this study as most of the pans in Mpumalanga had water during both surveys while only on the second survey water was present in North West. No water was present in the Free State although in previous seasons water was present throughout a whole year indicating the importance of rainfall in the pan catchment for filling of the pans. The more perennial a pan is the more stable the physico-chemical variables will be and vice versa for pans that are more ephemeral. The Electrical Conductivity (EC), for example, ranged between 0.19 Ms/cm and 9.06 Ms/cm in Mpumalanga. In comparison the EC in the Free State and North West provinces ranged from 0.81 Ms/cm to 110.56 Ms/cm.

In addition to the spatial variability, large temporal variability in water quality characteristics was also observed. The current study did initially attempt to classify the pans from Mpumalanga, Free State and North West based on their salinity and nutrient data based on the Hutchinson *et al.* (1932). However, as was seen in the statistical analysis no real classification or different groups were identified based on the water quality. The classification of pans based on their water quality variables were first attempted by Hutchinson *et al.* in 1932 on various pans in the Lake Chrissie area. Since then a few studies (Ferreira, 2010) have attempted to classify pans based on their water quality as well as their biological communities. However, no one method has really been successful. This is due to the inherent variability within these pan systems.

CONCLUSIONS

It can be concluded that Branchiopoda can successfully be hatched from sediment collected *in situ* under controlled laboratory conditions. The hatching patterns are also closely related to patterns observed during *in situ* studies. This clearly indicates that egg bank analysis can be used as a monitoring tool and can aid in the determination of diversity when pans are desiccated. This becomes essential in any impact assessment. It is further concluded that AMD has a negative impact on the egg banks within pans and causes a loss of biodiversity. Ultimately this demonstrates how AMD will degrade these unique environments should its disposal not be properly managed and should mining activities continue to encroach upon the vicinity of these wetlands. It is critical that the integrity of these ecosystems be maintained for all that depend on them.

Due to the inherent variability within endorheic wetlands, attempts to classify these systems according to trophic state or salinities have been unsuccessful. The variability observed in water quality was also observed in the invertebrate communities that inhabit these ecosystems. There was large spatial and temporal variation in biodiversity when comparing the communities from the various sites selected in the three provinces. The water quality dataset collected throughout this project was used to determine ranges within the water quality variables for pans from Mpumalanga as well as from the North West and Free State provinces. These ranges can potentially be used to look if any variation out of this range has occurred and whether it is due to an anthropogenic impact.

RECOMMENDATIONS FOR FUTURE RESEARCH

From chapter 3 and 4 it was evident that a number of variables may have a potential impact on the hatching success of egg banks from sediment collected *in situ*. It is therefore recommended that further studies be completed to refine the methodology for hatching experiments as egg banks can be an important consideration in biodiversity studies and impact assessments involving pans. It will also be important to complete future studies which compare the hatching success from egg banks collected from impacted sites.

From chapter 5 it was evident the AMD has a definite effect on the hatching success of Branchipoda and that this effect may be permanent. During the current study the effect of AMD was studied, but no dilution series was made for the AMD medium. This will allow the assessment of relevant end-points and further research is therefore recommended.

From chapter 6 it was evident that further research is required with regards to the water quality of pans and the possible classification of pans based on physico-chemical characteristics. Studies completed on the perennial pans of Mpumalanga produced similar results. It has become evident that the variability in water quality is of such an extent that each pan could be considered unique. This has major implications for water resource management and the protection of the ecosystems.

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LIST OF ABBREVIATIONS

AMD	Acid Mine Drainage
DO	Dissolved Oxygen
DWAF	Department of Water Affairs
EC	Electrical Conductivity
EIA	Environmental Impact Assessment
FS	Free State
IUCN	International Union for Conservation of Nature
KOSH	Klerksdorp/Orkney/Stilfontein/Hartebeesfontein
MLD	Mpumalanga Lake District
MP	Mpumalanga
NBF	Neutral Buffered Formalin
NMDS	Non-Metric Dimensional Scaling
NW	North West
NWA	National Water Act
OECD	Organization for Economic Cooperation and Development
PCA	Principle Component Analysis
RDA	Redundancy Analysis
RHP	River Health Programme
SANAS	South African National Accreditation System
SoER	State of the Environment Report
TDS	Total Dissolved Solids
UNESCO	United Nations Educational, Scientific and Cultural Organization

1 INTRODUCTION AND OBJECTIVES

Many of the endorheic wetlands (pans) in South Africa occur in areas where there has been an increase in mining activities and therefore, it has not been unexpected that the number of Environmental Impact Assessment (EIA) and monitoring programs have increased. As is the case with most biotic indices and methods used in water resource management, studies on pans are also reliant on the selection of relevant reference conditions. As studies have showed there is a large variability within the physico-chemical parameters of pans, the selection of appropriate reference conditions can be complicated. Hutchinson *et al.* (1932) has suggested that pans can be separated based on their trophic state and can be considered as alkaline dystrophic, saline eutrophic or eutrophic. The trophic state of a pan has a major influence on the presence and abundance of aquatic invertebrates and plants. It is thus very important when comparing pans to consider this classification. The classification systems suggested by Hutchinson *et al.* (1932) is based mainly on pans in Mpumalanga. Further studies into the different trophic states of pans in various provinces is very important and can contribute to a better understanding of the general ecology of these systems and the potential impacts of anthropogenic activities. Most importantly it can contribute to future selection of appropriate reference conditions, in turn ensuring the effective management and conservation of these ecosystems.

As many of these systems are already (and will in the future) be affected by mining activities, the effect of Acid Mine Drainage (AMD) on the biota is also of particular concern. The effect of AMD on the hatching success of egg banks has not been well studied, especially in South Africa. This study will thus contribute to our knowledge of the effect of water quality changes in particular on the branchiopod communities within these systems. This is very important as these branchiopod crustaceans are specifically adapted to these endorheic ecosystems. Many branchiopods (especially Anostraca) are also classified as being endangered or threatened according to the IUCN Red List (De Roeck *et al.,* 2007). A dormant egg phase is a dominant feature of most large branchiopod taxa. After production, eggs are deposited on the substrate ultimately forming egg banks. The conditions required to end this dormant stage varies between species and can even vary amongst a population of the same species (Brendonck and De Meester, 2003). As a result, a population of a particular species of branchiopod can often consist of different generations. Some may have hatched from eggs that where deposited the previous season while other can be from eggs that were deposited a decade ago (or more). Studies have shown the metals alone can have an effect on the hatching success of dormant egg stages (Jiang, *et al*. 2007; Sarabia *et al*., 2008). Changes in water quality may thus influence the hatching success of these branchiopods and with conductivity and pH having a major influence on hatching, AMD may lead to a loss in the biodiversity of branchiopod crustaceans in several provinces.

Aims

The aims of the project were as follows:

1. To develop methods to assess the hatching success of egg banks in pans – Chapter 4.
2. To determine the impact of AMD on the hatching of egg banks – Chapter 4.
3. To determine whether pans effected by Acid Mine Drainage can still sustain egg banks ("ecological sustainability") – Chapter 5.
4. To study the applicability of a trophic state based classification system for pans – Chapter 6.
5. To study the applicability of this classification system in selecting reference conditions – Chapter 6.

2 STUDY AREA AND SITE SELECTION

The South African climate and geology varies to a great extent from the west to the east of the country. This variability can have significant effects on the ecology of pan ecosystems and results in different biological communities and water quality conditions. The published research on pans in South Africa has mainly been limited to the Lake Chrissie area, Free State, Gauteng and northern KwaZulu-Natal provinces. Very little work has been completed in the North West Province apart from studies on Barberspan. As the pans do occur over a wide range of climates three areas with a high density of pans were selected as study areas. These areas are the Lake Chrissie area in Mpumalanga, Wesselsbron in the western Free State and Delareyville in the North West Province. These areas differ in climate and rainfall but still contain perennial and ephemeral pans. The following sections provide background information about each of the selected study areas.

2.1 Mpumalanga

Mpumalanga lies in the north eastern region of South Africa, approximately 100 km east of Johannesburg, and is characterised by spectacular natural beauty and a wealth of natural resources (DWAF, 2002). Three main wetland types have been identified in Mpumalanga. These include: endorheic pans, floodplain wetlands and seepage wetlands with most of the endorheic pans occurring in the wetter Highveld region with the main concentration in the Lake Chrissie area. It is estimated that there are a total of 4 628 endorheic pans that occur in Mpumalanga of which approximately 60% are ephemeral in nature and the remaining 40% are perennial. From Table 2-1 below it is evident that a total of 89% of perennial pans are still intact with 10% being altered. The non-perennial pans are more heavily transformed with 31% being transformed and 68% still intact (DWAF, 2002). These estimates are based on studies completed prior to and including 2002 and after a decade (2012) the situation may have changed.

Table 2-1: Status of Endorheic Pans in Mpumalanga (Adapted from DWAF, 2002).

Pan type	Status	Area hectare	Percentage
Perennial	Untransformed	117067.94	89.34
	Transformed	13973.67	10.66
Non-perennial	Untransformed	65873.96	68.84
	Degraded	29.44	0.03
	Transformed	29790.13	31.13

The pans that have been included from the Mpumalanga Province all occur in an area around Lake Chrissie known as the Mpumalanga Lake District (MLD). Pans do occur in a "belt" from the dry Northern Cape, the North West, Free Sate, Gauteng, Mpumalanga and down into northern Kwazulu-Natal. The MLD marks the eastern most edge of this belt. There are approximately 300 pans of various types in this area (McCarthy *et al.*, 2007). In addition, the area forms a watershed with the Vaal, Usuthu and Mpuluzi Rivers originating in the area. There are very few activities in the area that impact on the water quality of the selected pans. The area is mostly used for grazing by cattle and very few crops are planted in the vicinity of the pans. There is an estimated 300 pans within a 20 km radius in the MLD, most of which remain inundated throughout the year. As a result, the pans in the area are of particular importance for waterfowl while the near-threatened Lesser Flamingo (*Phoenicopterus minor*) is also often seen in the area. Along with the approximately 300 bird species that frequent the area, 10 species of frog has also been recorded in the MLD.

It is said that the MLD "provides a glimpse of the most ancient landforms in southern Africa" (McCarthy *et al.*, 2007). The pans show a variety of shapes and are exposed to wind action. This wind action has led to the pans having exposed bedrocks on their western margins and thick sand deposits on their eastern margins. The existence of the pans of Mpumalanga and especially those in the MLD has been used by several authors as proof that the climate of Mpumalanga was at some point in time much drier than it is today. This drier climate is reflected in other parts of the country as well (Rodgers, 1922; Hutchinson *et al.,* 1932).

2.1.1 Climate

Mpumalanga enjoys a sub-tropical climate with hot summers and mild to cool winters. The average daily temperature in January is 24°C, while in June the average daily temperature is 14.8°C. The average annual rainfall is 767 mm, with approximately 10 times more rain falling in summer than in winter (SoER, 2001; 2003). In dry years (<450 mm/annum) most of the pans dry up whereas in normal to wet years (>750 mm/annum) most of the pans retain their water throughout the year.

2.1.2 Geology

The southern part of Mpumalanga up to the southern half of Emalahleni (Witbank), Middelburg, Belfast, the western half of Carolina, Delmas, Kriel, Ermelo, Bethal, Balfour, Standerton, Amersfoort, Volksrust, Wakkerstroom, patches in Piet Retief district are covered by the Ecca Group of the Karoo Supergroup (Karoo rocks can be divided into the Dwyka, Ecca, Beaufort and Stormberg Groups). The major coal bearing strata in South Africa are associated with the Karoo Basin. The five recognized coal seams in the Emalahleni (Witbank) area are named, consecutively, 1 to 5, with the latter be the youngest (Figure 2-1).

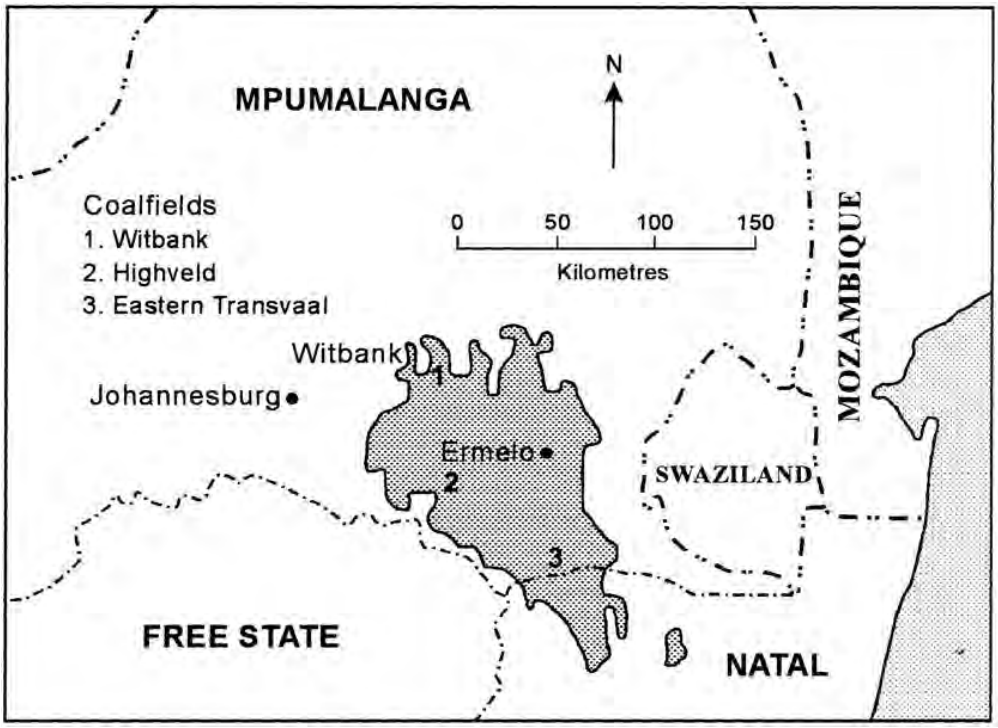


Figure 2-1: The position of the Witbank Coalfield. The Coalfield underlies all the pans selected for the study (Bullock and Bell, 1997).

2.2 Free State

The pans of the Free State are mostly situated in the western part of the province in a belt running from the Wesselsbron area in the northwest to the provincial border in the south west (Seaman, 1987). The pans in this area are mostly seasonal pans and only contain water during the rainy season (DWAF, 2003). It has been estimated that the area contain approximately 13 750 pans greater than one hectare in size with the largest exceeding a 100 hectares in size.

Pans in the Free State are important habitats for overwintering palearctic waders that frequent the area. Pans are also important as breeding grounds for ducks and geese. On average only 10% of pans receive water during the rainy season that would allow the breeding of ducks and geese (Seaman, 1987). The major threats on pans that have been identified by DWAF (2003) are the lack of conservation and incorrect use of the pan as a resource.

2.2.1 Climate

The Free State climate can be extremely variable but is generally hot and arid. The rainfall season is generally over the summer season but occasional winter showers can occur. The annual average rainfall is between 500-600 mm (South Africa Channel, 1995). The western area of the province is generally drier than the eastern area. The average summer temperature is +/- 23ºC and the average winter temperature is +/- 8ºC. The hottest month is generally January while the coldest is June. The province is situated more or less 1300 m above sea level (South Africa Channel, 1995).

2.2.2 Geology

The Free State pans are situated on the vast Karoo Supergroup that covers almost two-thirds of South Africa and was deposited between 200 and 300 million years ago. This supergroup are known to contain major coal and clay deposits. The underlying geology of the Free State can be further divided into the sedimentary rocks that belong to the Beaufort and Ecca groups (Grodner, 2002).

2.3 North West

The North West (NW) Province is situated at the center of the northern border of South Africa. The province shares a border with Botswana to the north and four South African provinces: Limpopo Province to the south-west, Free State to the south, Gauteng to the southeast and Limpopo to the east and northeast (North West Province Environmental Outlook Report, 2008). The altitude of the province ranges from 920-1782 meters above sea level (De Villiers and Mangold, 2002). The western and north western portions of the province are dominated by plains, with scattered hills running in an arc from the Northern Cape. The central and southern portion of the province has many landscape types which include: plains with pans; slightly undulating plains which feature in areas to the west of Vryburg, south of Lichtenburg; and parallel hills and lowlands of Potchefstroom and Rustenburg as well as the towns of Taung and Vryburg (North West Province Environmental Outlook Report, 2008).

There are an estimated 636 pans falling in the Western Plateau region, of the North West Province. The numerous pans occurring in the province provide important breeding and feeding habitats for waterfowl, amphibians and aquatic invertebrates which are adapted to the seasonal nature of these water bodies (North West Province Environmental Outlook Report, 2008).

2.3.1 Climate

The North West has a continental climate with temperatures range from 17 to 31°C in the summer and from 3 to 21°C in the winter. The total annual rainfall is approximately 360 mm, with the highest rainfall occurring in the summer months, between October and April. Due to the low precipitation levels the province is considered to be an arid region (North West Province Environmental Outlook Report, 2008).

The North West Province falls within the Savannah/Grassland biome, with roughly 71.5% falling within the Savannah biome and 28.5% falling in the Grassland biome. Many vegetation types make up these biomes, but more specifically Delareyville falls within the Sandy Highveld grassland type as described by Low and Rebelo (1998). This type of grassland is dominated by *Acacia caffra* and grasses of the *Cymbopogan* and *Themeda* types. These grasses consist mainly of the *Eragrostis* spp., *Panicum coloratum, Stiptagrostis uniplumis, Themeda triandra*, *Cymbopogon plurinodes, Anthephora pubescens, Aristida congesta* and *Digitaria eriantha* (Acocks, 1953).

2.3.2 Geology

The north-eastern and north-central regions of the province are largely dominated by igneous rock formations from the Bushveld Complex. The oldest rock formations in the province are those of the Archaean Granites which form basement rock, they occur in the south-eastern portion of the province as well as the north-central portion of the Vryburg and Ganyesa districts. The Transvaal sequence occurs discontinuously throughout the province with the western section of this sequence occurring in the region of Vryburg, Kuruman and Douglas, an area known as the Ghaap Plateau. The eastern section of the Transvaal sequence extends from Potchefstroom to Ventersdorp. The Ventersdorp Super Group covers an extensive area and ranges in thickness from 300 m to 5000 m. The formation is composed largely of volcanic andesitic larvas and related pyroclastics. Various conglomerates, tuffaceous, calcareous shales and pyroclastics are also constituents of this formation. This Super group outcrops extensively in the south eastern portions of the province, and is known to occur in all but the north eastern districts. This Super group forms undulating hilly areas in the central and eastern portions of the province, while extensive flat to slightly undulating plains dominate the landscapes towards the west (De Villiers and Mangold, 2002). Delareyville falls within the Ventersdorp Super Group.

Due to the high evaporation rates experienced in the province high concentrations of salts such as calcium and silica are present in the soil, particularly in pans. Thus pan soils tend to be alkaline with low levels of organic matter. The north-eastern portion of the province tends to have lithosols of arenaceous sediment, whereas the southern and central regions tend to have black and red clays and ferrisiallitic soils of sands, loams and clays. The western region which is much drier is characterized by red and yellow arenosols, and the south-western region of the province is characterized by calcareous sands, loams and arenaceous lithosols (De Villiers and Mangold, 2002).

2.4 Threats to pans

2.4.1 Agriculture

Pans that lie in agricultural regions are usually threatened by crop encroachment around the periphery of the pan. Crop encroachment can even impinge into the basins of smaller well vegetated pans (Allan *et al*., 1995). Agricultural activities such as ploughing, overgrazing and excessive trampling by livestock are known to damage the shore vegetation. A loss in natural vegetation increases wind evaporation leading to the siltation of the pan basin (Allan, 1987). Many pans are dammed for irrigation or excavated to provide a water supply for livestock (Allan *et al*., 1995), which poses the risk of secondary salinization due to water logging and interference with the natural drying regime (UNESCO, 2003; Ning *et al*., 2011).

In addition pans are subjected to contamination through the use of pesticides and fertilizers. Agricultural activities are the number one cause of eutrophication which is a common and growing problem in most wetlands (Jeffries and Mills, 1990; Smith, 1998). Eutrophication is caused by increased inputs of nutrients, nitrogen and phosphorous, in the aquatic environment and has many negative effects on aquatic ecosystems. The process of eutrophication causes an increase in the primary productivity of certain resident biota within the wetland (Rich, 1973). Phytoplankton species take over causing a reduction in water transparency (Cullen, 1984; Jeffries and Mills, 1990). The primary consumers mainly the zooplankton species that survive in these wetlands are unable to consume and process the excessive phytoplankton community. The phytoplankton then dies and gets decomposed by decomposers (Cullen, 1984). The decomposition process is an oxygen intensive process and thus leads to oxygen depletion of the water and its sediments. These anaerobic conditions influence the nutrient-cycling, causing an accumulation of products invariably to toxic levels (Welch, 1980; Mason, 1983; Cullen, 1984). Aquatic species are unable to handle these conditions and ultimately die, and the species that are able to cope, such as the macrophytes, then take over. This further exacerbates the problem as their consumers have been eliminated. Eutrophication is therefore a huge factor leading to the loss of aquatic biodiversity (Seehausen *et al*., 1997; Jeppesen *et al*., 1998).

2.4.2 Mining

The mining industry is the principal cause of the lowered pH levels of wetlands in South Africa (Coetzee, 1995). Acid mine drainage (AMD), a specific type of mine pollution, refers to the drainage resulting from the natural oxidation of sulphide minerals in mine rock or waste material. Acid mine drainage is responsible for both ground and surface water pollution (Geldenhuis and Bell, 1998). In the case of pans it is the surface run-off from mine dumps into pan basins that is responsible for the water contamination (Sharp and Allan, 1985; Sharp and Cowan, 1987).

Pyrite or iron disulphide (FeS_2) is the most important mineral associated with AMD (Riley, 1960; Barnes and Romberger, 1968). Acid mine drainage occurs in areas of coal, pyritic sulphur, copper, zinc, silver and lead mining (Sullivan *et al*., 1995). Acid mine drainage is produced when sulfidic material such as pyrite (FeS_2) contained in rock strata is exposed to oxygen and water, primarily due to anthropogenic activities. Through a number of reactions (some of which are catalysed by iron oxidizing bacteria), pyrite is oxidized eventually forming ferric hydroxides and sulphuric acid (Soucek *et al*., 2000). In addition to the formation of water with low pH and high iron content, the acid produced from the oxidation of pyrite may also dissolve other minerals which in themselves do not contribute to the formation of acid waters (Robb, 1994). Common among the chemical compounds in waters issuing from the coal mines are sulphuric acid and the acid salts of iron, aluminium, zinc, lead and copper (Parsons, 1957). The problem with this is that in acidic waters metals which are insoluble at a neutral or near neutral pH within the sediment are mobilized into solution. Heavy metals are of concern as they persist in ecosystems for an extended period of time and because they tend to accumulate up the food chain (Fuggle, 1983; Gilmour and Henry, 1991). Trace metals and lowered pH have been shown to adversely affect benthic invertebrate communities (Bell and Nebeker, 1969). Several studies have looked at the effects of AMD on benthic aquatic invertebrates and have revealed that there is a reduced diversity and abundance of aquatic invertebrates from impacted sites relative to un-impacted sites and species shift from intolerant taxa to tolerant taxa (Soucek *et al*., 2000; Gerhardt *et al*., 2004). It has been found that high concentrations of metals result in a decrease in hatching success in zooplankton communities (Kerfoot, 1999; Jiang *et al*., 2007; Sarabia *et al*., 2008).

The impacts of AMD on aquatic ecosystems are very difficult to predict due to the variability of the discharge from adits (an entrance to an underground mine which is horizontal or nearly horizontal), variation in adit strength and composition which varies seasonally, the effect of surface run-off from exposed areas of the mines during heavy rainfall, and the effect of the catchment discharge characteristics affecting dilution and the concentration of organic matter in the water chelating soluble metals present (Gray, 1997). Therefore it still remains unclear to what extent such activities are impacting aquatic ecosystems and the biota within.

Many pans in the Highveld region occur in areas where there have been recent increases in mining activities (North West, Free State and Mpumalanga provinces). As many of these pans are already (and will in the future) be affected by these activities, the effect of AMD on the biota is of particular concern. The effect of AMD, specifically on the hatching success of egg banks in pans has not been well studied previously. Studies have paid more attention firstly, to lotic systems impacted by AMD and secondly, focus has been put on the active community rather than the dormant community. However, egg bank studies have gained much attention over the past decade. In lotic environments it has been found that as the pH of water decreases so does the percentage of aquatic invertebrates to emerge successfully (Bell, 1971). With pH and conductivity being important contributors to the hatching of dormant eggs of branchiopod crustaceans, AMD may influence the hatching success of these aquatic invertebrates. Acid mine drainage may therefore lead to a loss in the biodiversity of branchiopod crustaceans in pans from areas where there is extensive mining. This would be detrimental to the pan’s ecology because of the flagship status of these aquatic invertebrates, and because they are the primary food source for many aquatic bird species. Their loss would have impacts further up the food chain. Many branchiopods (especially Anostraca) are already listed as endangered or threatened according to the IUCN Red List (De Roeck *et al*., 2007).

2.5 Site Selection

A site selection survey was carried out during May 2012 to the North West, Free State and Mpumalanga provinces. The aim was to select 30 pans that have no impacts of agriculture and mining activities. Agriculture was determined as an impact due to crops within the pan basin while livestock grazing was deemed as having a minimal impact. However, no pan was selected that showed indications of overgrazing due to livestock. The reason for the selection of unmodified pans is to ensure that the trophic state of the selected pans is not altered by human activities. This is important as the classification system for pans should be based on natural trophic states. It is also important that the invertebrate communities of the selected pan (especially the branchiopod communities) are not altered by anthropogenic impacts.

The site selection survey in Mpumalanga was done in the Lake Chrissie area (Figure 2-2), in the Free State the selected pans were in the Wesselsbron region and in the North West pans were situated near Delareyville. This represents three different regions based on the climate and pan types present as was identified in the previous section. As a signatory of the RAMSAR convention South Africa is obligated to protect and manage any RAMSAR site as is the case with Barberspan close to Delareyville. Barberspan is not a pan as it has an outlet but due to its RAMSAR status it was included in the study.

The pans that were selected in the three provinces are presented in Table 2-2 with their GPS coordinates and the hydrological state during the three field surveys. Figure 2-2 - Figure 2-4 depicts the location of the sampling sites graphically. A short description of each pan is provided in the following section to indicate the general condition and habitat available at each pan.

Table 2-2: GPS coordinates of the selected pans in the Free State (FS), North West (NW) and Mpumalanga (MP) region.

Site Name	GPS coordinate		Hydrological State May 2012	Hydrological State December/January 2012/2013	Hydrological State March/April 2013
	South	East			
FSpanA	-28.03594	26.62722	Dry	Dry	Dry
FSpanB	-27.934	26.53281	Dry	Dry	Dry
FSpanC	-27.82997	26.41219	Dry	Dry	Dry
FSpanD	-27.84344	26.40142	Dry	Dry	Dry
FSpanE	-27.89466	26.35641	Dry	Dry	Dry
FSpanF	-27.91275	26.43726	Dry	Dry	Dry
FSpanG	-27.85454	26.29013	Dry	Dry	Dry
FSpanH	-27.89646	26.53324	Dry	Dry	Dry
FSpanI	-27.91759	26.55128	Dry	Dry	Dry
FSpanJ	-27.88847	26.46089	Dry	Dry	Dry
FSpanK	-28.33096	26.24425	Not sampled	Wet	Dry
FSpanL	-28.39097	26.1839	Not sampled	Wet	Dry
FSpanM	-28.30321	26.15914	Not sampled	Wet	Wet
FSpanN	-28.22252	26.22951	Not sampled	Wet	Wet
FSpanO	-28.17545	26.23727	Not sampled	Wet	Dry
NWpanA	-26.54822	25.30036	Dry	Wet	Wet
NWpanB	-26.61613	25.34218	Dry	Wet	Dry
NWpanC	-26.63553	25.37402	Dry	Dry	Dry

Table 2-2: Continued.

NWpanD	-26.66221	25.40725	Dry	Dry	Dry
NWpanE	-26.77504	25.30532	Wet	Wet	Dry
NWpanF	-26.80451	25.49156	Dry	Wet	Dry
NWpanG	-26.7692	25.48975	Dry	Wet	Dry
NWpanH	-26.69913	25.4566	Dry	Wet	Wet
NWpanI	-26.59834	25.58096	Wet	Wet	Wet
NWpan J	-26.80149	25.49281	-	Wet	Dry
MPpanA	-26.26997	30.26477	Wet	Wet	Wet
MPpanB	-26.27777	30.26957	Wet	Wet	Wet
MPpanC	-26.26871	30.29175	Wet	Wet	Wet
MPpanD	-26.32602	30.28577	Wet	Wet	Wet
MPpanE	-26.32674	30.26735	Wet	Wet	Wet
MPpanF	-26.37426	30.28127	Wet	Not sampled	Not sampled
MPpanG	-26.36087	30.31407	Wet	Wet	Wet
MPpanH	-26.34822	30.3299	Wet	Wet	Wet
MPpanI	-26.36309	30.35846	Wet	Wet	Wet
MPpanJ	-26.35973	30.25344	Wet	Wet	Wet
MPpanK	-26.28903	30.25752	Wet	Wet	Wet
MPpanL	-26.27023	30.28007	Wet	Wet	Wet
MPpanM	-26.27051	30.24452	Wet	Wet	Wet
MPpanO	-26.36156	30.24622	Wet	Wet	Wet
MPpanP	-26.337552	30.272606	Not sampled	Not sampled	Wet
MPpanQ	-26.321633	0.225957	Not sampled	Not sampled	Wet
MPpanR	-26.599845	9.979029	Not sampled	Not sampled	Wet

MP pan A, MP pan B and MP pan C

MP pan A, MP pan B and MP pan C MP pan A, MP pan B and MP pan C (Table 2-2) are all situated on the farm Blaauwater. Although there is a marked difference in size, the habitat of MP pan A and MP pan B are quite similar. In both pans there was a lack of vegetation (marginal, emergent and submerged) and open water and sediment was the dominant habitat available in these pans. In both pans the sediment layer covering the bedrock was relatively thin. The water in MP pan A and MP pan B was very turbid and almost black in colour. The habitat of MP pan C (Table 2-2) was different. Reeds were abundant along the margins of the pan and submerged vegetation was also present in large quantities. The substrate consisted of clay and the water, although still very turbid, was not as dark when compared to MP pan A and MP pan B. Almost no anthropogenic activities occurred in the vicinity of the pans. Activities were limited to some livestock grazing within the watershed of the pan, but there was no evidence of overgrazing. A gravel road runs along the eastern embankment of MP pan A.

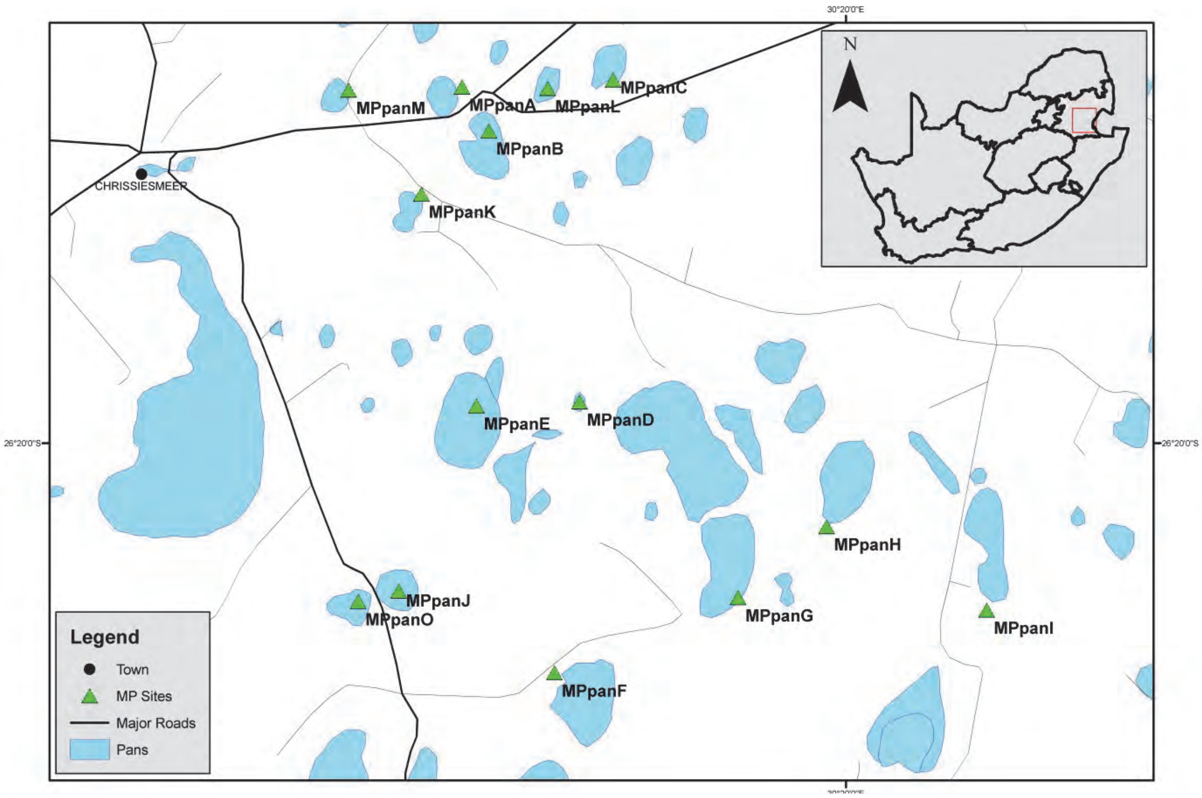


Figure 2-2: Site localities in the Mpumalanga region near Lake Chrissie.

MP pan D and MP pan E

Both MP pan D and MP pan E (Table 2-2) are located on the farm Goedehoop. Anthropogenic activities were limited within the catchment of these pans with the grassland surrounding these pans mainly used for grazing. Forestry activities occurred in the region near MP pan D, which was a new activity as the forestry was in its beginning stages. The forestry activities remained the same and did not expand throughout the duration of the project. The habitat of MP pan D was dominated by open water and clay as a substrate. The water was very turbid and like MP pan A and MP pan B almost black in colour. The habitat of MP pan E consisted of open water and sand as a substrate. Very little clay was present in the pan covering the bedrock. There were a few patches of submerged vegetation, yet marginal vegetation was absent. The water was very turbid and greyish in colour.

MP pan F and MP pan G

MP pan F (Table 2-2)) is also known as Magdalenas Lake and MP pan G is known as Eilands Lake. Both these pans have formed part of historical studies (Hutchinson *et al*., 1932) and were included in the study due to the availability of their historical data. The habitat at MP pan F was dominated by open water and clay as a substrate. There was no vegetation in the pan. The water was very turbid and greyish in colour. There was evidence of some agricultural activities near the eastern side of the pan, but these activities were limited. MP pan G 9 (Table 2-2) was one of the largest pans surveyed in the study. The pan consisted of clay as a substrate, with some emergent and submerged vegetation present along the margins of the pan. The water was very turbid and dark in colour. The anthropogenic activities surrounding MP pan G were limited to livestock grazing.

MP pan H, MP pan I and MP pan J

MP pan H (Table 2-2) is situated very close to MP pan G. Habitat availability was again limited to open water and clay as a substrate. The water was very turbid and dark in colour. Apart from grazing, there were almost no anthropogenic activities within the catchment of the pan. MP pan I (Table 2-2**) is** also known as Lake Banaghar and historical data is also available for this pan. The pan is a large kidney shaped pan. Water in the pan was dark and very turbid. Habitat availability in the pan consisted of submerged and emergent vegetation, open water and clay as a substrate. Apart from some forestation towards the south-western edge of the pan, there was no evidence of any anthropogenic activities within the catchment of the pan. MP pan J (Table 2-2) is situated near Lake Chrissie, the largest pan in the study area. There was some emergent vegetation available as habitat, but habitat was dominated by open water and sand as a substrate. The water, although very turbid was light brown in colour. Apart from the dirt road crossing along the western side of the pan, there are no other activities within the watershed of the pan.

MP P, Q, R

A further three pans were added to the study to further increase water quality data. These pans were MP pan P, MP pan Q and MP pan R. MP pan P is situated on the farm Goedehoop and is in close proximity to MP pan D and MP pan E. It is situated within a dense *Eucalyptus* plantation and therefore some impacts on the water quality are potentially expected. The substrate was found to be mostly clay with some macrophyte and algal growth present in the pan. MP pan Q was selected as Lake br and it is the biggest pan within the area. The habitat at the site was mainly open water with clay substrate and some reedbeds have also formed at the site. Overall little reeds can be found around the pan and the catchment is mostly dominated by grasslands. MP pan R was situated on the outskirts of Ermelo on the N11 road to Volksrust. This pan falls outside of the Lake Chrissie area and was therefore included as a potential representative of other pans in Mpumalanga. The catchment of MP pan R was mostly dominated by grasslands and some macrophyte and algal growth was present within the pan. Site photographs of all the pans sampled in the Mpumalanga Province is presented in Table 2-2.

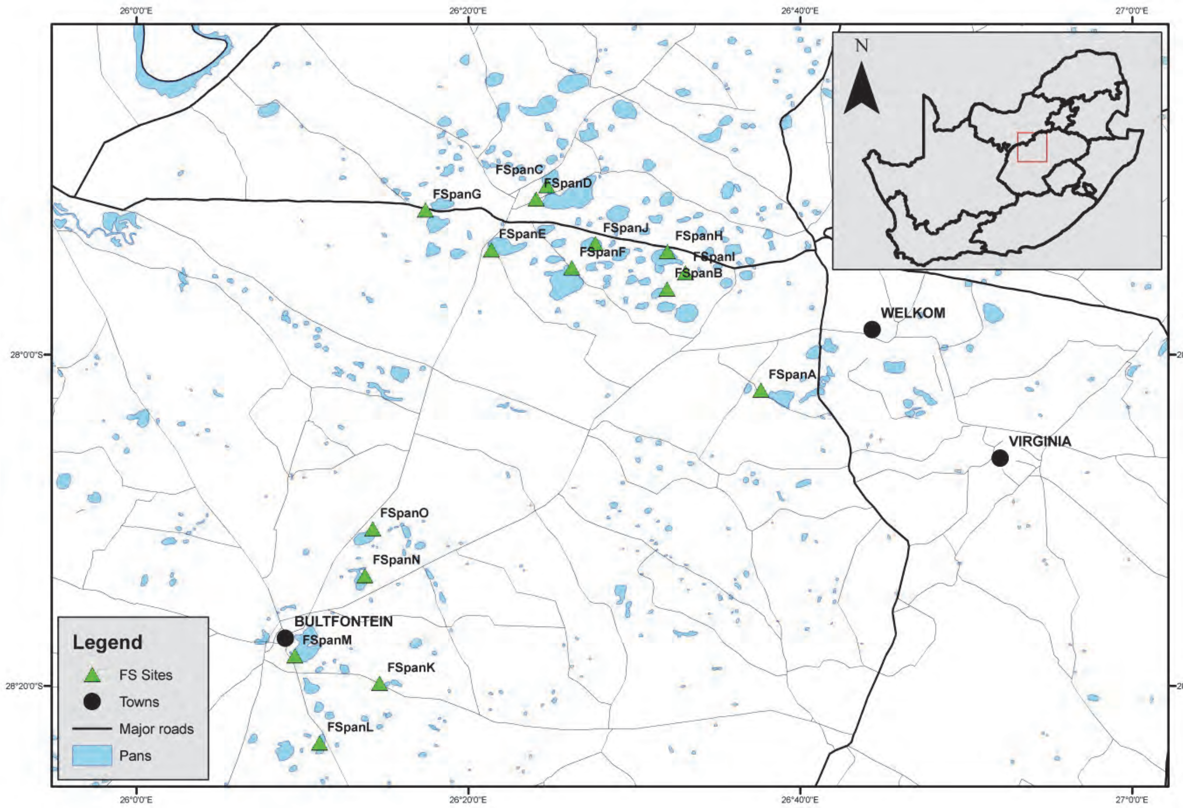


Figure 2-3: Site localities in the Free State region near Welkom, Wesselsbron and Bultfontein

FS pan A and FS pan B

FS pan A (Table 2-2) is situated the closest to Welkom and its associated impacts with regards to mining. The pan is an open pan and is one of the smaller pans in the study area. The pan surface was mostly open with little to no grass covering. The pan margin consisted mostly of grassland species with no noticeable impacts present. FS pan B (Table 2-2) is an oval pan and is classified as an open pan. The pan is of a moderate size. The surface of the pan consisted of patches of grass and open substrate, in the form of clay and silt. Anthropogenic activities within the pan catchment were limited to some grazing of livestock.

FS pan C

This pan (Table 2-2) is situated to the northeast of Wesselsbron. The pan is circular in shape with the substrate consisting mostly of clay and silt. The pan surface did contain a slight grass cover. The catchment and margins had numerous grass species present. Livestock activities were present within the catchment, but no overgrazing was evident.

FS pan D

FS pan D (Table 2-2) is one of the largest pans in the area and is classified as an open pan. The grass covering of the substrate was dense with few open patches visible. The substrate consisted of clay and silt. The margins of the pan were covered in grassland and no significant impacts were noticeable in the vicinity of the pan.

FS pan E and FS pan F

FS pan E (Table 2-2) is situated close to Wesselsbron and is an open pan. The pan surface consisted of grass cover along the periphery and an open surface towards the centre of the pan. The sediment consisted of clay and silt. The pan margins were covered in grassland. Some grazing activities were present around the pan but these were limited. FS pan F (Table 2-2) is situated approximately 10 km from Wesselsbron and is an open pan. The surface consisted of a combination of grass and open substrate. The substrate was mostly clay and silt. The pan margins consisted of open grassland. No anthropogenic impacts were evident.

FS pan G

This pan (Table 2-2) is situated to the west of Wesselsbron and is an open grass pan. The surface of the pan was completely covered with grass with no open surface present. The sediment in the pan was mostly sand and clay while the margins were dominated by grassland species. Some fences were present within the pan with evidence of livestock grazing, but no overgrazing was apparent.

FS pan H

FS pan H (Table 2-2) is situated between Wesselsbron and Odendaalsrus. The pan surface was a combination of grass and open surface. The sediment was mostly clay and sand. The pan is fed by a spring on the southern side that is also used for livestock watering. The water flow from the spring is determined by the groundwater level. The margins of the pan consisted of grassland. Some livestock activity was present.

FS pan I and FS pan J

FS pan I (Table 2-2) is an open pan with grass covering some of its surface. The pan surface consisted of sand and clay as sediment. The catchment was covered in grassland with a few shrubs present in the upper catchment. There was a fence running through the pan and some indicators of livestock activity. FS pan J (Table 2-2) is situated approximately 10 km from Wesselsbron in the direction of Odendaalsrus. It is a medium sized pan and had grass covering the majority of its surface. The sediment composition was of sand and clay. The margins of the pan were covered in open grassland with a few shrubs present. There was some evidence of livestock activity in the surrounding catchment.

The pans in the Free State have been experiencing a low rainfall season and as such most of the pans in the Wesselsbron and Welkom area have been dry for an extended period. Therefore it was decided to move in a south westerly direction to Bultfontein where numerous pans are also known to occur. It was evident that more rain had fallen in this area as most of the pans contained some water – even if it was only a few centimetres. An additional five pans were sampled from this pan for inclusion in the trophic state classification part of the project.

FS pan K

This pan is situated around 10 km outside of Bultfontein on the road to Theunissen. The pan is a small oval shaped pan with substrate composed mostly of mud and clay. There is a significant amount of reeds and sedges on the western side of the pan during this survey (Table 2-2). The pan was host to numerous birds and flamingos as it contained more water than other pans in the area. The catchment consists mostly of grassland with some evidence of grazing while the access road is also situated within the pan catchment.

FS pan L

This pan is of a rectangular shape with a very steep catchment that consists of grassland with evidence of cattle grazing (Table 2-2). The pan is significantly longer than its width with no marginal vegetation while the substrate is mostly mud and clay. The water level was low with only approximately 3cm of water. There was a large amount of Branchiopoda visible within the water column suggestion hatching had occurred recently. Very little bird life was present at the pan as compared to other pans in the Bultfontein area.

FS pan M

This pan is situated on the outskirts of Bultfontein and is the largest pan in the area as well as in the selected pans for the study. The catchment was mostly grassland with a few smaller trees and shrubs present further away from actual water boundary Table 2-2). The substrate consisted mostly of mud and clay while only a 2cm covering of water was present during the survey indicating that the pan was drying out. Inside the pan there was no significant vegetation while the marginal vegetation was also limited. The pan had an estimated 5000 flamingos that was feeding in the shallow water.

FS pan N

This pan is situated on the road from Welkom to Bultfontein approximately 10 km from Bultfontein. The pan is large and rectangular in shape (Table 2-2). The water level was significantly higher than any other pan in the Bultfontein and Wesselsbron area due to the larger catchment area and an inflow from the southern side. The catchment consisted mostly of grassland with evidence of cattle grazing. Some agriculture was present at the outer extremes of the catchment. No marginal vegetation or macrophytes were present while the substrate was mostly mud and clay.

FS pan O

This pan was situated on the road linking Bultfontein and Wesselsbron. The pan is mostly oval with a mud and clay substrate (Table 2-2). The water level was very low during the survey while many flamingos were also present at the site. There was no marginal or macrophyte vegetation present but some algal growth were present on the substrate. The surrounding catchment is mostly grassland with some shrubs present in the upper catchment. No agriculture was visible in the catchment but signs of cattle grazing were present.

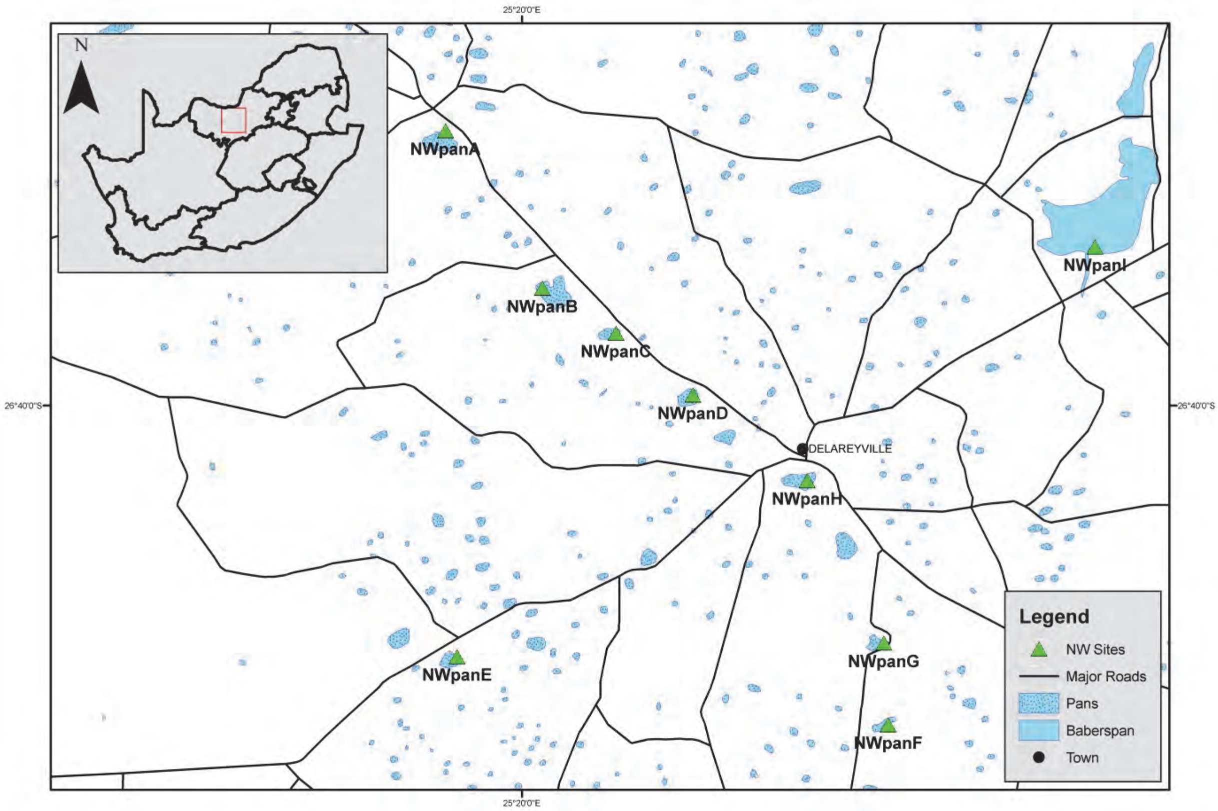


Figure 2-4: Site localities in the North West near Delareyville and Baberspan.

NW pan A and NW pan B

NW pan A (Table 2-2) is a large oval pan. The vegetation consisted of grassland species, and covered an extensive area around the pan before the nearest road. The pan surface was open and flat. Sediment composition was that of clay. The catchment of the pan was undisturbed with no agricultural activity or human settlements within the immediate vicinity. NW Pan B (Table 2-2) is oval in shape. The vegetation was much the same as NW pan A however more shrubs and trees were present on both embankments. This pan is situated on agricultural land. Livestock were fenced off in regions far from the pan vicinity. The pan bed showed signs of wild game activity as hoof prints were present. The pan bed was dry and had deep sediments composed of clay.

NW pan C

NW pan C (Table 2-2) is oval in shape. This pan has the same vegetation type as NW pan A and NW pan B with the grass species on the near embankment being showing farm land. The vegetation on the far embankment was covered in dense shrubbery. The pan was far less impacted by animal activity than NW pan B. The pan surface was open, flat and dry, with clay based sediments.

NW pan D

NW pan D (Table 2-2) is oval in shape. The vegetation varied from the previous three pans in that there was a diversity of grass species surrounding the pan. There was a forest of Eucalyptus trees occurring on the far embankment of the pan. No other anthropogenic impacts were present. The surface of the pan was dry with clay sediments.

NW pan E

NW pan E (Table 2-2) is large and round in shape. It is situated on agricultural land. Trees were present in the catchment but were scattered. The far embankment of the pan was close to a main road with sparse vegetation. The pan surface was open, flat and dry. There were waterfowl tracks present in the pan indicating that the pan was recently inundated.

NW pan F

NW pan F (Table 2-2) is round in shape. It was inundated with water during the survey. Waterfowl were present in large numbers mainly of the family Phoenicopteridae. The water was turbid and greenish in colour. Submerged macrophytes were present. The vegetation surrounding the pan consisted of a diversity of grass species with some reed species present along the water margin. This pan is situated on farm land, but was fenced off from livestock.

NW pan G

NW pan G (Table 2-2) is oval in shape and is relatively small. The vegetation with the catchment was that of grassland species, some encroachment of alien species of the Asteraceae family was evident. The substrate of the pan was hard, dry and of clay composition. Bedrock was exposed towards the periphery of the pan. No anthropogenic activities were present in the catchment of the pan.

NW pan H

NW pan H (Table 2-2) was situated very close to the town of Delareyville. The pan was large and round in shape. The vegetation was that of mixed grass species. The pan bed was semi-dry, with sediments consisting of clay. The water was dark in colour and rich in organic content, possibly due to municipal effluent. The pan however was fenced off from all sides and showed no signs of agricultural activity. There were no human settlements within the immediate catchment.

NW pan I

NW pan I or Barberspan (Table 2-2) is irregularly shaped. This pan is not a true pan as it has a freshwater inlet. The pan is however a RAMSAR site, and thus was incorporated into the study for its status. The pan was inundated with water which was turbid and green in colour. There were both submerged and emergent macrophytes present within the pan. The pan had sandy sediment. Waterfowl from the family Rallidae were abundant during the survey.

3 HATCHING METHODOLOGY

A brief summary of some of the literature which shaped this project design and the method which lead to optimal hatching success is discussed here.

3.1 The advantages of hatching experiments

Dormant eggs of temporary wetland inhabitants can be invaluable in studies of ecological biogeography, biodiversity, evolutionary ecology and community and population ecology. This is because emergence of individuals from egg banks, especially in isolated wetlands, can be the most important colonization process after first in inundation of that wetland (Leibowitz, 2003). Individuals hatching from egg banks form the primary colonizers of wetlands while macroinvertebrates, such as Coleoptera and Hemiptera, form the secondary colonizers and appear when water levels are more stable. Therefore, the emergence of individuals from egg banks has impacts on future population, community and ecosystem structures (Brendonck and De Meester, 2003). For instance, egg banks can be responsible for the recruitment of old genotypes into the water column that might have been lost over time, therefore restoring genetic variability for certain environmental responses (Hairston et al., 1996; Hairston and Kearns, 2002).

In addition dormant eggs have been suggested as useful tools in indication of anthropogenic stress in environmental monitoring practices. Anthropogenic activities have the potential to affect emergence patterns from dormant eggs. Therefore the emergence of individuals from egg banks can be used as indicators of ecological stress (Angeler and García, 2005). This may prove difficult at first because of the large variability in hatching patterns these organisms show making it hard to pin point changes on natural variability or anthropogenic stressors. However, should reference conditions be determined the ability to distinguish changes is improved. The emerged community from a non-impacted site should differ from that of an impacted site as sensitive species would fail to emerge in the presence of the stressor and so the diversity, abundance and taxonomic richness should differ (Gleason et al., 2003; Nielsen et al., 2003; Angeler and García, 2005). The downfall to this is that the egg bank structure can vary between wetland types, implying that it would be better to compare impacted and non-impacted wetlands of the same type to get an accurate interpretation of impacts. This can be achieved by using historical information of the wetland (Innis et al., 2000). Consequently this is another field in which egg banks prove useful, and is known as reconstruction ecology. Since egg banks provide an accessible record of local history and integrate annual and seasonal variation the genetic structure of the egg bank can be used to reconstruct evolutionary changes in the past (Weider et al., 1997; Brendonck and De

Meester, 2003). Thereby a thorough analysis of an egg bank from a single wetland can provide the required information to set a reference condition for the future monitoring of that wetland itself. Overall, making egg bank studies that assess the alterations in community assemblages useful tools in determining ecological integrity (Angeler and García, 2005).

3.2 The disadvantages of hatching experiments

The majority of hatching experiments in the past were concerned with determining the hatching characteristics of certain species, to aid in future toxicology testing in which specific and standard hatching conditions are a requirement. One of the most studied branchiopods is the brine shrimp Artemia as it is used in toxicology and aquaculture. The problem with hatching experiments is that only a fraction of the viable eggs hatch during any one period of incubation (Vanderkerkhove et al., 2004). The most probable reason for this erratic hatching pattern has to do with the different egg types i.e. the diapausing and quiescent fraction of eggs within the sediment. Both egg types respond differently to hatching cues (Brendonck, 1996). Many studies have indicated the variability in hatching under identical culture conditions with eggs that have been treated in the same manner, even from the same brood (Moore, 1957, 1959, 1967; Seaman et al., 1991; Scott and Grigarick, 1979; Van Dooren and Brendonck, 1998). Therefore, the standardization of conditions can be difficult. Ecotoxicology studies have proven to be problematic due to this variation and lack of standardization.

Diversity studies are challenging as determining the right exposure conditions to obtain maximal hatching of a wide range of species from a mixed egg bank within the sediment, as opposed to singular test species, is difficult. It is also impossible to evaluate the full diversity of the egg bank from a single hatching attempt as anything that hatches will be dependent on the conditions they were exposed to. Therefore laboratory experiments should be carried out using a wide range of conditions as close as possible to the environmental conditions from which the eggs came to make extrapolation of results more relevant (Vanderkerkhove et al., 2005).

The major downfall with laboratory based experiments, especially when it comes to assessing the cues that terminate dormancy and induce hatching, is that they overlook the relevance in nature. Laboratory experiments use artificial conditions. For example: the decapsulation of eggs, constant light exposure and using a limited number of clones. Laboratory results as a consequence do not adequately predict how the termination of dormancy influences the natural population (Cáceres and Schwalbach, 2001). Studies that have investigated diapause termination in the field have invariably only documented the timing and rate of hatching without directly testing the mechanisms involved driving the patterns of emergence (Cáceres and Schwalbach, 2001). Mesocosm experiments are now being used more frequently in egg bank studies as they offer improved environmental realism with a better predictive potential, while still being replicable and enabling laboratory-like manipulations (McCullough and Horwitz, 2010). Therefore the study design and the appropriate use of hatching experiments are dependent on the outcome of the experiment. There a numerous ways to approach such experiments and a fair amount of manipulation of test conditions can be involved in obtaining the desired outcomes. Such determining factors should be considered before the start of the experiment.

3.3 Variables to consider

3.3.1 Salinity

The salinity of temporary waters increases over time as water evaporates from the surface. This makes salinity an important variable to consider when conducting hatching experiments, especially since different groups of organisms have different tolerances to salinity (Belk and Cole, 1975; Williams, 1985). Salinity refers to the amount of dissolved salts and conductivity refers to the charge of these salts. Both terms are related and are used interchangeably in the literature, but are one in the same concept and imply the same thing when it comes to hatching cues and conditions. Different groups of wetland inhabitants have different tolerances to salinity (Belk and Cole, 1975), with Ostracods having one of the greatest salinity tolerance range (Martens, 1985; Kerfoort et al., 2007).

Ortells et al. (2005) studied the salinity tolerance in Daphnia magna by incubating D. magna eggs at different salinities to test if they showed any preferences under the different salt conditions. To do this distilled water was used and adjusted to 3 different salinities with commercially available sea salt. The salinities were 0, 3 and 6 g/l respectfully, with the 0 g/l serving as a control. The eggs were incubated at 20°C under constant light conditions for a period of 30 days. Hatchlings were transferred to culture jars filled with oxygenated lake water adjusted to the experimental salinity levels. The study found that there was an absence of a salinity preference for hatching, as well as no differentiation in growth and egg production between the individuals surviving and growing under the different saline conditions. However there was a difference in the number of individuals surviving, with more mortalities occurring in the 6 g/l salinity treatment.

Waterkeyn et al. (2009) found that salinity was the most important variable in explaining the distribution of large branchiopods in the Camargue wetlands in France. Laboratory results revealed that the total number of species hatching from sediment declined significantly with increasing salinity, with salinities of 5000 mg/l proving fatal for the few individuals. This corresponds with other studies such as those by Ketley (2007) and Nielson et al. (2003). Hatching experiments by Ketley (2007) revealed that salinity affected the hatching rate with the fastest hatching of individuals occurring at the lowest salinity with minimal differences in hatching abundance between salinities of 0 mg/l and 1000 mg/l. Salinity only affected hatching abundance above 1000 mg/l, with no hatching taking place at 5000 mg/l. This concurred with the study by Nielson et al. (2003) whom also found that salinities between 1000 and 5000 mg/l decreased species richness and abundance of organisms in both a temporary and semi-permanent wetland in Australia. They concluded that increasing salinities in wetlands results in a loss of biodiversity. It was also noted that the salinity effect was not permanent, as hatchlings that did not appear at high salinities did start to appear when they were dried and re-inundated in water of a lower salinity. Nielsen et al. (2012) further demonstrated that dormant eggs of zooplankton can survive in sediment exposed to high salinities that would kill off the adult population for up to 22 months. But eggs could only hatch once the stressor was removed. While the dormant eggs seem to be resilient to high salinities adult stages are not as tolerant for many species. Thus it would seem that low salinities are good triggers for hatching on a broad scale.

Vanschoenwinkel et al. (2010) emphasized the importance of low initial conductivities as hatching cues as conductivity is a predictor for the length of inundation in a wetland. Some laboratory studies have shown that low conductivities promote hatching and elevated conductivities inhibit hatching (Brown and Carpelan, 1971; Sam and Krishnaswamy 1979; Brendonck et al., 1998). This is, however, very much dependent on the test species as different species have different ranges of tolerance and thus different conductivities initiate hatching depending on the habitat from which individuals come. Ito (1960) found that for the rotifer Brachionus plicatilis the salinity at which egg production took place determined the optimal salinity concentration at which hatching occurred.

3.3.2 Temperature and photoperiod

Temperature is one of the most important environmental variables for stimulating quiescent eggs to hatch along with growth and survival as temperature can affect the oxygen concentration of water systems as well as the metabolic rate of organisms (Horne, 1971; Brendonck et al., 1996; Ali and Dumont, 1995).

Vanderkerkhove et al. (2005) conducted a study to investigate how the variations in optimal hatching conditions for Cladoceran resting eggs from different (temperate) regions interfere

with the interpretation of species richness patterns in hatching assemblages. Sediment samples were incubated under four different temperature regimes (10, 15, 20 and 25°C respectively) and two different photoperiods. The sediment samples had a pre-incubation period of four months at 4°C. The resting eggs were isolated from the sediment by means of the sugar flotation method (Onbé, 1978). The resting eggs were incubated in 2 litre aquaria filled with diluted ADaM medium (200 µS/cm). The incubation medium was renewed every nine days. The aquaria were randomly selected and exposed to different temperature regimes and photoperiods. In this study it was found that the hatchling abundance was highest at 10-15°C and declined with increasing temperature, and by incubating the eggs at lower temperatures a larger portion of the regional species pool was able to be detected.
Ketley (2007) tested the hatching abundances of dormant eggs exposed to temperatures of 10, 15 and 25°C respectively from sediment collected in the Western Cape, South Africa. Results indicated that at 10°C the hatching abundance was lower and the time taken for hatchlings to first appear was much longer, as compared to 15 and 25°C. The greatest hatching abundance, however, occurred at 15°C. Various species of branchiopods have been studied and optimal temperature regimes recorded (Triops, Streptocephalus, Artemia and Daphnia species respectively), but in some species it is not the optimal temperature range but the optimal regime of successive temperature conditions that best enable hatching. Moore (1967) found that in Streptocephalus seali fluctuating temperatures in the range of 19-23°C increased the numbers hatching, compared to when constant temperatures within this range were used. Vanderkerkhove et al. (2005) found that the effect of photoperiod on the overall hatchling abundance varied among the cladocerans from the different regions sampled.

Regarding photoperiod Ketley (2007) also found that there was no significant difference between the number of hatchlings incubated under constant light conditions and those incubated under a 12 hour light/dark regime. The constant light conditions did have a significant effect on the time it took for maximum numbers of invertebrates to hatch. Constant light therefore has a positive effect on the rate of hatching and not the abundance of hatching. Mitchell (1990) had similar findings with regards to constant light conditions when determining the factors affecting the hatching of Streptocephalus macrourus eggs. It has been said that light is required to trigger the onset of embryonic metabolism (Sorgeloos and Persoone, 1975). Constant light was recommended by Ketley (2007) for future studies as it allows a faster growth rate of algae, which in turn provides more food for the growing larvae.

It is important to note that the study by Vanderkerkhove et al. (2005) was performed on Cladoceran resting eggs (an order of Branchiopoda) and so these exact conditions for hatching cannot exactly be applied when trying to hatch a mixed egg bank of the greater Class. The study also took place in temperate regions using temperate species, therefore caution needs to be applied when using tropical species as they respond to conditions differently. A similar point can be made regarding the study by Ketley (2007) where hatching experiments were performed on sediment collected from pans in the Western Cape, which is a winter rainfall region. Therefore inundation and henceforth hatching of invertebrates occurs in winter where the water temperature of the pans are substantially lower, which explains why low experimental temperatures proved optimal. These temperatures cannot be applied with certainty to summer rainfall regions.
Vanderkerkhove et al. (2005) cautioned against the use and interpretation of laboratory conditions and stated that, “the stimuli for induction and termination of diapause are adjusted to the prevailing environmental conditions by natural selection in order to maximize survival, growth and reproduction of the local population. This makes the natural conditions of the wetland itself more important in determining the right hatching cues in the laboratory when compared to the conditions used in other studies”. In this regard making use of mesocosm studies can provide more accurate results with dormant eggs being exposed to natural temperature and light regimes (Ning et al., 2011). It has also been noted that good hatching conditions do not necessarily promote growth and survival because of the natural variation in environmental conditions during a hydroperiod (Waterkyn et al., 2009) a change in conditions may be required to promote growth of individuals.

3.3.3 Desiccation

As ephemeral wetlands experience a wet and dry phase their inhabitants have to be well adapted to surviving the dry phase. The dormant eggs produced by the inhabitants are thus desiccation resistant. Furthermore because desiccation is an integral part of the life cycle of such inhabitants it is also therefore an important prerequisite to egg hatching in most species, and is thus considered a hatching cue.

Mura (2005) tested the possible effects of pre-incubation treatment on the hatching success of Anostraca egg banks. Sediment cores were divided into two halves (transects), with half being incubated immediately and the other half being oven dried at 30°C prior to incubation. The samples were incubated at 18°C with a 12 hour light/dark photoperiod and were monitored daily for a period of one month. It was found that there was a marked difference in the hatching success of the two transects. The wet sediment showed a much more erratic hatching pattern. Statistical analysis revealed that the time at which cysts of the same age hatched was significantly affected by the initial sediment conditions (dry or wet), with dry sediment producing better results.

In Artemia species drying the eggs at temperatures of up to ± 35°C has been found to have a beneficial effect on terminating dormancy (Sorgeloos et al., 1976). Takahashi (1977) found that desiccation of Triops longicaudatus and Triops granarius eggs increased the percentage of eggs hatching, with the length of the desiccation period being a significant factor. Mitchell (1990) found using S. macrourus that longer periods of desiccation lead to higher numbers hatching up to one year, thereafter hatching decreased with longer desiccation periods. It has also been found that in some instances several desiccation and hydration cycles are required to induce hatching of dormant eggs (Fox, 1949; Takahashi, 1977). Not all species require desiccation as a prerequisite for hatching (Brendonck, 1996), but for those species that do desiccation benefits the rate of hatching.

3.3.4 Turbidity

In various field studies turbidity has been positively correlated to species richness. Turbid waters are composed mainly of clay particles which have an increased surface area for the attachment of organic matter and bacteria, thus increasing the diversity of food types available for filter feeding organisms (Thiéry, 1991, Waterkeyn et al., 2009). However, laboratory based hatching experiments of Day et al. (2010) indicated that the higher the turbidity, the longer it took for nauplii to hatch. This is possibly because increased turbidity reduces light penetration and darkness has a negative effect on hatching (Brendonck et al., 1993; 1998). If light is a cue for hatching, then turbidity will have a significant impact on the hatching response. Reduced light penetration also leads to reduced primary production which could impact on feeding success thereby slowing development and productivity rates (Day et al., 2010). Turbidity within pans tends to only increase as the hydroperiod progresses after each successive inundation, and thus turbidities are usually quite low at the beginning of the season (Meintjes et al., 1994). Low turbidity would thus prove better for laboratory based hatching experiments and would serve to make the identification of individuals easier.

3.3.5 Sediment depth

Sediment depth is an important factor to consider in hatching experiments, as it has been found that hatching is mainly restricted to dormant eggs situated in the top 2 cm of sediment. The largest fraction of viable eggs generally occurs in the top 4-6 cm of sediment (Herzig, 1985). Natural disturbance of the sediment through bioturbation of fish and burrowing organisms, as well as aquatic birds and large game determines the extent to which buried eggs are re-suspended and exposed to suitable hatching cues (Cáceres and Hairston, 1998; Brendonck and De Meester, 2003). Eggs in the top 10 cm of the sediment therefore have a reasonable chance of being exposed to hatching cues (Hairston et al., 2000).

Gleason et al. (2003) assessed the effects of sediment burial on the emergence of individuals from egg banks. To assess this 1 cm of sediment containing eggs was placed in containers with dimensions of 19.5 x 19.5 x 6 cm to form a composite layer. The composite sediment layer was then overlain with 0, 0.5, 1 and 2 cm respectively of sterilized soil in order to create different burial depths for the egg banks in the composite layer. The eggs were incubated for a period of six weeks at a constant temperature of 10°C for the first three weeks and then raised to 20°C for the next three weeks. A 12 hour light/dark photoperiod was used. Distilled water was added to the containers to maintain the water levels and the salt concentrations. The hatched individuals were removed from the containers every two weeks by siphoning the water through a 0.1 mm screen. The results obtained from this study indicated that a sediment overburden of as little as 0.5 cm can inhibit hatching success. Burial may affect hatching cues or may provide a physical barrier to emergence (Gleason et al., 2003).

3.3.6 Oxygen

The amount of oxygen that can dissolve in water varies with temperature, as high temperatures reduce the oxygen holding capacity of water and enhance the metabolic rate of inhabitants. As water evaporates the inhabitants become more concentrated in space, further increasing the oxygen demand. Temporary wetland inhabitants frequently experience hypoxic conditions, and use physiological and behavioural adaptations to cope with this (Horne, 1971). Data obtained by Day et al. (2010) indicated that temporary wetland crustaceans can occur at relatively low levels of dissolved oxygen. In situ data showed that oxygen levels ranged from 2.3 to 1.5 mg/l amongst the different sites. Cladocerans, ostracods and copepods were the most abundant between about 2 and 8 mg/l, and conchostracans and anostracans between 4 and 8 mg/L. Higher concentrations of oxygen are not a limiting factor, but minimum concentrations often are.

3.3.7 pH

pH can play a role in the efficiency of hatching with different species once again having different tolerances depending on the environment from which they come. Most species have a preference for an alkaline pH, in particularly Artemia species have an optimum hatching pH in the range of 8-8.5. Triops species have been found to have a preference for the acidic side of the pH scale (Scott and Grigarick, 1979). The pH of the hatching medium is said to affect the optimal functioning of the hatching enzyme which is responsible for the digestion of the inner cuticular membrane thus allowing the release of the free-swimming nauplius (Sato, 1967).

3.3.8 Decapsulation

Studies have also used a decapsulation technique in which the outer membrane of the egg is chemically removed by placing the eggs in a hypochlorite solution. This enhances the hatching rate and abundance because embryos have to expend less energy to hatch. Lower intra-cystic osmotic pressures are required to break the tertiary membrane. Decapsulation has also been said to make eggs more sensitive to light, and if light is a requirement for hatching then this will have a positive effect on hatching (Brendonck et al., 1996). This would make laboratory experiments more successful as one can extrapolate a larger portion of the egg bank at any given time. However, decapsulation, if exposed to hypochlorite for too long, can negatively affect hatching success through chemical damage to the outer cuticular membrane or embryonic cuticle. Decapsulation, if not done properly, poses a risk to the successful hatching of egg banks (Brendonck et al., 1996).

3.3.9 Isolation

Decapsulation of eggs requires isolating them from the surrounding sediment. There are various isolation methods (De Stasio, 1989; Duggan, 2002) but the most commonly used is the Onbé-Marcus method (Onbé, 1978; Marcus, 1990). Vanderkerhove et al. (2004) evaluated the hatching rate and hatching success of zooplankton resting eggs with and without isolation of the resting eggs. Results indicated that isolation by means of the Onbé-Marcus method is very effective and advantageous in terms of overall hatching success and hatching synchronization. Isolation however can be a time consuming and tedious process that requires large volumes of sediment in order to increase prospects of finding eggs as eggs could easily be lost in the process given their small size. If eggs have been isolated from the surrounding sediment, a culture medium is then required to supply the nutrients that have been lost with the removal of the sediment. However, the medium is unable to provide food for the developing hatchlings, and thus supplementation is required (Ketley, 2007). Isolation is therefore not very feasible and is dependent on the experimental outcomes.

3.3.10 Summary

In summary most hatching experiments have been conducted using:

- 2 litre aquaria
- Low salinities/conductivities

- A culture medium (ADaM medium)
- Temperatures ranging from 10-20°C
- Alternating photoperiods
- Single test species
- An exposure period of one month

3.4 Materials and methods

3.4.1 Sediment preparation

Sediment samples were collected from selected pans in the North West, Free State and Mpumalanga Provinces. These regions included Delareyville, Wesselsbron and Chrissiesmeer respectively. An auger was used to obtain five core samples from each pan with care being taken to sample the top 10cm of sediment from each area. The core samples were collected at randomly selected areas of the pan, working from the periphery towards the centre of the pan. Core samples were taken from the deepest section of the pans when these sections could be identified. The deepest section of the pan should be the last to dry up and the sediment should contain newly deposited branchiopod egg banks if any are present (Day et al., 2010). Newly deposited eggs should be more viable. The core samples were transported back to the laboratories at the University of Johannesburg where hatching experiments were performed. In the laboratory the five core samples taken from each pan were combined to form a composite sediment sample. The composite samples (Figure 3.1) from each pan were desiccated for a minimum period of four weeks. This was done by placing the composite samples in an environmental room (controlled laboratory conditions). On a weekly basis the sediment was turned to allow mixing of the eggs in order to get a random distribution throughout the sample.

3.4.2 Hatching experiments

After the four week desiccation period five pans were selected at a time to perform the hatching experiments. The pans selected in the Free State Province were chosen to undergo the hatching experiments first as they were the driest after the four week desiccation period. Following the hatching experiments of the Free State pans were the North West and Mpumalanga pans respectively.

During the experiment, 2 L plastic containers were used and 25 g of sediment was added to each container in replicates of five (n=5). Each replicate was allocated a different treatment (Figure 3.1). Three treatments were used which included distilled water adjusted to a salinity of 1000 mg/l, distilled water adjusted to a salinity of 1500 mg/l, and AMD. The two salt solutions (1000 mg/l and 1500 mg/l) were adjusted using commercially available sea salt and served as control solutions. The salinities of the controls were decided upon based on hatching experiments performed by Ketley (2007). The AMD was collected from a current decant point near the Krugersdorp Game Reserve in Gauteng, as close to point of origin as possible to prevent the mobilization of heavy metals from the sediment being a factor. Water quality variables of the AMD were analysed prior of its addition to the sediment to ensure that chemistry was standard. The same decant AMD was also used throughout the course of the experiment to ensure that no external factors such as chemical treatment could influence the chemistry. The containers were filled with 1 L of their respective solutions. At intervals during the course of the experiment the containers were topped up using distilled water when it was observed that the water had evaporated below the 1 L point. The containers treated with AMD were topped up with the decant AMD to avoid dilution of the contents, which would interfere with recovery experiments to be run at a later stage.

The hatching experiments were conducted over a period of 28 days using a temperature exposure of 18°C and a 24 hour photoperiod. Every fourth day the physico-chemical variables of all the containers were measured using an EXTECH DO 700 multimeter. During the measurement of the physico-chemical variables each container was also examined for the presence of any hatchlings (nauplii emerging), and the abundance of the hatchlings, if any, were recorded. Any hatchlings which were observed were removed from the containers and placed in polyethylene jars containing 300 ml of ADaM medium (Kluttgen et al., 2004). This was done to prevent cumulative counts of emerged individuals. It was also done to allow the development of the hatchlings where survival and growth would be easier to monitor. The hatchlings in these containers were fed a diet of “Daphnia food” (Truter, 1994). After the 28 day exposure period all the hatchlings were fixed and preserved in 96% ethanol for identification using a standard light microscope.

Recovery experiments were performed after the initial hatching experiments on the sediment exposed to AMD only. This was done to test whether the egg banks exposed to the AMD could recover from its exposure, given that not many individuals hatched upon first exposure. These experiments were performed by allowing the AMD to completely evaporate from the containers. Once this had occurred, the sediment was again left to undergo desiccation for a period of two weeks which was substantial enough for the sediment to be completely dry given the quantity within the containers (25 g). After the desiccation period 1 L distilled water was added to the AMD containers. Once again the physico-chemical variables were recorded and the number of hatchlings counted every fourth day, along with evaporation being monitored.

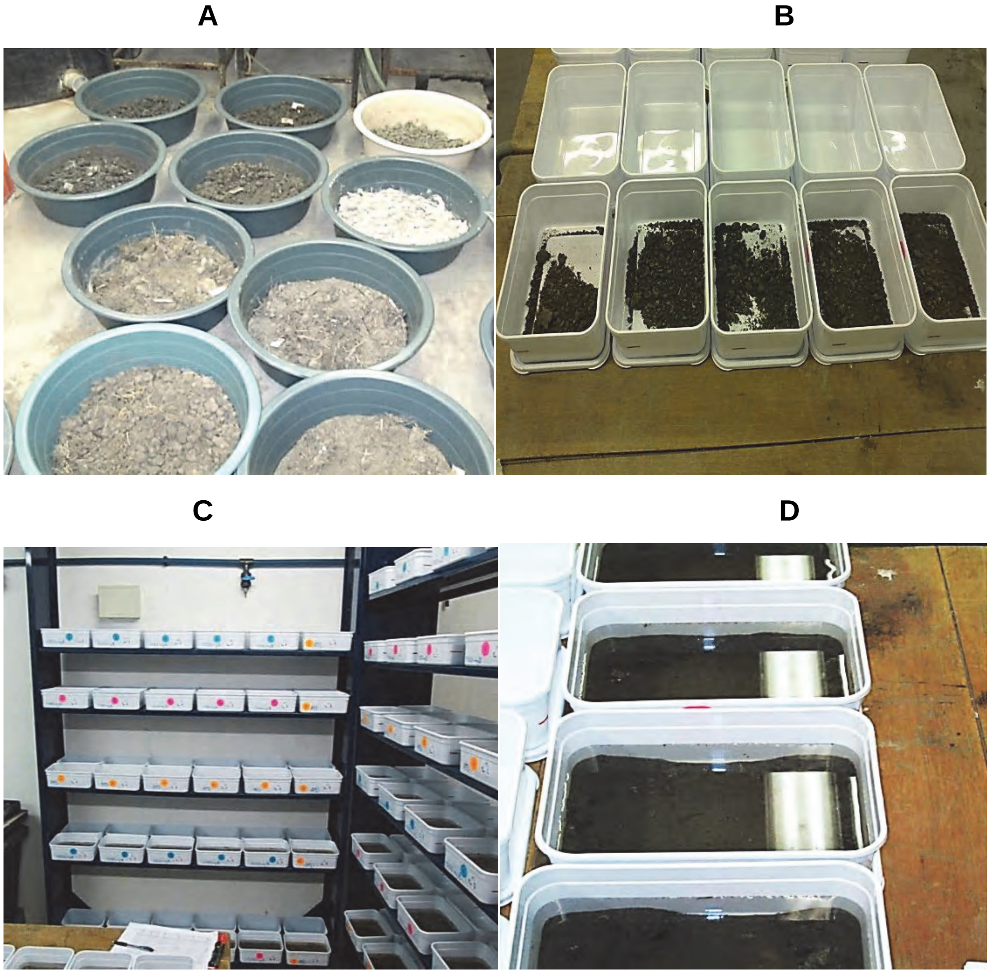


Figure 3-1: Photographs of sediment from selected pans undergoing desiccation (A) and the experimental setup (B-D).

3.5 Statistical analysis

3.5.1 Hatching success

Non-parametric statistics were used to determine the overall hatching success of nauplii between treatments and between pans. SPSS statistical software, version 21 was used to run these univariate statistics. Kruskal-Wallis H tests were used to test for significant differences in hatching abundances between pans with the separate pans used as the dependent variable and the number of hatchlings used as the independent variable. Mann-Whitney U tests were used to test for significant differences in hatching abundance between the separate treatments within each pan. For this test the treatment was used as the dependent variable and the number of hatchlings the independent variable. Assumptions of normality for all univariate statistics were tested for using Shapiro-Wilks goodness of fit test (Pallant, 2007; Bless and Kathuria, 1993).

3.5.2 Diversity indices

Indices of diversity were applied to describe the abundance relationships among the communities of the separate pans, and were calculated using PRIMER 6 software (Clarke and Warwick, 1994). Species richness was expressed as Margalef's index (Margalef, 1968), while the evenness was expressed as Pielou's evenness index (Pielou, 1975). Species richness is a measure of total number of species in community. Evenness measures how evenly individuals in a community are distributed over the number of different species (Heip et al., 1998). Shannons index (Shannon-Wiener, 1963) was incorporated as it combines both species richness and evenness components (Clarke and Warwick, 1994).

3.5.3 Temporal analysis

Multivariate analyses were used to assess the spatial variation in the community structure of hatched individuals from the selected pans. PRIMER version 6 and CANOCO version 4.5 software was used. Analyses included Bray-Curtis similarity matrices subjected to group average clustering, constructed from the hatchling abundance data and the community data of each pan (Clarke and Warwick, 1994). Principal Component Analyses (PCA) bi-plots were also constructed. PCA is based on a linear response model which relates species and environmental variables (Van den Brink et al., 2003). The resulting ordination is a map of the samples analysed on a 2-dimensional bases. The placement of the samples on the bi-plot reflects the (dis)similarities between them. The separate pans from all regions represented the different sampling sites. The placement of the taxa on the bi-plot reflects the taxa responsible for pan groupings.

4 BRANCHIOPOD HATCHING SUCCESS

4.1 Introduction

Pans are endorheic wetlands, which refers to the closed nature of the drainage system. There is no inflow, outflow or ground water recharge. Water within the pan is a result of direct precipitation and evaporation rates usually exceed that of precipitation. As a result pans are prone to drying out at least once a year. Temporary wetland is another term commonly used to describe such environments because of their ephemeral nature (Hutchinson *et al.,* 1932; Wiggins *et al*., 1980; Geldenhuys, 1982). Temporary wetlands are highly variable ecosystems and undergo changes in physical and chemical characteristics on a regular basis. These variations are brought about by changes and differences in the substrate present in a wetland, inundation and desiccation patterns, local climate and the physical dimensions of the wetland. As a result, the invertebrate fauna that inhabit these environments have various physiological, behavioural and structural adaptations (De Roeck *et al*., 2007), enabling their survival in a constantly changing environment.

The diversity of fauna is known to differ in species composition from site to site and therefore contributes disproportionately to regional diversity (Oertli *et al*., 2002; Williams *et al*., 2004). The pan community and the development thereof during the first few days of inundation is characterised by the presence of r-selected species which are small in size, emerge shortly after inundation, and mature and reproduce rapidly (Wiggins *et al*., 1980; Williams, 1998). These species are usually referred to as the primary colonisers. As the inundation period progresses the community shifts towards the k-selected species. These species form the secondary colonisers and consist of migratory species (Lahr *et al*., 1999). Due to the short inundation periods, k-selected organisms such as fish are unable to survive and reproduce within the time constraints, and are usually absent from pans (Wellborn *et al*., 1996). The most unique group of fauna inhabiting pans, which are considered a flagship group, are the branchiopod crustaceans. The branchiopods form part of the primary colonisers and are well adapted to surviving the alternating wet and dry phase of the pan environment (Belk and Cole, 1975).

The class Branchiopoda, descriptively termed phyllopods, consist of the orders Anostraca (fairy and brine shrimp), Notostraca (tadpole shrimp), Conchostraca (clam shrimp) and Cladocera (water fleas). Branchiopoda without the cladocerans are commonly referred to as the “large branchiopods” (Brendonck *et al*., 2008). The Anostracans are elongated and lack a carapace. The Anostracans are omnivorous filter feeders feeding on particles suspended in the water column (Brendonck, 1993; Brendonck *et al*., 2008). The Notostracans have a shield shaped carapace which is attached only at the head region (Fryer, 1988). These shrimp are predominantly benthic and are omnivorous, feeding on detritus and smaller organisms, living or dead, such as cladocerans (Martin, 1992). The Conchostracans are laterally compressed bodies which are enclosed in a bivalve carapace (Brendonck *et al*., 2008). Conchostracans are benthic and feed on detritus and algae in suspension (Belk, 1982). The Anostraca are the most taxonomically diverse group, and in southern Africa 46 species of Anostraca have been recorded from the subcontinent with *Streptocephalus* and *Branchipodopsis* being the most specious genera (Hamer and Appleton, 1996; Hamer and Brendonck, 1997; Brendonck *et al*., 2008).

Branchiopods and a few other zooplankton taxa make use of the escape in time survival strategy, achieved through the production of an egg bank (Nourisson, 1964; Hairston *et al*., 1996). The egg bank consists of desiccation resistant eggs which lie dormant in the sediment during the dry phase, and only hatch upon the return of favourable conditions when the pan is once again inundated with water (Thiery, 1996; Brendonck *et al*., 1998a). These dormant eggs have also been referred to as resting eggs. The egg bank creates a storage effect which has the sole function of buffering against species loss during periods of unfavourable growth when conditions are not satisfactory enough to allow full development of the emerged population (Chesson, 1983; Brendonck *et al*., 1998a). This means that the community dynamics are uncoupled from the immediate effects of the previous growing season (de Stasio, 1989). The egg bank functions as a filter whereby different species are favoured for hatching at different times, enabling coexistence (Tempelton and Levi, 1979), and not all individuals from a single species will hatch during a single inundation, thereby creating a generation overlap (Ellner and Hairston, 1994). The egg bank therefore plays an important role in the reestablishment of invertebrate communities, ensuring the continuation of biotic diversity and delaying population extinctions (Nelson and Hairston, 1996; Euliss *et al*., 1999). Cohen (1966) made the prediction that in variable environments the theoretical fraction of eggs to hatch at a given site per generation should be proportional to the frequency of a good season for reproduction and the chance for successful development.

4.1.1 Eggs, Dormancy and Hatching Cues

The eggs of branchiopods occur in different shapes and sizes and have an outer protective cover in most species (Fryer, 1996). In large branchiopods the shape of the eggs ranges from sub-spherical to disc-like and cylindrical (Brendonck *et al*., 1998b). The shape of the eggs can be used for identification in some groups at a higher taxonomic level (Brendonck and De Meester, 2003). Studies that have investigated egg biology have focused on *Artemia* species as a test organism. Eggs consist of three layers. The first and outer most layer of the egg is the alveolar layer which consists of lipoproteins. This layer protects the egg from mechanical disruption. The second layer is the outer cuticular membrane which protects the embryo from penetration by molecules larger than carbon dioxide. The third layer is the embryonic cuticle which is a transparent elastic layer which covers the inner cuticular membrane, the membrane in direct contact with the embryo. Hatching occurs in eggs through the uptake of water in which case the egg swells and becomes spherical in shape. Eggs are very hygroscopic and take up water rapidly. Eggs are said to become active at a 60 % level of water uptake provided conditions are favourable. Thus at this level the ametabolic egg regains its metabolic activity. Through aerobic metabolism the embryo converts its carbohydrate reserve of trehalose into glycogen and glycerol (Clegg, 1964). The increased levels of glycogen and glycerol, which are also hygroscopic, result in the further uptake of water by the embryo. The water build up creates an osmotic pressure inside the outer cuticular membrane which builds up until a critical level is reached. The obtainment of such results in the breaking of the alveolar and outer cuticular membrane which releases the embryo still encased in the embryonic cuticle which is also referred to as the hatching membrane. The embryo which is a metanauplius then differentiates into an instar I nauplius which starts to move about. A hatching enzyme secreted in the head region of the nauplius weakens the hatching membrane and through the mechanical efforts of the nauplius it eventually breaks through the membrane and is released to the surrounding water (Hall and MacDonald, 1975; Van Stappen, 1996).

Branchiopod eggs have been found to exhibit different states of dormancy. Diapause is one state of dormancy where the arrest in development is initiated by internal factors. Eggs do not hatch even when environmental conditions are favourable as diapause termination is also internally controlled (Lavens and Sorgeloos, 1987; Drinkwater and Clegg, 1991; Brendonck *et al*., 1993). Quiescence is an alternate state of dormancy where the arrest in development is initiated by external factors. Quiescence is induced by unfavourable external conditions and is terminated as soon as conditions are permissible (Lavens and Sorgeloos, 1987; Drinkwater and Clegg, 1991; Brendonck, 1996). Both forms of dormancy have been found to occur in a single brood of eggs. Quiescent eggs respond rapidly to a change in environmental conditions giving species a quick start to colonisation before the pan dries up (Brendonck, 1996). Diapause is most likely the phenomenon which ensures some eggs always remain dormant in the sediment to insure the continuation of the species over long periods of time and is most likely responsible for the long term viability of eggs in the egg bank. Viable zooplankton eggs have been found in sediment dated between 40 and 125 years old (Brendonck and De Meester, 2003).

In order to synchronise the life cycle with suitable conditions for growth, species require mechanisms for detecting the appropriate time of year and stage within the inundation period to hatch. The cues for hatching differ according to the species and the predictability of the habitat (Brendonck and De Meester, 2003), and even differ between conspecifics from different geographic origins (Marcus, 1984). Environmental cues that have been linked to hatching are salinity, oxygen tension, illumination and water temperature (Vanschoenwinkel *et al*., 2010). Once branchiopods have hatched they still have to survive the fluctuating conditions of the pan environment during the inundation period. This is done through adaptive behavioural and physiological mechanisms. To survive temperature extremes phyllopods have been found to make use of vertical migration of the water column. When temperatures are at their highest aquatic invertebrates can be found close to the substratum where temperatures are lower. The same vertical migration is used to cope with low oxygen concentrations except the phyllopods move closer to the surface i.e. the air-water interface. Organisms can also increase their ventilation activity (Hamer and Appleton, 1991; Lahr, 1997). Many species of branchiopod have been found to be equipped with respiratory pigments enabling tolerance to hypoxic conditions (Fox, 1950; Horne, 1971).

A very important adaptation of these crustaceans is the production of drought-resistant eggs enabling the Branchiopod community within a particular wetland to survive a dry phase. Apart from allowing this dormant phase to survive dry periods, this adaptation also allows for surviving periods of low or high temperature or low oxygen, or periods of increased predation and competition (Brendonck *et al*., 1998; Brendonck and de Meester, 2003). During these unfavourable conditions, the dormant egg stages remain in a state of suppressed development (dormancy). Dormancy is a term that is used regardless of what conditions prompted the induction or termination thereof. The term includes a wide range of physiological states with quiescence and diapause at opposite ends of the spectrum (Brendonck and de Meester, 2003). Quiescence refers to an immediate response to a factor that is limiting in nature (externally induced), whereas diapause refers to an internal initiation of development arrestment (internally induced) (Brendonck, 1996). With eggs or cysts that are in a quiescent stage, metabolism and development are resumed as soon as favourable conditions are present, compared to eggs or cyst that are in diapauses where diapause needs to be broken even if favourable conditions are present. It is well known that not all eggs hatch and reach maturity at the same time. A small fraction becomes metabolically active when favourable conditions are present, yet other remains in diapauses for several filling and drying phases (Mura and Zarattini, 1999; Maffei *et al*., 2005). This delay in hatching enables adult life-phases of these branchiopod crustaceans to overcome seasonal changes that could be fatal. This behaviour is a common survival strategy in unpredictable environments and is also referred to as bet-hedging (Philippi and Seger, 1989). Once produced, many of the resting stages sink to the bottom of the wetland where they are able to survive extreme conditions, including temperature extremes and fluctuations in oxygen concentrations (Brendonck and de Meester, 2003). The resting stages that sinks to the bottom or floats to the surface together forms the egg bank. Persistent egg banks form when different resting stages produced at different time periods start to mix. This type of egg banks often form when long-term dormancy occurs. Transient egg banks can also form from resting stages that do not survive long-term dormancy. The carry-over of eggs from a generation during multiple seasons allows the continuous presence of a population in a geographic area despite possible unfavourable conditions during a particular season (Brendonck and de Meester, 2003). The conditions that are required to induce hatching from these egg banks may vary between species and individuals from a specific population (Wyngaard, 1988; Brendonck and de Meester, 2003). Apart from ensuring the carry-over of resting stages through multiple seasons, this also ensures the coexistence of multiple species.

Various environmental variables are known to play an important role in inducing hatching or breaking dormancy that includes water level, light regime (Mitchell, 1990), oxygen concentrations, conductivity and temperature (Brendonck and de Meester, 2003). However, some resting eggs do not respond to these cues but rather need a specific time, a cold shock or a desiccation period for hatching to occur. Numerous studies (Mitchell, 1990; Seaman, 1991; Brendonck et al., 1998; Brendonck and de Meester, 2003) have been completed on these resting eggs from temporary waters to determine the exact environmental cues for hatching. These studies have been mainly focussed on European waters but a limited few studies have been carried out in Southern Africa. Results indicated that environmental hatching cues are species-specific and variation in the cues for European species compared to Southern African species was noted (Mitchell, 1990; Brendonck *et al*., 1998; Vandekerkhove *et al*., 2005). These differences are related to the general environmental conditions present within the pan ecosystems in Southern Africa compared to the general environmental conditions in the European systems. The most important condition regarding the environmental variables is that the conditions should be favourable for growth and reproduction of the organisms to ensure survival of the species (Brendonck, 1996; Day *et al*., 2010).

The egg banks present in these environments provide an ecological and evolutionary reservoir that may influence the population, community and ecosystem processes (Brendonck and de Meester, 2003). It is evident that the deterioration off egg banks in response to various human activities may ultimately influence the ecological integrity of temporary wetlands. Colonisation from egg banks has been identified as the single most important process during succession in a temporary wetland, especially in wetlands that are biogeographically isolated (Angeler and Alvarez-Cobelas, 2005; Jenkins and Boulton, 2007). Despite the importance of these egg banks, environmental toxicology has focussed on the effects of selected compounds on various species. Most of the organisms that have a dormant stage as part of their life history rely on these egg banks for recovery, yet little information is available on the actual effect of toxicants on the dormant fraction of a given population (Brendonck and de Meester, 2003). Studies have shown that changes in habitat in the form of an increase in sediment load (Gleason *et al*., 2003) and changes in water quality (Jiang *et al*., 2007) can all have a detrimental effect on the hatching success of resting egg banks.

4.1.2 Hatching experiments as a monitoring tool

Ecological integrity is the capacity to support and maintain a balanced, adaptive biological system which is able to support the full range of elements and processes which make up the natural habitat of a region (Karr and Dudley, 1991). Traditional indicators of wetland health such as water quality variables, vegetation, aquatic invertebrates and birds yield little information on pan integrity due to the variability and seasonality of such indicators in a temporary environment (Angeler and García, 2005).

Egg banks present an accessible record of local history and have an ecological role in population dynamics where the recruitment of different genotypes at different times can serve to buffer the population against environmental catastrophes (Hairston *et al*., 1999, 1999; Hairston and Kearns, 2002). Therefore monitoring of pan ecosystems through the analysis of egg bank diversity could be more advantageous than field diversity sampling alone. The egg banks are responsible for recolonizing the water column and species could be lying dormant in the sediment for years while absent from the water column. Also due to the ephemeral nature of most pans, water column sampling is not always possible, therefore indicators of integrity that are accessible during both phases of a pan lifecycle make for useful alternatives (Skinner *et al*., 2001). Egg bank analyses along with enabling monitoring during dry conditions have also been found to provide fairly accurate representations of the natural community structure (Day *et al*., 2010).

4.2 Materials and methods

The materials and methods used to complete the hatching success study are indicated in sections 3.4 and 3.5.

4.3 Results

4.3.1 Physico-chemical variables

4.3.1.1 North West Province

The means and standard deviations of the physico-chemical variables recorded every four days over the 28 day exposure period are presented in Table 4.1 for pans from the North West (NW). The variables shown are temperature, pH, electrical conductivity (EC), total dissolved solutes, oxygen concentration and oxygen saturation. The variables of main concern for the hatching success of branchiopods were the temperature, pH and the EC readings. Oxygen concentrations were considered negligible as they were above the minimum thresholds that have been recorded and considered lethal (Chapter 3).

The mean temperature, pH and EC readings for all pans were calculated statistically. Treatments were kept separate. Table 4.1, 4.2 and 4.3 give a general view of how stable the variables were between the treatments and between the pans on average, evident in the minimum and maximum values observed. The 1000 mg/l treatments had a mean temperature of 17.82°C (± 1.36°C) and a minimum and maximum of 15.24°C and 19.77°C respectively. The 1500 mg/l treatments had a mean of 17.50°C (± 1.60°C); a minimum of 14.44°C and a maximum temperature of 19.20°C. The Acid Mine Drainage (AMD) treatments overall experienced a mean temperature of 17.56°C (± 1.44°C) and a minimum and maximum of 13.76°C and 18.84°C respectively.

The mean pH of all the pans for the 1000 mg/l treatments were on average 8.25 (± 0.45) with minimum and maximum values of 7.24 and 8.90 respectively. The 1500 mg/l on average had a pH of 8.24 (± 0.41). The minimum pH measured for a single pan was 7.41 and the maximum was 8.81. Regarding the AMD treatments pH on average was 7.20 (± 0.25), which was only slightly lower than the 1000 mg/l and 1500 mg/l control treatments. A minimum and maximum of 6.70 and 7.63 were recorded respectively.

The mean EC for the 1000 mg/l treatments were 4.48 mS/cm (± 1.91 mS/cm) with a minimum of 1.99 mS/cm and a maximum of 8.58 mS/cm. The 1500 mg/l treatments averaged 5.41 mS/cm (± 2.15 mS/cm). A minimum of 2.59 mS/cm and maximum of 11.03 mS/cm was recorded. The AMD treatments for all pans averaged 6.70 mS/cm (± 2.09 mS/cm) with a minimum and maximum of 4.01 mS/cm and 12.04 mS/cm respectively.

Table 4-1: The averages and standard deviations of all physico-chemical variables recorded during the course of the experiment for North West pans.

		TEMP (°C)		pH		EC (mS/cm)		TDS (ppt)		O_2 CONC. (mg/l)		O_2 SAT (%)	
		AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD
NW pan A	1000 mg/l	19.10	1.53	8.75	0.16	3.32	0.48	2.12	0.45	4.52	0.91	49.71	9.92
	1500 mg/l	18.70	1.41	8.71	0.25	4.27	0.60	2.83	0.56	4.43	0.93	48.01	9.84
	AMD	17.00	1.77	7.39	0.66	6.17	0.78	4.30	0.95	4.74	1.09	51.08	11.33
NW pan B	1000 mg/l	15.56	1.77	8.86	0.49	7.60	0.93	5.17	0.45	4.89	0.95	50.32	9.42
	1500 mg/l	14.56	1.43	8.65	0.18	8.21	0.73	5.78	1.52	4.85	0.97	50.80	8.90
	AMD	18.19	1.56	7.40	0.57	8.79	1.25	6.10	1.57	6.67	11.51	43.08	12.24
NW pan C	1000 mg/l	18.74	1.68	8.29	0.33	5.08	1.02	3.29	0.45	4.55	0.75	50.26	7.78
	1500 mg/l	19.03	1.62	8.31	0.15	5.41	0.87	3.61	0.81	4.43	0.71	48.74	7.15
	AMD	18.39	1.50	7.58	0.43	5.36	0.49	3.51	0.59	4.02	0.75	44.32	8.60
NW pan D	1000 mg/l	17.53	1.63	8.35	0.26	6.83	0.90	4.57	0.45	4.50	0.85	47.95	10.15
	1500 mg/l	15.61	1.67	8.35	0.25	8.78	1.46	6.04	1.57	4.57	1.06	48.48	11.28
	AMD	14.65	2.10	6.95	1.16	10.70	2.12	7.56	1.83	5.17	1.16	54.07	11.56
NW panE	1000 mg/l	15.52	1.59	8.56	0.37	3.75	0.58	2.43	0.45	4.81	1.16	50.42	11.29
	1500 mg/l	15.84	1.57	8.69	0.25	5.09	0.96	3.33	0.66	6.64	11.77	47.73	12.78
	AMD	15.74	1.55	7.04	0.84	6.94	0.89	4.80	0.99	4.94	1.54	52.20	15.51
NW pan F	1000 mg/l	18.57	0.30	7.39	0.11	2.51	0.29	1.40	0.45	5.92	0.45	62.64	3.68
	1500 mg/l	18.20	0.19	7.45	0.07	2.92	0.19	1.64	0.15	5.78	0.21	61.35	1.97
	AMD	18.67	0.27	7.24	0.13	4.59	0.67	2.59	0.27	5.92	0.26	63.02	2.69
NW pan G	1000 mg/l	18.25	0.14	7.77	0.11	3.43	0.32	1.93	0.45	5.83	0.20	61.80	2.09
	1500 mg/l	18.35	0.09	7.80	0.11	4.14	0.40	2.33	0.19	5.88	0.26	62.46	2.71
	AMD	18.51	0.21	7.22	0.13	5.65	1.05	3.22	0.52	5.78	0.28	61.46	2.66
NW pan H	1000 mg/l	18.58	0.11	8.10	0.33	5.71	1.12	3.35	0.45	5.82	0.32	62.41	3.28
	1500 mg/l	18.60	0.11	8.19	0.27	6.98	0.94	4.03	0.59	5.90	0.31	63.28	3.14
	AMD	18.47	0.17	7.03	0.32	7.98	1.48	4.67	1.48	5.77	0.23	60.98	2.82
NW pan I	1000 mg/l	18.57	0.11	8.15	0.22	2.06	0.21	1.13	0.45	5.88	0.24	63.00	2.58
	1500 mg/l	18.59	0.11	8.01	0.18	2.85	0.30	1.57	0.12	5.86	0.26	62.76	2.73
	AMD	18.43	0.18	6.98	0.42	4.17	0.54	2.32	0.16	5.85	0.22	62.18	2.18

4.3.1.2 Free State Province

Means of the physico-chemical variables taken during the course of the experiment for pans in the Free State (FS) Province are presented in Table 4.2. The mean temperature reading of all the pans for the 1000 mg/l treatment was 20.51°C (± 2.11°C) with a minimum of 17.37°C and a maximum of 23.40°C. The 1500 mg/l treatments had a mean temperature of 20.34°C (± 1.63°C) and minimum and maximum readings of 18.64°C and 23.17°C respectively. The AMD temperatures averaged at 19.92°C (± 0.84°C) with a minimum of 18.57°C and maximum of 21.71°C.

The mean pH for the 1000 mg/l treatments was 7.40 (± 0.70) with a minimum of 6.33 and a maximum of 8.34. The mean pH of the 1500 mg/l treatments was 7.44 (± 0.99) and had minimum and maximum values of 6.05 and 8.02 respectively. AMD treatments on average had a pH of 3.80 (± 0.96) and a minimum and maximum of 2.68 and 6.28 respectively.

The EC readings averaged at 2.44 mS/cm (± 0.63 mS/cm) for the 1000 mg/l treatment and experienced a minimum and maximum of 1.81 mS/cm and 3.91 mS/cm respectively. The 1500 mg/l treatments had a mean of 3.18 mS/cm (± 0.69 mS/cm), a minimum reading of 2.27 mS/cm and a maximum reading of 4.78 mS/cm. The AMD had a mean EC of 5.97 mS/cm (± 1.44 mS/cm) and a minimum and maximum of 2.41 mS/cm and 8.60 mS/cm respectively.

4.3.1.3 Mpumalanga Province

The mean physico-chemical variables for the Mpumalanga (MP) Province are shown in Table 4.3. The mean temperature for the 1000 mg/l treatments was 20.54°C (± 1.35°C) with a minimum of 19.21°C and a maximum of 23.31°C. Mean temperature for the 1500 mg/l treatments was 20.10°C (± 1.34°C). The minimum reading taken for the 1500 mg/l treatment was 18.61°C and the maximum 23.06°C. The AMD treatments had a mean of 20.50°C (± 1.38°C) with a minimum and maximum of 19.01°C and 22.51°C respectively.

Mean pH of the 1000 mg/l treatments was 7.15 (± 1.19) with a minimum and maximum of 5.44 and 9.10 respectively. The 1500 mg/l treatments had a mean pH of 7.17 (± 1.23) along with a minimum of 5.49 and a maximum of 9.05. The mean pH of the AMD treatments was 3.25 (± 1.35) with a minimum and maximum of 2.43 and 6.28 respectively.

The EC of the 1000 mg/l treatments had a mean of 1.63 mS/cm (± 0.47 mS/cm) and a minimum and maximum EC of 0.06 mS/cm and 2.07 mS/cm respectively. For the 1500 mg/l treatments the mean was 2.49 mS/cm (± 0.76 mS/cm) and the minimum and maximum EC was 0.08 mS/cm and 3.52 mS/cm respectively. The mean EC of the AMD treatments was 4.40 mS/cm (± 0.81 mS/cm), with a minimum of 2.93 mS/cm and maximum of 5.32 mS/cm.

Table 4-2: The averages and standard deviations of the physico-chemical variables recorded during the course of the experiment for the Free State pans.

		TEMP (°C)		pH		EC (mS/cm)		TDS (ppt)		O_2 CONC. (mg/l)		O_2 SAT (%)	
		AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD
FS pan A	1000 mg/l	19.73	2.63	7.66	0.23	3.67	1.16	1.93	0.45	5.20	0.68	60.33	5.60
	1500 mg/l	19.31	2.20	7.73	0.22	4.33	1.74	2.26	0.83	5.06	0.58	60.05	7.53
	AMD	19.56	2.01	3.80	0.23	8.46	0.52	4.23	0.27	4.80	0.40	64.05	5.04
FS pan B	1000 mg/l	18.79	0.09	8.26	0.22	1.96	0.17	1.06	0.45	5.79	0.23	62.11	2.51
	1500 mg/l	18.74	0.10	7.99	0.17	2.62	0.33	1.45	0.15	5.78	0.25	62.11	2.72
	AMD	19.93	1.70	3.82	0.24	5.55	0.35	2.78	0.18	4.89	0.40	66.16	5.31
FS pan C	1000 mg/l	18.88	0.30	6.43	0.49	2.04	0.18	1.03	0.45	6.16	0.20	66.92	2.03
	1500 mg/l	18.69	0.27	6.32	0.44	2.78	0.34	1.42	0.18	5.92	0.36	64.07	3.79
	AMD	20.14	1.87	4.21	0.30	5.55	0.29	2.76	0.15	4.89	0.35	66.10	4.72
FS pan D	1000 mg/l	18.62	0.22	6.39	0.39	1.88	0.12	0.94	0.45	5.99	0.27	64.94	2.95
	1500 mg/l	18.65	0.15	6.23	0.38	2.84	0.23	1.46	0.13	5.87	0.28	63.64	2.93
	AMD	18.74	1.30	3.86	0.10	6.34	0.70	3.14	0.36	5.11	0.39	66.81	5.17
FS pan E	1000 mg/l	18.84	0.15	6.36	0.35	2.08	0.12	1.05	0.45	5.95	0.31	64.19	2.70
	1500 mg/l	18.81	0.16	6.30	0.33	2.73	0.35	1.40	0.18	5.84	0.19	63.21	2.00
	AMD	19.25	1.22	3.95	0.18	6.17	0.42	3.08	0.20	4.64	0.79	63.38	5.42
FS pan F	1000 mg/l	22.65	2.32	7.69	0.16	3.34	0.49	1.83	0.45	5.16	0.59	60.54	6.28
	1500 mg/l	22.56	2.26	7.89	0.12	4.29	0.49	2.39	0.32	4.99	0.70	57.85	7.66
	AMD	20.35	2.69	4.31	0.34	6.61	0.70	3.75	0.44	5.21	0.51	58.88	4.16
FS pan G	1000 mg/l	21.54	8.51	7.63	0.98	2.13	0.24	1.15	0.45	5.12	0.63	58.31	6.00
	1500 mg/l	20.55	2.84	7.60	0.10	2.86	0.34	1.56	0.17	5.13	0.52	58.11	4.65
	AMD	20.29	2.83	3.08	0.20	5.54	0.85	3.11	0.49	5.13	0.76	58.20	6.94
FS pan H	1000 mg/l	19.45	4.04	7.78	0.99	2.78	0.41	1.51	0.45	5.12	0.82	71.61	83.65
	1500 mg/l	21.18	2.38	8.64	5.03	3.69	0.35	2.04	1.19	5.21	0.63	59.92	5.88
	AMD	20.42	2.91	3.24	0.25	5.91	1.08	3.29	0.66	5.29	0.53	60.48	4.87
FS pan I	1000 mg/l	22.62	2.25	7.93	0.16	2.51	0.43	1.35	0.45	4.95	0.67	57.97	7.09
	1500 mg/l	23.02	2.15	7.91	0.07	3.10	0.40	1.70	0.24	4.93	0.64	58.49	5.22
	AMD	21.57	2.33	2.99	0.11	5.58	0.57	3.25	0.61	5.21	0.36	60.51	2.99
FS pan J	1000 mg/l	22.50	2.25	7.94	0.08	1.99	0.21	1.06	0.45	4.77	0.43	55.53	4.08
	1500 mg/l	21.69	2.46	7.79	0.05	2.85	0.36	1.55	0.20	4.83	0.61	55.86	6.49
	AMD	20.26	3.17	2.70	0.19	6.92	0.90	3.91	0.63	5.37	1.00	60.70	9.18

Table 4-3: The averages and standard deviations of the physico-chemical variables recorded during the course of the experiment for the Mpumalanga pans.

		TEMP (°C)		pH		EC (mS/cm)		TDS (ppt)		O_2 CONC. (mg/l)		O_2 SAT (%)	
		AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD	AVG	SD
MP pan A	1000 mg/l	22.64	1.34	7.75	0.96	1.65	0.74	0.89	0.45	3.92	0.76	45.14	8.31
	1500 mg/l	22.13	1.94	8.22	0.27	2.01	0.97	1.15	0.61	4.11	0.67	47.64	7.84
	AMD	21.21	1.57	6.15	0.84	3.76	0.57	2.26	0.35	6.06	8.03	48.52	8.08
MP pan B	1000 mg/l	20.00	1.49	8.31	0.17	1.53	0.73	0.90	0.45	4.19	0.65	47.48	6.39
	1500 mg/l	18.81	1.37	8.34	0.22	2.25	1.10	1.36	0.68	4.31	0.53	47.51	5.25
	AMD	21.98	1.48	5.63	1.02	3.03	0.79	1.80	0.49	3.91	0.79	44.62	8.81
MP pan C	1000 mg/l	22.44	1.61	8.40	0.30	1.50	0.81	0.86	0.45	4.33	0.67	50.44	7.32
	1500 mg/l	22.99	1.24	8.40	0.24	1.92	1.40	1.05	0.60	4.14	0.53	48.42	5.69
	AMD	22.08	1.55	2.94	0.13	3.47	0.82	2.10	0.51	4.25	0.35	48.85	4.35
MP pan D	1000 mg/l	21.62	1.43	8.40	0.21	1.31	0.70	0.77	0.45	4.25	0.50	48.53	5.15
	1500 mg/l	20.33	1.42	8.41	0.19	2.26	1.03	1.36	0.63	4.20	0.58	47.59	6.15
	AMD	22.45	1.39	2.77	0.16	3.70	0.87	2.21	0.53	4.41	0.44	50.50	4.89
MP pan E	1000 mg/l	19.86	1.32	8.50	0.32	1.42	0.77	0.85	0.45	5.35	5.88	48.48	4.64
	1500 mg/l	19.96	1.32	8.43	0.34	2.17	1.20	1.28	0.71	4.08	0.56	45.60	6.03
	AMD	21.27	1.45	2.58	0.18	4.49	0.78	2.89	0.62	4.37	0.59	49.33	6.53
MP pan F	1000 mg/l	19.35	2.25	5.50	0.16	1.92	0.24	1.10	0.45	6.14	0.44	67.86	5.25
	1500 mg/l	19.24	2.28	5.58	0.13	2.66	0.42	1.51	0.25	5.93	0.58	65.49	6.44
	AMD	19.47	1.86	2.51	0.11	4.88	0.85	2.95	0.26	6.01	0.31	66.00	6.64
MP pan G	1000 mg/l	19.27	2.30	5.83	0.14	1.77	0.24	0.98	0.45	5.83	0.38	64.07	6.04
	1500 mg/l	19.29	2.27	5.82	0.15	2.88	0.16	1.65	0.10	5.96	0.45	65.57	6.78
	AMD	19.17	1.92	2.47	0.11	5.20	0.36	3.06	0.22	8.46	11.19	62.28	14.77
MP pan H	1000 mg/l	19.37	2.19	6.09	0.14	1.82	0.14	1.02	0.45	5.95	0.27	66.25	6.13
	1500 mg/l	19.40	2.18	6.04	0.14	2.96	0.86	1.61	0.17	5.97	0.27	66.13	6.29
	AMD	19.14	1.91	2.47	0.10	5.18	0.35	3.05	0.21	5.83	0.35	63.74	7.76
MP pan I	1000 mg/l	19.38	2.17	6.32	0.11	1.74	0.15	0.97	0.45	5.98	0.28	66.04	6.61
	1500 mg/l	19.36	2.09	6.22	0.12	3.01	0.32	1.73	0.19	5.86	0.29	64.71	6.77
	AMD	19.09	1.89	2.72	1.03	5.20	0.34	3.06	0.21	5.90	0.21	64.72	6.76
MP pan J	1000 mg/l	19.50	1.92	6.39	0.10	1.80	0.18	1.01	0.45	6.04	0.32	67.21	7.48
	1500 mg/l	19.46	2.04	6.23	0.12	2.80	0.28	1.60	0.16	5.97	0.28	66.46	6.69
	AMD	19.05	1.92	2.45	0.09	5.07	0.43	2.97	0.26	5.86	0.18	64.18	6.82

4.3.2 Hatching abundance and timing of hatch

4.3.2.1 North West Province

A total of 1870 individuals hatched from North West Province (NW) pans over the 28 day exposure period. The total numbers of individuals to hatch from the pans are presented in Table 4.4. These values are the sum of hatchlings (nauplii) to hatch from both the 1000 mg/l and 1500 mg/l control treatments, with all five replicates added together. No hatchlings occurred in the AMD treatment, and thus AMD is not listed in Table 4.4. North West pan H had the greatest number of hatchlings (692 individuals). North West pan I had the lowest abundance with only 8 individuals hatching from the laboratory experiments in total.

In testing whether there were any significant differences in hatchling abundance between pans for separate treatments, Kruskal-Wallis tests indicated that there were significant differences between the pans for separate treatments (1000 mg/l: χ^2=31.738; p = 0.000 and 1500 mg/l: χ^2= 37.370; p= 0.000). Mann- Whitney U tests, using an α=0.002, were however unable to detect between which pans the significant differences were situated. The AMD treatments were excluded from these tests as no hatchlings were present. North West pan H had the highest mean rank for the 1000 and 1500 mg/l treatment (41.20 and 42.40) respectively, while conversely NW pan I had the lowest mean rank for the 1000 mg/l and 1500 mg/l control treatments (5.70 and 5.00) respectively.

Table 4-4: The total abundance of hatchlings to hatch from the North West pans.

Pans	Total hatchling abundance
NW pan A	162
NW pan B	187
NW pan C	54
NW pan D	328
NW pan E	111
NW pan F	264
NW pan G	64
NW pan H	692
NW pan I	8

Figure 4.1 visually represents the cumulative abundances of hatchlings to emerge from each treatment per pan. The hatchling abundance of the replicates for each treatment were added together to obtain these values. In NW pan A, D, E and F the greatest cumulative hatchling abundance occurred in the 1500 mg/l. In NW pan B, C, F, G and I the greatest abundance of hatchlings hatched in the 1000 mg/l treatment. Five out of the nine pans had greater hatchling abundances in the 1000 mg/l treatment.

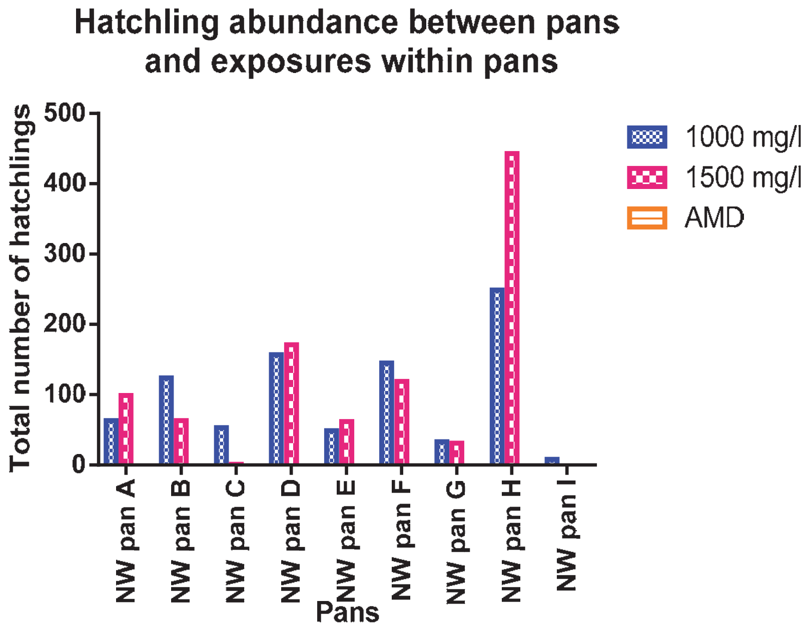


Figure 4-1: The total hatchling abundance from the 9 pans in the North West (NW pan A-I) and the difference in hatching abundance between the three different treatments for each pan (1000 mg/l, 1500 mg/l and AMD).

Table 4-5: The mean number of hatchlings for the separate concentrations.

Pans	1000 mg/l	1500 mg/l
NW pan A	12.60	19.80
NW pan B	24.80	12.60
NW pan C	10.60	0.20
NW pan D	31.40	34.20
NW pan E	9.80	12.40
NW pan F	29.00	23.80
NW pan G	6.60	6.20
NW pan H	49.80	88.60
NW pan I	1.60	0

The mean numbers of hatchlings to emerge from the 1000 mg/l and 1500 mg/l treatments are given in Table 4.5. North West pan H, D, F and B had the highest means for both control concentrations compared to all other pans. Mann-Whitney U tests revealed that statistically there were no significant differences in hatchling abundances between the 1000 mg/l and 1500 mg/l treatments within the pans, except for NW pan C ($U = 3.000$; $Z = -2.117$; $p = 0.034$). In NW pan C the 1000 mg/l concentration had a total abundance of 53 hatchlings (Mean rank= 7.40; $n=5$) over the five replicates, whereas the 1500 mg/l concentration only had 1 hatchling (Mean rank=3.60; $n=5$) over the five replicates.

The mean hatching graphs (Figure 4.2) present the time interval at which hatching started; at which hatching peaked and the fluctuations in between. Hatching peak refers to the time interval at which maximum hatching occurred. Hatching peaks varied from day 4 to 28, however the majority of hatching peaked within the 4 to 16 day time period. In NW pan A, B, F, G and H hatching started at the four day interval for both control treatments, and thus there was a short delay between inundation and hatching. These pans reached their peak hatching in the 4 to 12 day interval. In NW pan C, D and I hatching only started during the 8 to 16 day interval, and hatching only peaked around day 24.

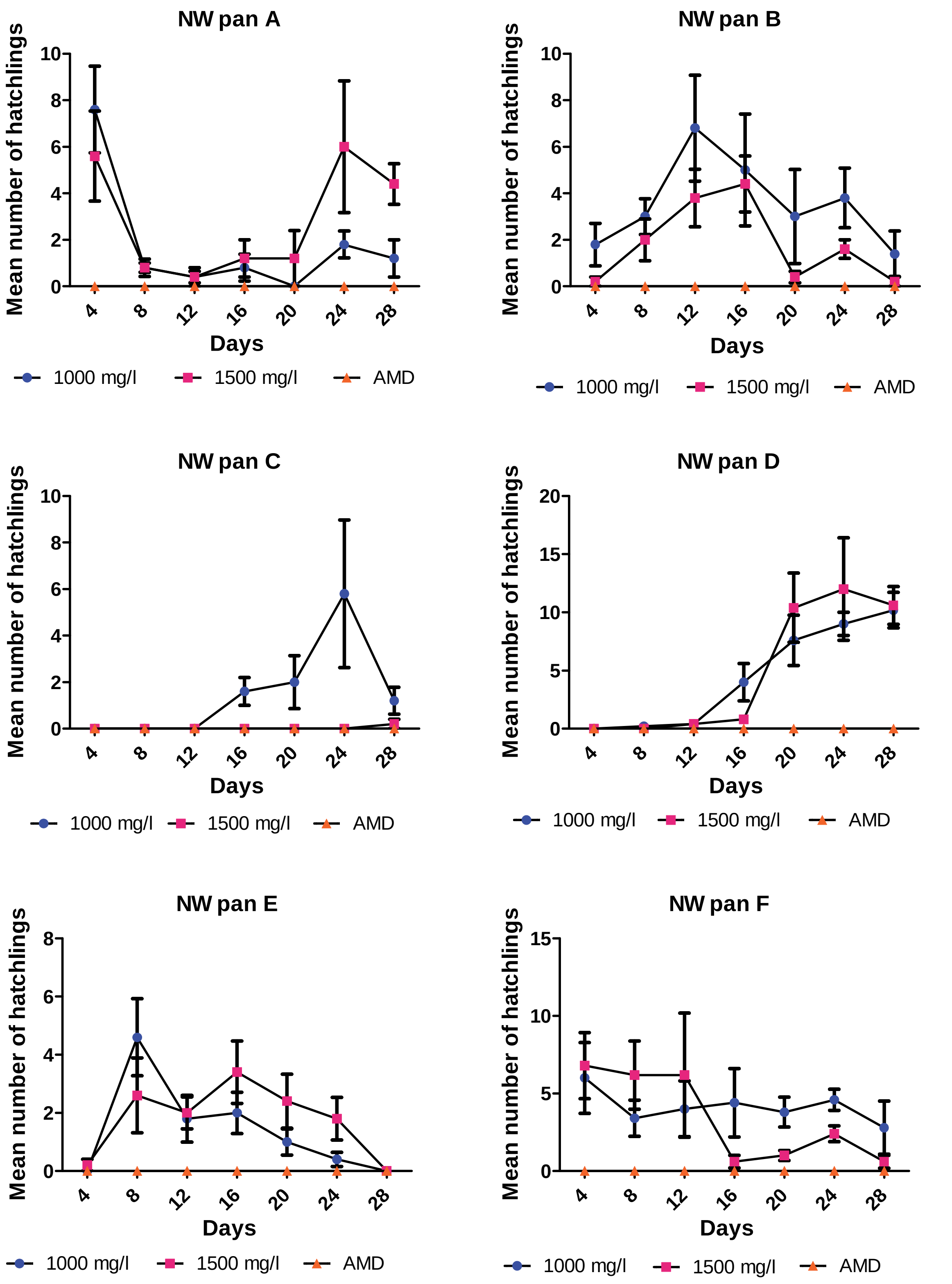


Figure 4-2: Graphs indicating the mean number of hatchlings per 4 day interval for the North West Province pans (NW pan A-I). (Note the scale differences)

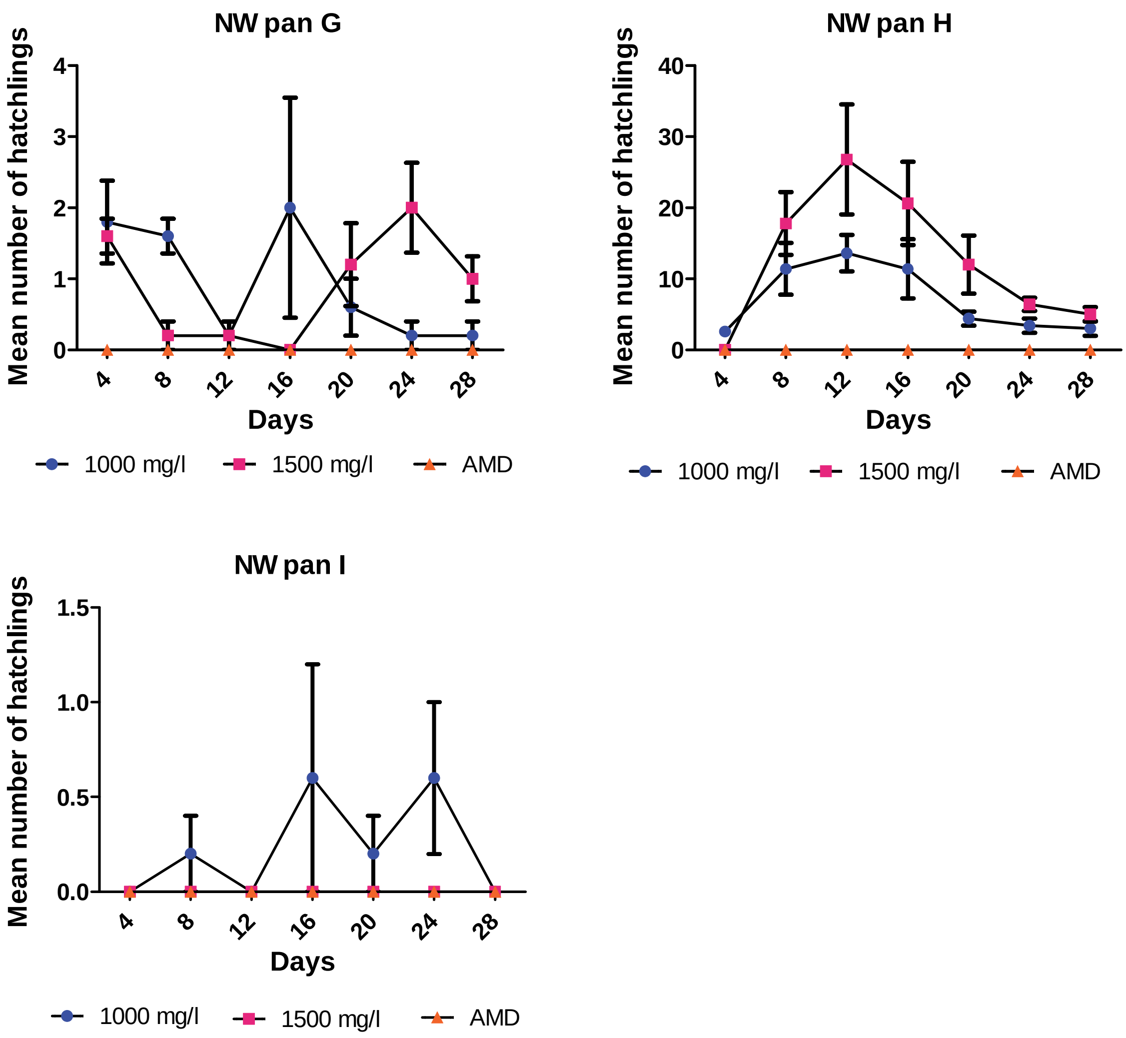


Figure 4.2: Mean hatching graphs (continued).

4.3.2.2 Free State Province

A total of 2188 individuals hatched from the 10 Free State pans during the hatching experiments. Free State pan A had the greatest number of hatchlings with 719 individuals hatching in total from both the 1000 mg/l and 1500 mg/l treatments added together. Table 4.6 specifies the total number of hatchlings to hatch from each pan over the course of 28 days. Free State pans A, J, G and D had the highest abundance of total hatchlings respectively. Free State pan C had the least number of nauplii, with 10 individuals hatching in total. The cumulative abundance of hatchlings per treatment per pan is better illustrated in Figure 4.3.

Statistically there were significant differences between the hatchling abundances that hatched in 1000 mg/l (χ^2=33.75; n=5; p=0.000) and 1500 mg/l (χ^2 = 39.48; n=5; p=0.000) between the separate pans. The non-parametric tests were unable to determine which pans the differences fell between. Free State pan A had the highest mean rank for both the 1000 mg/l and 1500 mg/l treatments (47.20 and 45.20 respectively) and FS pan C had the lowest mean rank for both treatments (10.00 and 6.50 respectively). The mean abundances of hatchlings to occur in the separate control treatments are presented in Table 4.7. Free State pan A had the highest mean hatching abundance for the 1000 mg/l and 1500 mg/l treatments. Free State pan C had the lowest mean hatching abundance for both the 1000 mg/l and 1500 mg/l treatments. There was an absence of hatchlings in the AMD exposures for all pans and were thus excluded from statistical analysis.

Table 4-6: The total abundance of hatchlings to hatch from separate pans.

Pans	Total hatchling abundance
FS pan A	719
FS pan B	54
FS pan C	10
FS pan D	228
FS pan E	192
FS pan F	42
FS pan G	289
FS pan H	25
FS pan I	109
FS pan J	520

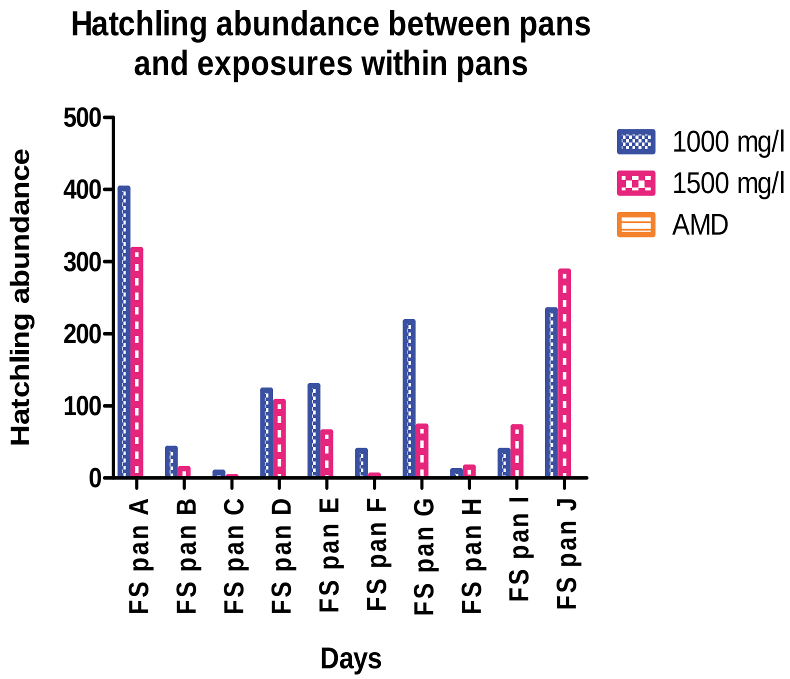


Figure 4-3: The total hatching abundance of branchiopods from the 10 pans in the Free State (FS pan A-J) and the difference in hatching abundance between the three different treatments for each pan (1000 mg/l, 1500 mg/l and AMD). Means and standard deviations excluded.

Figure 4.3 illustrates that in FS pan A, B, C, D, E, F and G a greater number of hatchlings occurred in the 1000 mg/l treatment. Alternatively, in FS pan H, I and J hatchlings were present in larger abundances in the 1500 mg/l treatment. A greater abundance of hatchlings emerged in the 1000 mg/l treatment for the majority of pans, 7 out of 10 pans.

In the Free State Province, although the majority of pans had a greater hatchling abundance in the 1000 mg/l treatment, the only pans to show significant differences in the mean hatchling (Table 4.7) abundances between the 1000 mg/l and 1500 mg/l treatments were: FS pan B ($U=2.00$; $Z=2.207$; $p = 0.027$), FS pan C ($U=3.500$; $Z= 1.972$; $p = 0.049$) and FS pan E ($U=2.00$; $Z= 2.200$; $p = 0.028$).

Table 4-7: The mean number of hatchlings for the separate concentrations.

Pans	1000 mg/l	1500 mg/l
FS pan A	80.40	63.40
FS pan B	8.20	2.60
FS pan C	1.60	0.40
FS pan D	4.60	4.20
FS pan E	25.60	12.80
FS pan F	7.60	0.80
FS pan G	43.40	14.40
FS pan H	2.00	3.00
FS pan I	7.60	14.20
FS pan J	46.60	57.40

In the Free State pans hatching began in the 4 to 8 day time interval for the majority of the pans (FS pan D, E, G, I and J) for both treatments (Figure 4.4). In FS pan B and H hatchlings only started to occur from day 16 onwards for both concentrations, while in FS pan A and C hatching started at different times for the separate treatments. In FS pan A hatching started early in the 1500 mg/l treatment (day 4) and late in the 1000 mg/l treatment (day 12). In FS pan C hatching started early in the 1000 mg/l (day 4) and late in the 1500 mg/l concentration (day 20). Despite early hatching in most of the pans, hatching generally peaked from day 16 to 28.

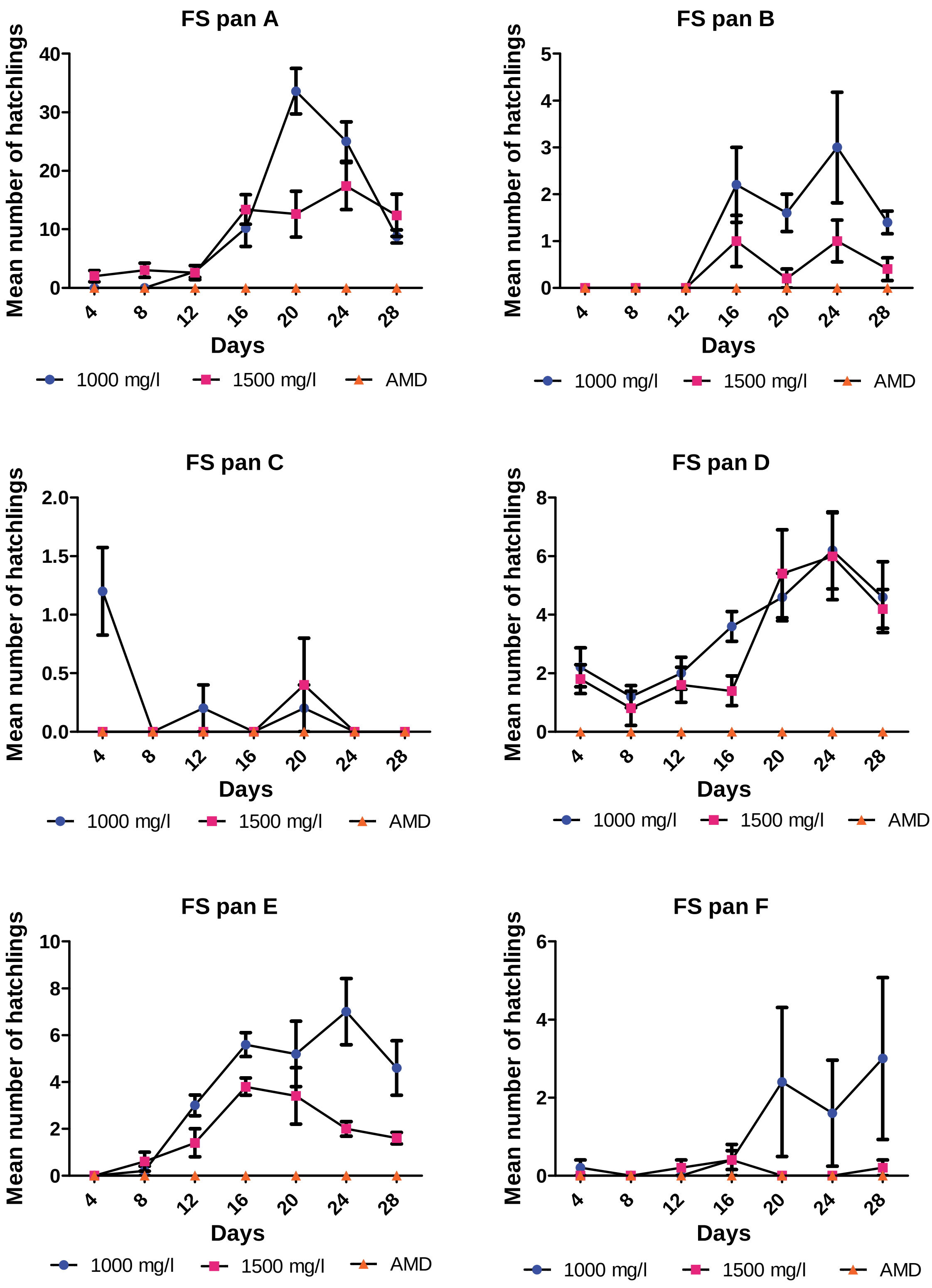


Figure 4-4: Graphs indicating the mean number of hatchlings per interval for the Free State Province pans.

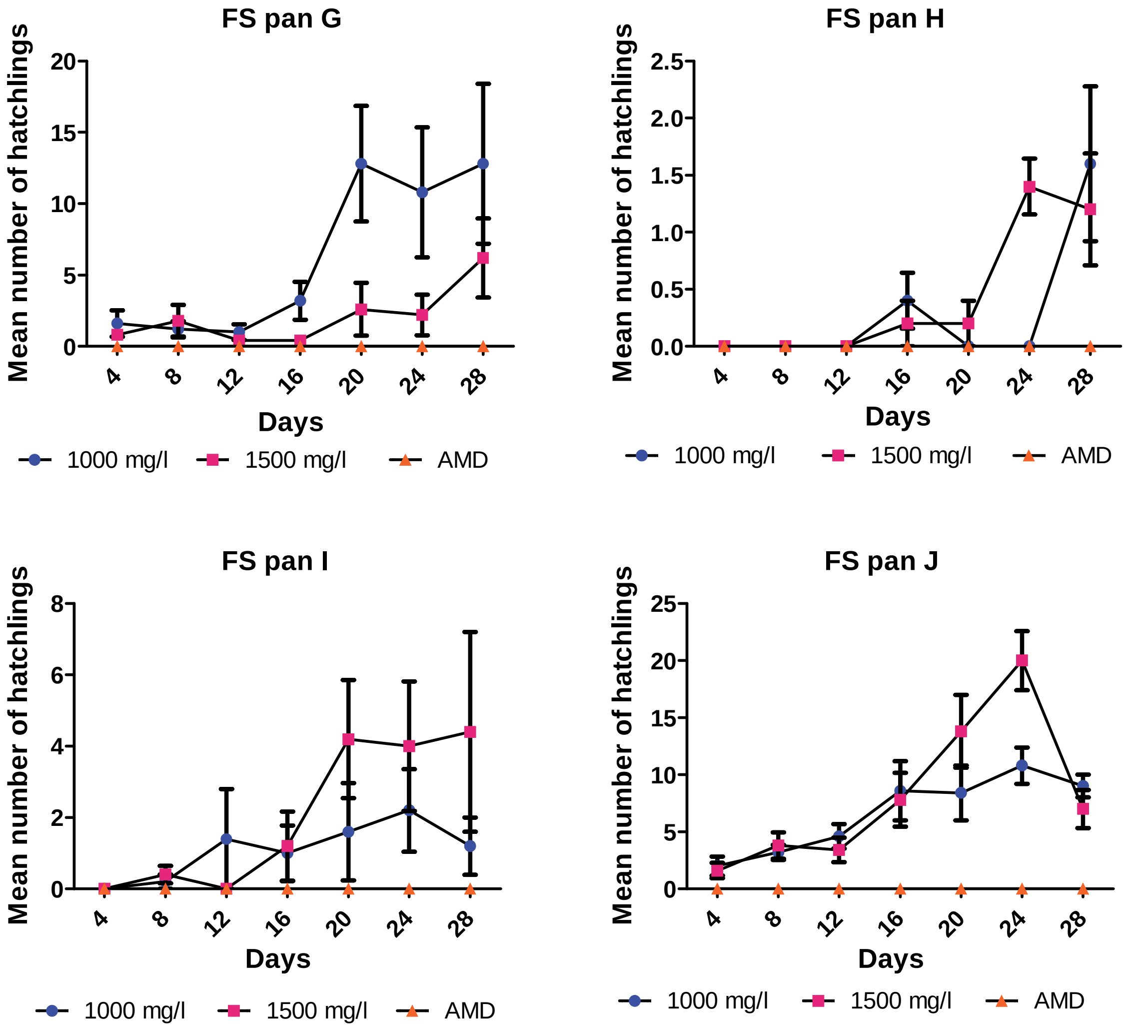


Figure 4.4: Mean hatching graphs (continued).

4.3.2.3 Mpumalanga Province

In the Mpumalanga (MP) Province a sum of 476 individuals hatched from the sediment collected from the 10 pans. Table 4.8 presents the total number of individuals that hatched from each pan (treatments combined). Mpumalanga pan C had the highest abundance of individuals hatching (268 individuals), while MP pan E, F and I had the lowest abundance with no individuals emerging from the sediment of these pans.

Regarding the hatchling abundances, statistically there were significant differences between pans of the 1000 mg/l treatments (χ^2= 33.593; p= 0.000) and the 1500 mg/l treatments (χ^2= 38.723; p= 0.000). Non-parametric Mann Whitney U tests were unable to determine between which pans the differences were situated.

Table 4-8: The total abundance of hatchlings to hatch from individual pans.

Pans	Total hatchling abundance
MP pan A	36
MP pan B	58
MP pan C	268
MP pan D	84
MP pan E	0
MP pan F	0
MP pan G	1
MP pan H	26
MP pan I	0
MP pan J	3

Figure 4.5 illustrates the total abundance of hatchlings per treatment per pan. In MP B, C, D, G, H and J a higher abundance of hatchlings emerged from the 1000 mg/l treatment. The only pan to have a larger abundance of hatchlings in the 1500 mg/l treatment was MP pan B. There was an absence of hatchlings to emerge from the sediment of MP pan E, F and I. Hatchling abundances were low overall in the Mpumalanga Province.

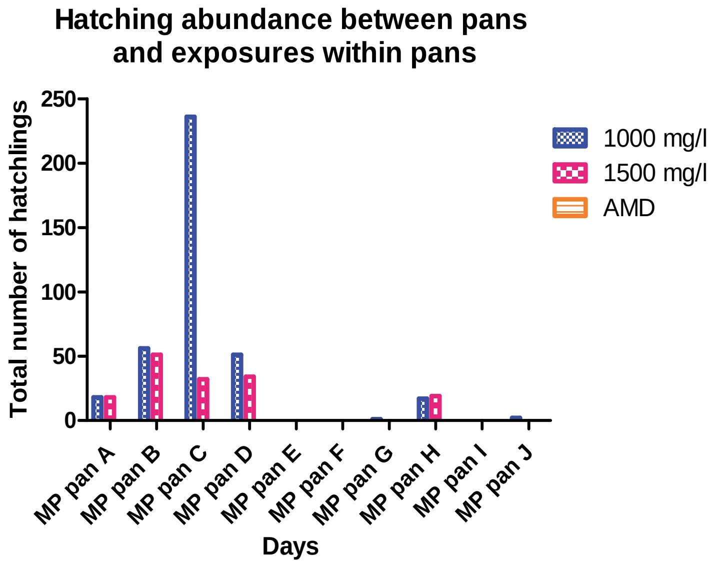


Figure 4-5: The total hatching abundance of branchiopods from the 10 Mpumalanga pans (MP pan A-J) and the difference in hatching abundance between the three different treatments for each pan (1000 mg/l, 1500 mg/l and AMD).

The mean numbers of hatchlings to occur in the 1000 mg/l and 1500 mg/l treatments are presented in Table 4.9. The abundance of hatchlings over the five replicates were added together to obtain such means. Mann Whitney U tests found no significant differences between the 1000 mg/l and 1500 mg/l treatments with regards to hatching abundance in the pans that had the presence of hatchlings. Pans that had no hatchlings occurring from both treatments were omitted from these tests.

Table 4-9: The mean number of hatchlings for the separate concentrations.

Pans	1000 mg/l	1500 mg/l
MP pan A	3.80	3.80
MP pan B	11.20	10.20
MP pan C	47.20	6.40
MP pan D	10.20	6.80
MP pan E	0	0
MP pan F	0	0
MP pan G	0.20	0
MP pan H	3.40	1.80
MP pan I	0	0
MP pan J	0.20	0.20

The means of hatchlings counted over the course of the experiment per time interval are presented in Figure 4.6. The figure shows that hatching started early, around the four day interval in most pans. Hatching peaked at the four day interval in MP pan D, and was the only to experience a peak in this period. In MP pan A, B, C and J hatching peaks occurred later in the inundation period from day 20 onwards.

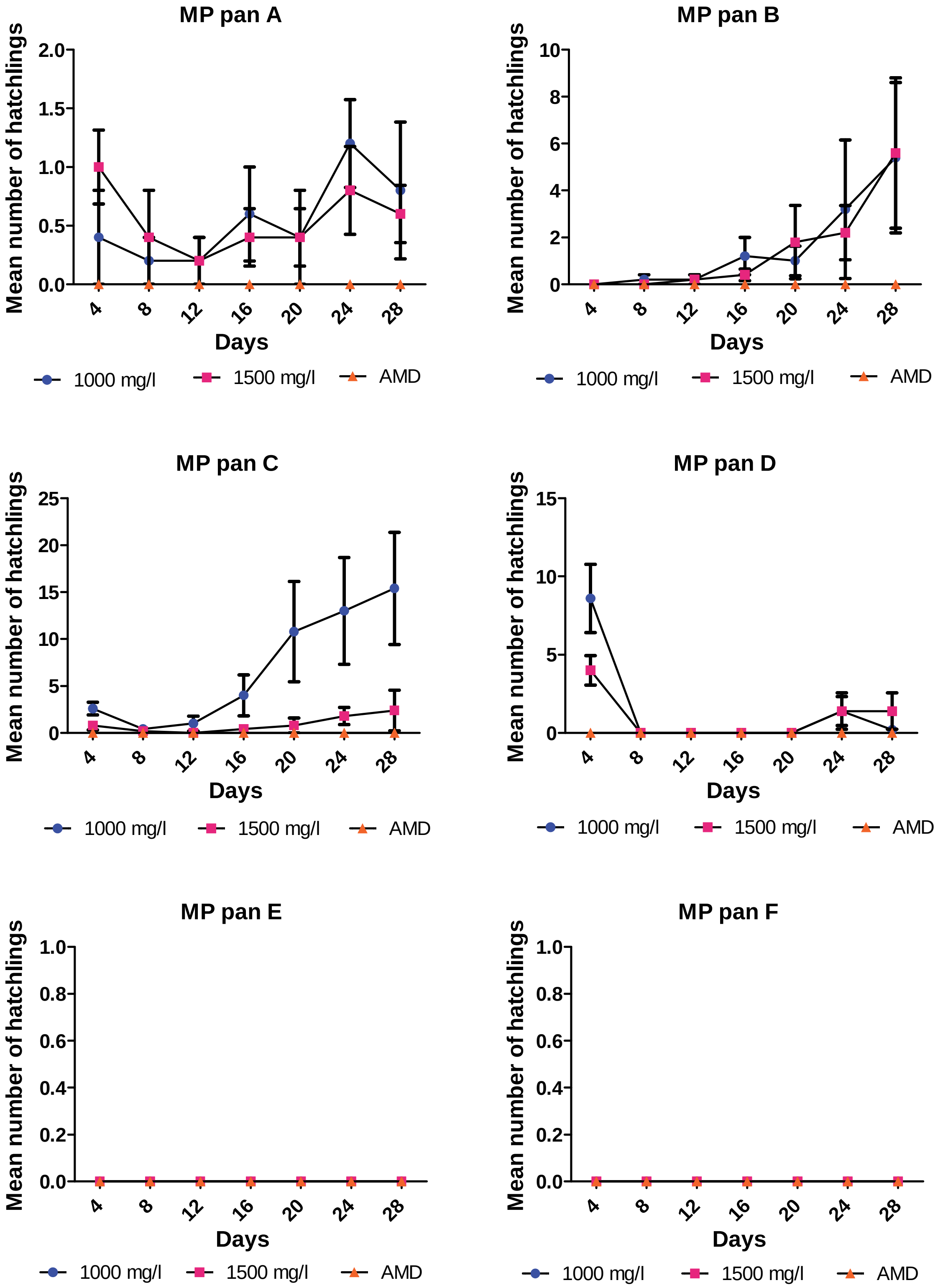


Figure 4-6: Graphs indicating the mean number of hatchlings per interval for the Mpumalanga Province pans (MP pan A-J).

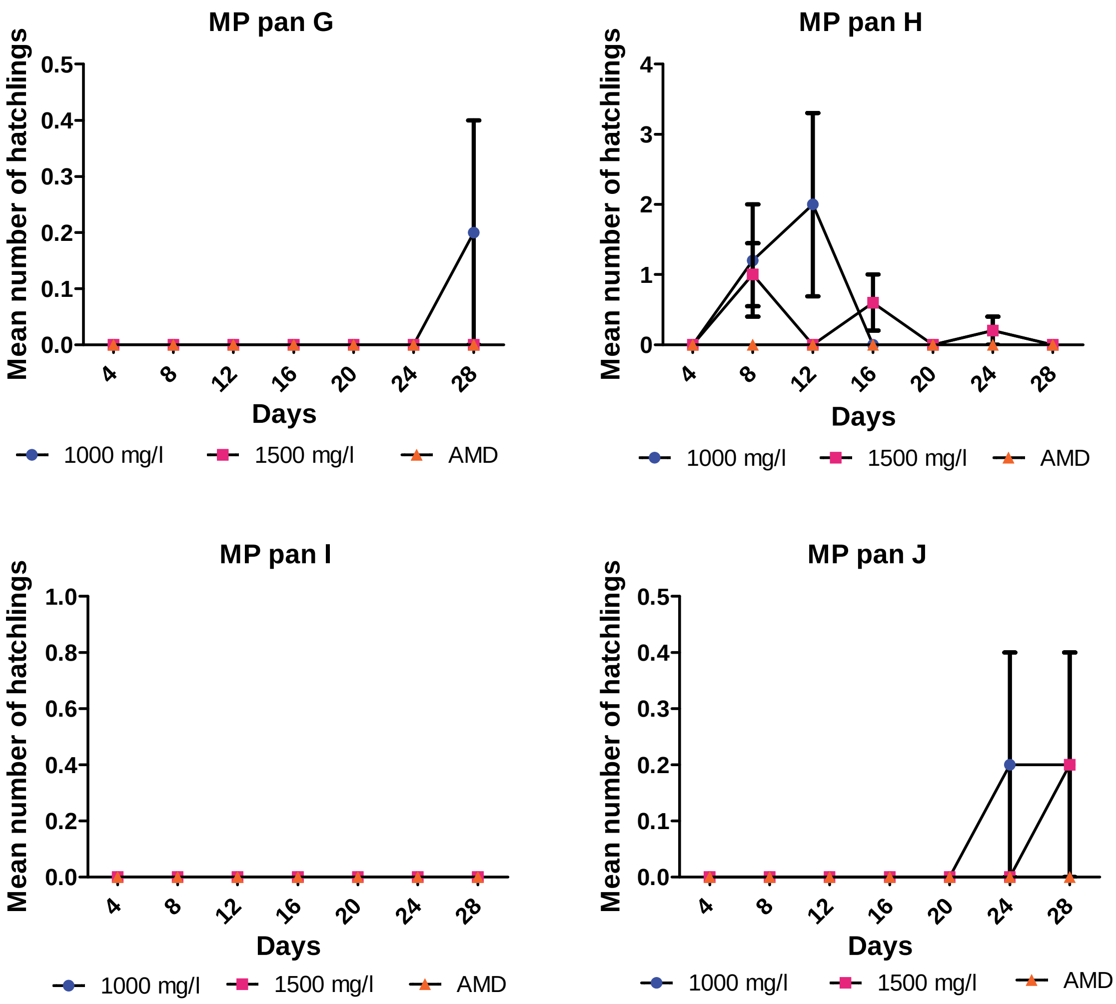


Figure 4.6: Mean hatching graphs (continued).

4.3.3 Hatching diversity

4.3.3.1 North West

A total of 964 individuals from the order Anostraca, ten from the genus *Triops*, five from the order Conchostraca, four from the family Chydoridae, 30 from the family Daphniidae, 24 from the family Moinidae, three from the subclass Copepoda and 183 individuals from the class Ostracoda were identified from the hatching experiments in the North West Province pans (Table 4.10). Nauplii did not develop to a stage that allowed their accurate identification in all pans. The majority of hatchlings could only be positively identified to family level, except in NW pan A where the genus *Triops* was identified, NW pan F where the genus *Streptocephalus* was identified and NW pan H where the species *Moinia belli* was identified. Only six individuals of the *Streptocephalus* and 20 of the *Moinia belli* could be clearly distinguished. As a result the diversity data indicated in Table 4.6 is based on family level identifications, except for the *Triops* in NW pan A as there were no other pans that contained this taxon. To avoid skewing the results of univariate and multivariate statistical tests all sites were compared at family or order level.

The hatching results indicated that NW pan A was the only pan to show the presence of *Triops* species. North West pan F and G were the only two pans that had hatchlings from the order Conchostraca. North West pan D was the only pan to have Daphniidae and pan H the only pan to have Moinidae. North West pan I was the only one to have Chydoridae. All pans except NW pan A, C and I had the presence of Anostraca and all pans except NW pan B and E had the presence of Ostracoda. The most common group to hatch from the North West pans were the Anostraca and Ostracoda occurring in six out of nine, and seven out of nine pans respectively.

Table 4-10: Diversity and abundance of taxa that hatched from the North West pans.

Taxa	NW A	NW B	NW C	NW D	NW E	NW F	NW G	NW H	NW I
Anostraca	0	61	0	3	16	223	39	622	0
Triops	10	0	0	0	0	0	0	0	0
Conchostraca	0	0	0	0	0	3	2	0	0
Chydoridae	0	0	0	0	0	0	0	0	4
Daphniidae	0	0	0	30	0	0	0	0	0
Moinidae	0	0	0	0	0	0	0	24	0
Copepoda	0	0	0	0	0	2	1	0	0
Ostracoda	47	0	1	101	0	12	16	2	4

Univariate statistics were used to compare the diversity of hatchlings between the pans. Margalef's diversity index asses species richness, and takes into account the total number of species and the number of individuals (Clarke and Warwick, 1994). The Margalef's index in Figure 4.7 shows that, in consecutive order, NW pan G, F, I, D and H had high species richness' and NW pan A, B, C and E had low species richness'. North West pan F and NW pan G both had four taxa, NW pan D and NW pan H had three taxa and NW pan I only had two taxa. Based purely on the number of different taxa NW pan D is more diverse. The index is slightly skewed for NW pan I, because the index takes into account the total number of individuals. North West pan D has less taxa per individuals present compared to NW pan I, making it look less diverse.

Pielou's evenness index calculates how evenly the individual hatchlings were distributed over different taxa (Clarke and Warwick, 1994). In consecutive order NW pan I, A, D and G had high evenness scores. North West pan F, H, B, C and E had low evenness scores indicating that there was a dominance of certain taxa hatching from these pans. North West pan F for instance had 240 hatchlings of which 223 (93%) individuals belonged to the Order Anostraca. North West pan H, B and E also had a dominance of Anostraca while NW pan C had a dominance of Ostracoda hatching (Table 4.10).

The Shannon-Wiener diversity index (Figure 4.7) takes both species richness and evenness into account (Clarke and Warwick, 1994), therefore summarising the total diversity of hatchlings to hatch under the laboratory conditions. Shannon-Wiener indices revealed that, in order, NW pans G, I, D, A and F, had a high diversity and NW pans H, B, C and E had a low diversity. Pans G, D and F were in actual fact the most diverse pans having both high abundances and species richness, and moderate evenness scores. The low abundance values and high evenness scores of NW pan I skewed the diversity results for this pan as only two taxa were present, none of which were the large branchiopoda.

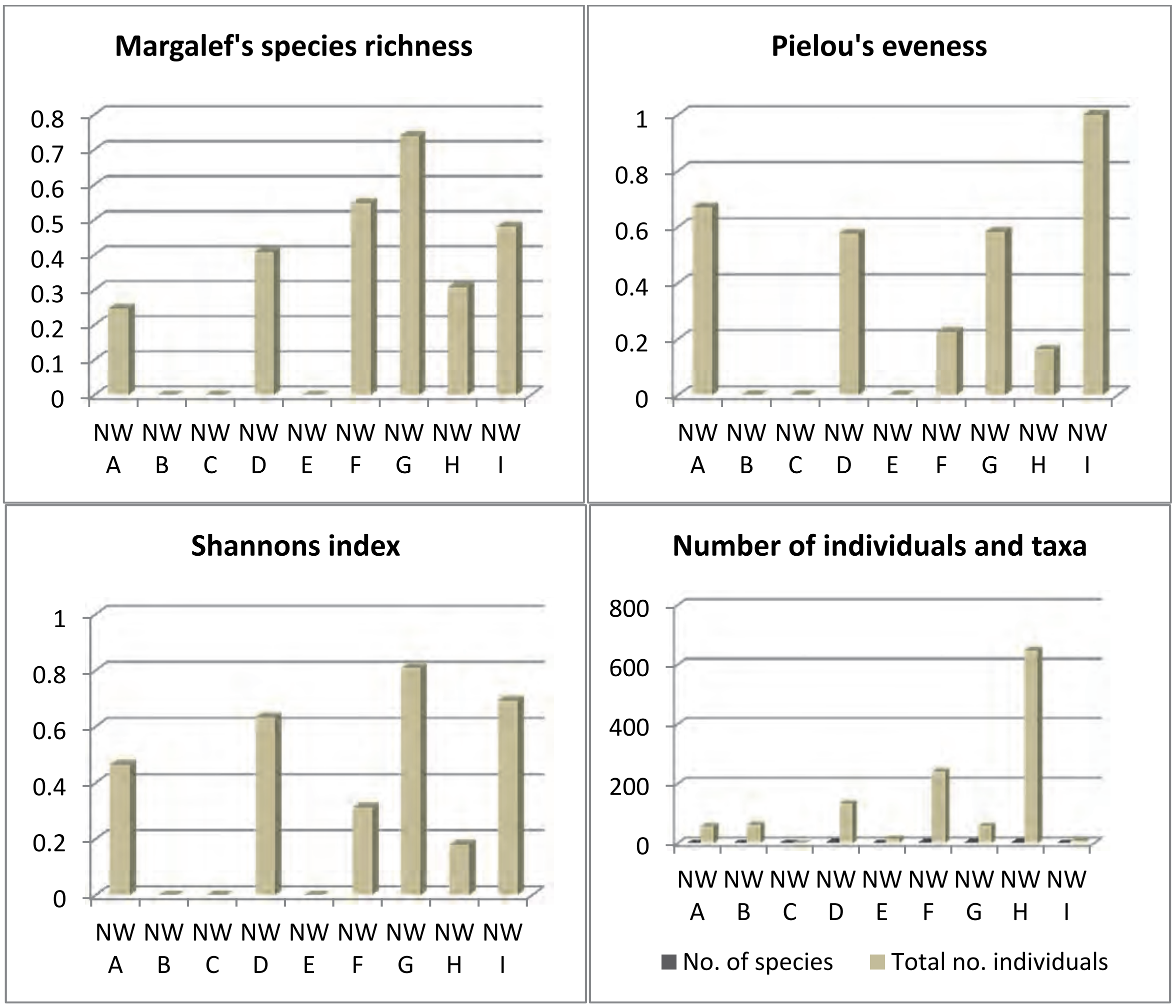


Figure 4-7: Univariate diversity indices of hatchling data for pans in the North West Province.

4.3.3.2 Free State Province

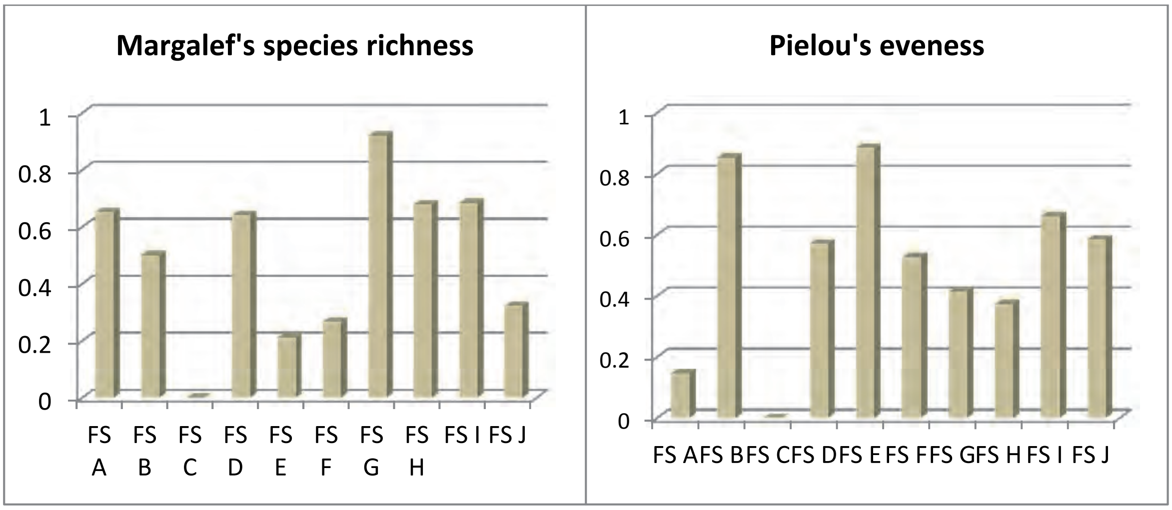
Of all the hatchlings identified eight belonged to the order Anostraca, 13 to the genus *Triops*, 1288 to the family Chydoridae, 117 to the family Daphniidae, five to the family Macrothricidae, seven to the subclass Copepoda and 157 to the class Ostracoda (Table 4.11). Free State pans A, F and G were the only pans to have the presence of Anostraca. Free State pans A and G were the only two to have *Triops* species hatching from the sediment. Free State pan D was the only one to have the occurrence of the Macrothricidae and Moinidae family. Free State pan G and I had Copepods hatching from the sediment, and FS pan A, B, G, H, I and J all had Daphniidae hatchlings. In the Free State pans only six individuals were able to be identified to species level. These individuals were *Daphnia barbata* and all occurred in FS pan B. All pans had the presence of Chydoridae, and all except FS pan C and F had the presence of Ostracoda. Chydorids and Ostracods were the most common species across these pans.

Table 4-11: Diversity and abundances of different taxa to hatch from Free State pans.

Taxa	FS A	FS B	FS C	FS D	FS E	FS F	FS G	FS H	FS I	FS J
Anostraca	1	0	0	0	0	5	2	0	0	0
Triops	7	0	0	0	0	0	6	0	0	0
Chydoridae	436	30	6	81	78	37	181	1	52	386
Daphniidae	2	6	0	0	0	0	3	1	18	87
Moinidae	0	0	0	6	0	0	0	0	0	0
Macro thricidae	0	0	0	5	0	0	0	0	0	0
Copepoda	0	0	0	0	0	0	6	0	1	0
Ostracoda	11	18	0	15	34	0	30	17	9	23

Margalef's species richness index (Figure 4.8) shows that in the Free State pans G, I, H, A and D had high species richness values while FS pan J, F, E and C had low species richness scores. Free State pan C had the lowest richness score as only one taxa hatched. Free State pan G had 6 taxa, FS pan A had five taxa, FS pan I and D had four taxa and FS pan H had three taxa present. Free State pan I and pan H were ranked above FS pan A, as these pans had more taxa over the total number of individuals compared to FS pan A. Regarding the evenness scores, FS pan E and B had high evenness scores signifying that there were no dominant taxa present in these pans. The individuals were more or less equally distributed across the taxa present in these pans. Free State pan C had the lowest evenness score. All 6 individuals that hatched from FS pan C belonged to the family Chydoridae. Free State pan A also had a low evenness score despite having 5 taxa present in the pan. The dominant taxon was again the Chydoridae with 436 out of the 475 individuals hatching belonging to this order. Free State pan G and H had moderate evenness scores. However, FS pan G had a high occurrence of Chydorids and FS pan H had a high occurrence of Ostracods.

The Shannon-Wiener index shows that FS pan B, I, D, G and J had a high diversity based on the combination of species richness and evenness scores, while FS pan E, H, F, A and C were less diverse. The lowest diversity of hatchlings was found in FS pan C. According the graph FS pan B had the highest diversity, although pan B only had 3 taxa, two of them belonging to the order Cladocera. FS pan G and A had a high diversity of hatchlings both having the highest species richness scores, with 6 and 5 taxa respectively. They were also the only two pans to have the presence of Anostracan and Notostracan species (large branchiopoda) as well as Cladocera and Ostracoda, making them taxonomically more diverse pans.



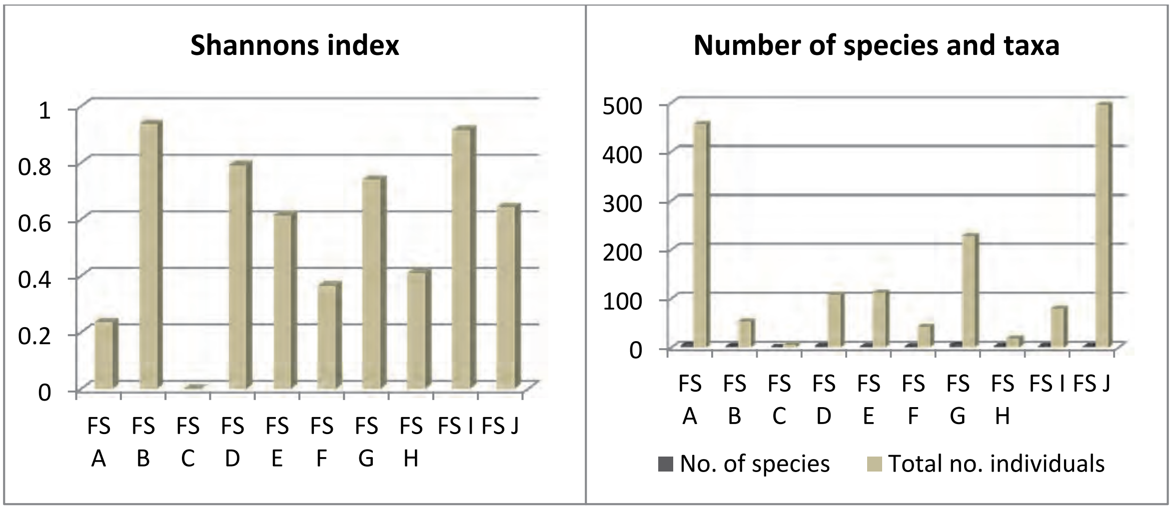


Figure 4-8: Univariate diversity indices for pans in the Free State Province

4.3.3.3 Mpumalanga Province

In the Mpumalanga Province, over all the pans, 369 individuals were identified. Of these individuals 18 belonged to the order Anostraca; 240 to the family Chydoridae; two individuals belonged to the family Daphniidae; 26 to the family Moinidae; four individuals were identified from the subclass Copepoda, 78 from the class Ostracoda and three from the subclass Hirudinea (Table 4.12). One individual was able to be identified to species level. This was *Daphnia carinata* and was found in MP pan H. Freshwater zooplankton (Cladocera, Ostracoda and Copepoda) were common and more abundant taxa occurring in the Mpumalanga pans rather than the large branchiopoda.

Table 4-12: The abundance and diversity of the different taxa to hatch from the Mpumalanga pans.

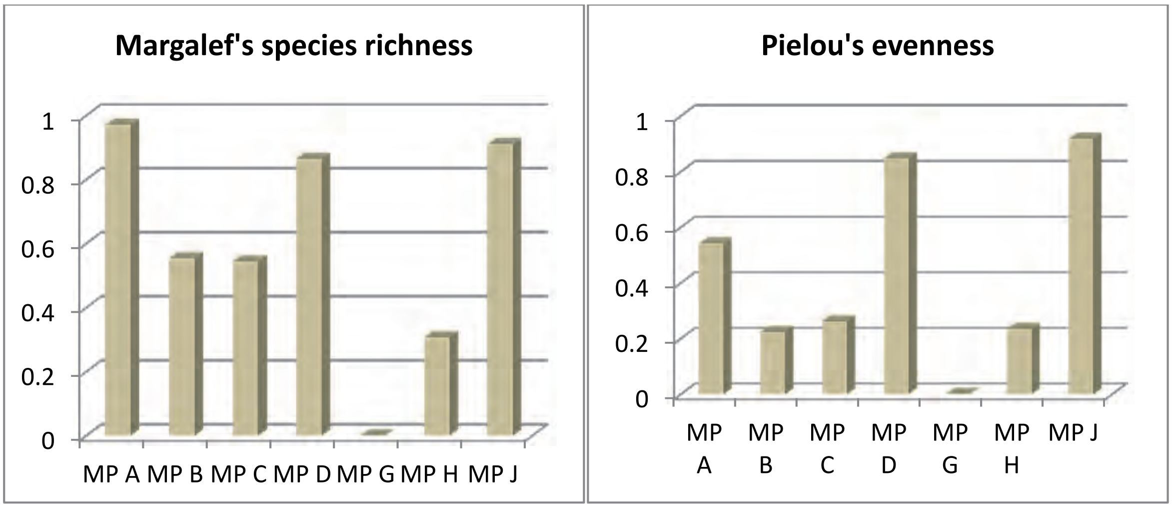
Taxa	MP A	MP B	MP C	MP D	MP G	MP H	MP J
Anostraca	1	1	4	12	0	0	0
Chydoridae	0	0	226	12	0	0	2
Daphniidae	0	0	1	0	0	1	0
Moinidae	0	0	0	0	0	25	1
Copepoda	3	1	0	0	0	0	0
Ostracoda	17	35	18	7	1	0	0
Hirudinea	1	0	0	1	0	0	0

Figure 4.9 presents the univariate statistics results comparing pan diversity. Mpumalanga pan E, F and I were omitted from the univariate analysis due to the absence of hatchlings in these pans. The Margalef's species richness shows that MP pan A, D and J had a high species richness. MP pan A had 4 taxa along with MP pan D. However MP pan J had only 2

taxa hatching from the sediment. Mpumalanga pan C scored lower than MP pan J but MP pan C had the presence of 4 taxa. The spread of the taxa across the total number of individuals brought the score down in MP pan C. Therefore the pans that had the greatest species richness were actually MP pan A, C and D.

Pielou's evenness index (Figure 4.9) shows that MP pan D and J had a high evenness score relative to the other pans. Mpumalanga pan B, C, H and G had low evenness scores indicating a dominance of taxa present. Mpumalanga pan B had a dominance of Ostracoda; MP pan C had a dominance of Chydoridae; MP pan H had a dominance of Moinidae and MP pan G had a single hatchling belonging to the class Ostracoda (Table 4.12).

The Shannon-Wiener diversity index indicated that MP pan D was the most diverse pan with regards to sediment diversity, as this pan had a high species richness and a relatively even spread of the individuals across the 4 taxa. Mpumalanga pan A also had a high diversity of individuals hatching from the sediment. Mpumalanga pan J, based on the total number of individuals and number of taxa (two taxa), was a low diversity pan but had a high index score due to skewed Margalef's richness scores. Despite the dominance of the Chydorid taxon (Table 4.12) in MP pan C, the total number of individuals together with the number of taxa (four taxa) places this pan in the high diversity category rather, in place of MP pan J. Thus pans that showed relatively good sediment diversity were MP pan D, A and C.



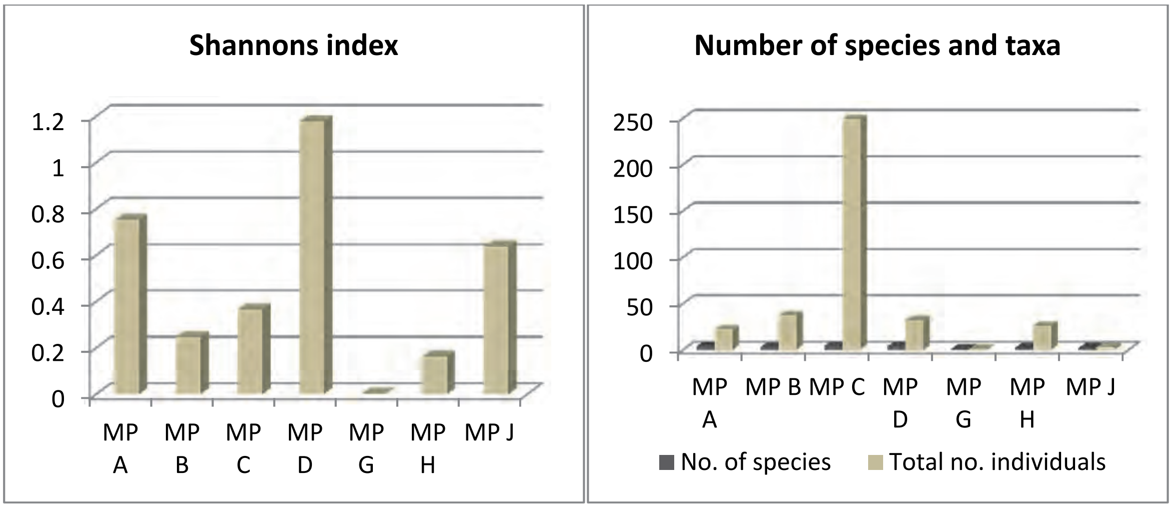


Figure 4-9: Univariate diversity indices for pans in the Mpumalanga Province.

4.3.4 Spatial variation

4.3.4.1 North West Province

The Bray-Curtis similarity coefficient-based analysis looking only at North West pans (Figure 4.10) shows that NW pan B and NW pan G were the most similar pans in terms of species assemblage (>60 %). NW Pan F and H were also similar but at a lower percentage (>50 %). The rest of the groupings have a high dissimilarity. All pans together have a similarity of less than 10 %. Emphasising that despite pans having only four taxa at most, the abundances and occurrences of taxa differed greatly between pans.

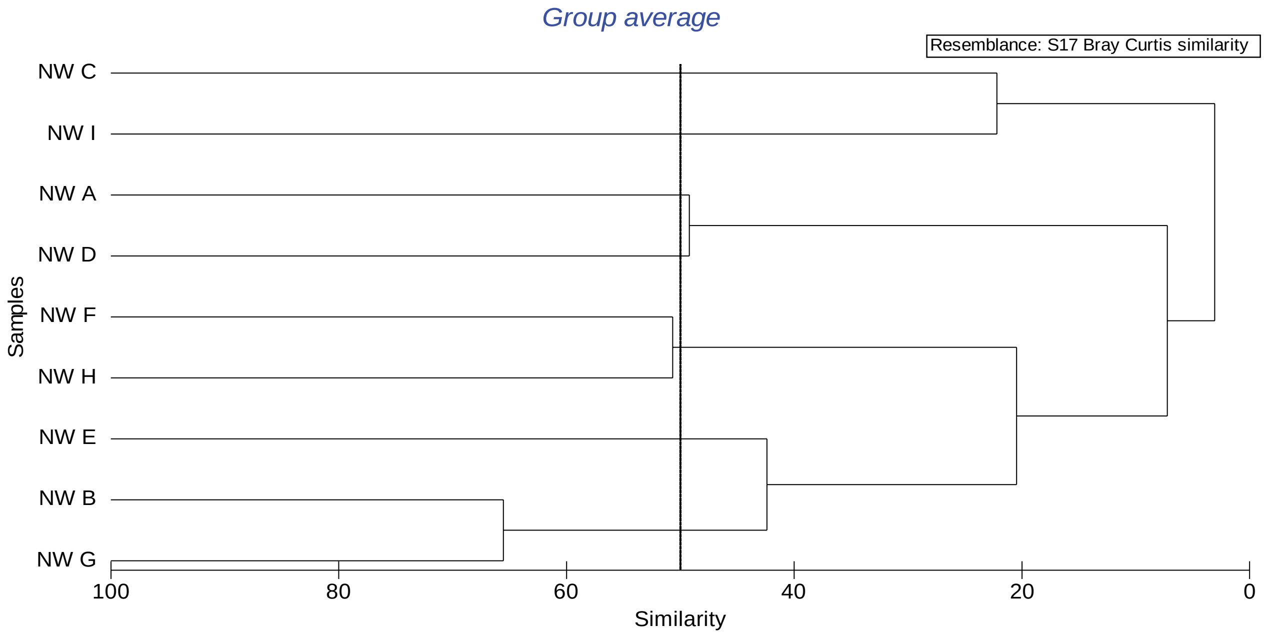


Figure 4-10: Bray-Curtis similarity coefficient-based cluster analysis of hatchlings identified from the different pans during the 28 day exposures.

4.3.4.2 Free State Province

The Bray-Curtis similarity graph (Figure 4.11) highlights that FS pan A and J are very similar (>90 %), and FS pan D and E are also very similar (>90 %). Free State pan B, F, I, D and E were all similar with similarity falling above 60 %. Free State pan A, J and G had a separate grouping on the graph (>60 %). Free State pan C was totally dissimilar to all the other pans (<20 %). The reason for such groupings was based mainly on the Chydoridae taxon abundance with FS pan B, F, I, D and E having similar abundances. Similar numbers of Chydorids were found in FS pan A and J, but these two pans also had the occurrence of the family Daphniidae in common which resulted in a separate grouping to FS pan B, F, I, D and E. Free State pan A and G shared similarities in their Anostraca and *Triops* taxonomic composition. Groupings in the Free State pans were thus based largely on Cladoceran abundance.

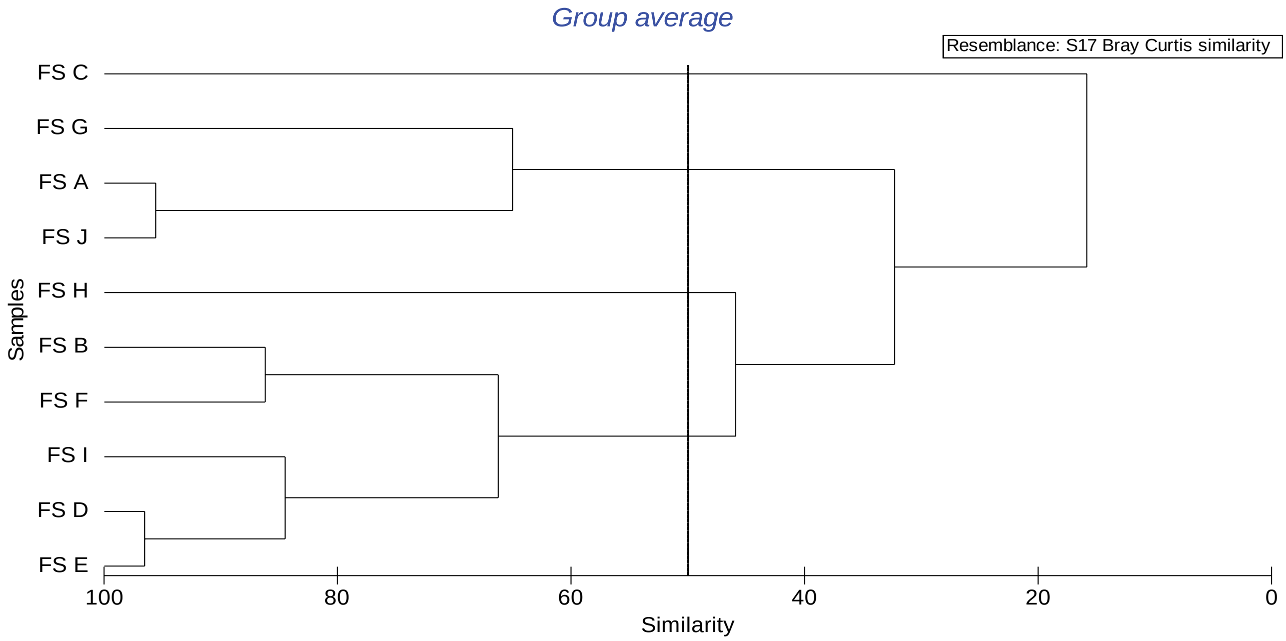


Figure 4-11: Bray-Curtis similarity coefficient-based cluster analysis of hatchlings identified from the different pans during the 28 day exposures.

4.3.4.3 Mpumalanga Province

The Bray-Curtis hierarchical cluster (Figure 4.12) shows that MP pan A and MP pan B are the most similar with regards to taxonomic composition (> 60 %) compared to all the pans that had the presence of hatchlings. Both pans had a similar number of Anostraca, Copepoda and Ostracoda. All the other pans were dissimilar in composition as all pans together were less than 10 % similar. There were therefore no significant groupings of pans in the Mpumalanga Province with variation in abundance and occurrence of taxa between the pans.

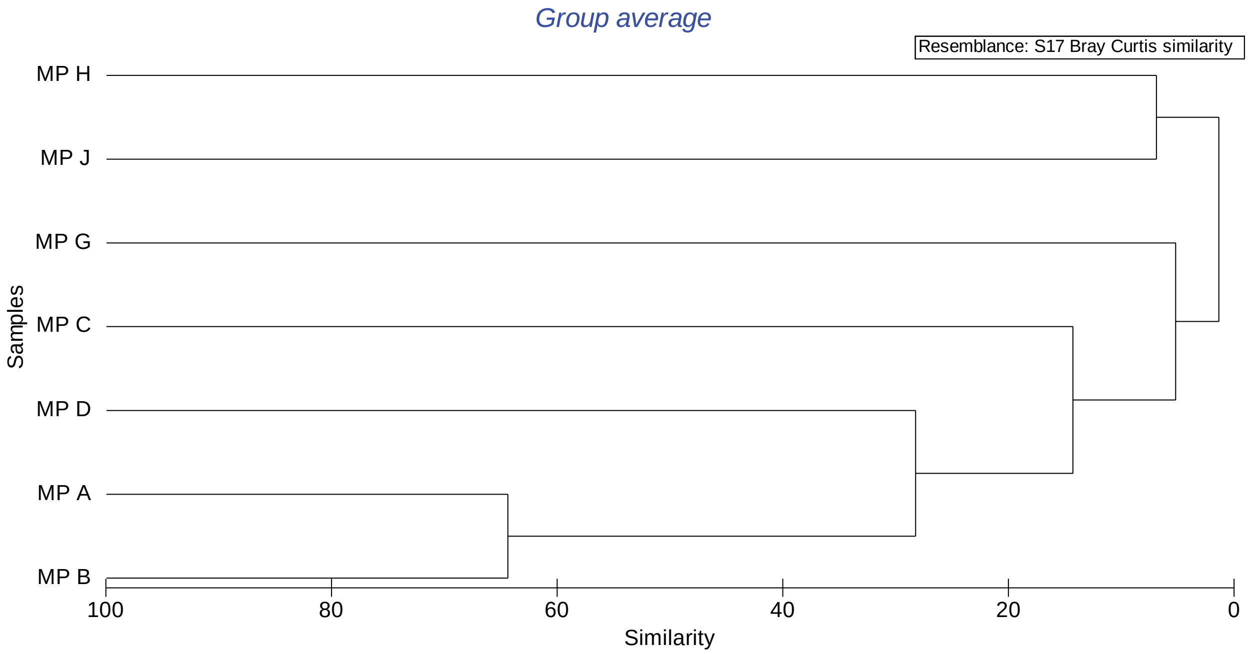


Figure 4-12: Bray-Curtis similarity coefficient-based cluster analysis of hatchlings identified from the different pans during the 28 day exposures.

4.3.4.4 Overview

A PCA biplot (Figure 4.13) was constructed to compare the community assemblages of the pans in all three provinces to assess for spatial differences based on the hatching data. The triangle geometric shapes on the biplot represent species data. The circle, diamond and square geometric shapes represent the pan data for the North West, Mpumalanga and Free State provinces respectively. Species data consisted of the different taxa to hatch from the 1000 mg/l and 1500 mg/l control treatments combined for each pan. Preliminary PCA's were constructed with the control treatments kept as separate entities, but results indicated a high degree of overlap between the 1000 mg/l and 1500 mg/l treatments for each pan. These separate points therefore had the same position in space on the graph and did not affect the groupings, thus it was decided to combine the two treatments to reduce clutter on the graph.

A total of 66.5 % of the spatial variation is described on the biplot. The PCA indicates that the majority of pans group together within their respective provinces i.e. pans in the North West group together; pans in the Free State group together and pans in Mpumalanga group together. Only a few pans had similar taxonomic compositions with pans in other provinces. Mpumalanga pan C had some similarities with some of the Free State pans. Free State pan H and C were similar to some of the Mpumalanga pans and two of the North West pans, while NW pan A, C and I were similar to the Mpumalanga pans. The PCA biplot confirms the trends observed in the cluster analysis in sections (4.1.4.1, 4.1.4.2 and 4.1.4.3). The biplot highlights that the characteristic taxa in the North West pans that differentiate these pans from pans in the Free State and Mpumalanga are the Anostraca and Conchostraca. The Free State pans grouped together, and separately from the North West and Mpumalanga pans, due to the Chydoridae and Daphniidae taxonomic composition. Mpumalanga pans grouped together as a result of a combination of taxa with no one taxon being responsible for the differentiation. This is because the component taxa of the Mpumalanga pans are situated towards the centre of the biplot (the intersect of the x-axis and y-axis). Taxa which are situated near the center do not contribute to groupings in a significant way, as they occur in low abundances and have a low occurrence among the pans. These taxa were the Moinidae, Hirudinea, Macrothricidae, *Triops* and Copepoda.

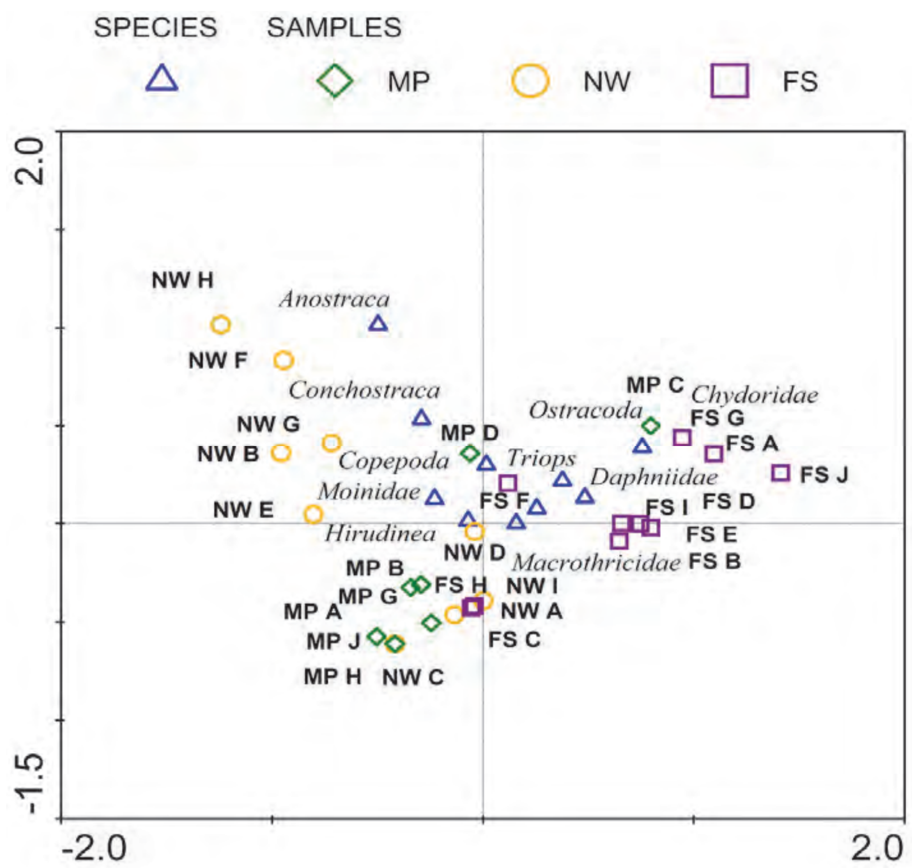


Figure 4-13: PCA bi-plot representing the taxon and abundance of pans in all three provinces. The PCA describes 66.5% of the total variation, with 46.9 % of the variation being described on the first axis and 19.6 % of the variation described by the second axis.

The Bray-Curtis hierarchical cluster for all pans together (Figure 4.14) emphasizes the similarity seen between pans in the three provinces. The cluster analysis reveals that the North West pans, although grouping together and showing similarities in the PCA (Figure 4.17), were less than 20 % similar. The Free State pans had similarities of less than 40 % and the Mpumalanga pans were the least similar, grouping together at less than 10 %. Pans in the Mpumalanga Province shared greater similarities with the low diversity pans in the North West and Free State provinces (NW pan C, I, A, E, and FS pan G and H).

Overall the PCA and Bray-Curtis data show that the taxonomic composition varied between pans and between provinces. Pans in their respective provinces were similar to a small extent but differed greatly from province to province. There were greater inter-pan similarities in the North West and Free State provinces respectively although very low, while the Mpumalanga pans had more inter-provincial similarities.

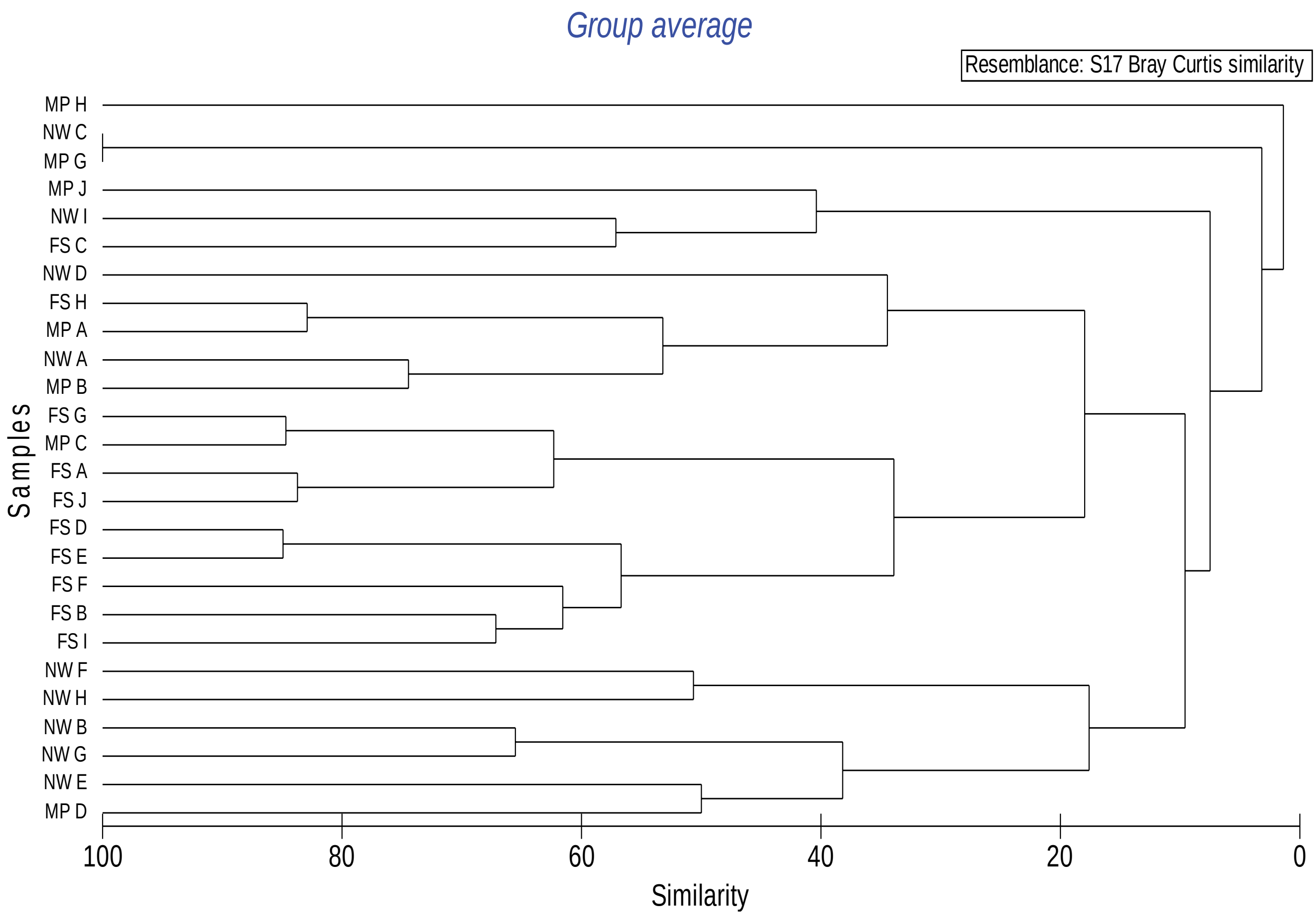


Figure 4-14: Bray-Curtis similarity coefficient-based cluster analysis of hatchlings identified from the all the pans in the North West, Free State and Mpumalanga provinces.

4.4 Discussion

4.4.1 Physico-chemical variables

The physico-chemical variables indicate that the mean temperatures differed slightly between the provinces; however, temperatures were relatively stable between treatments and between pans within their respective provinces. This is made evident by the range between the minimum and maximum temperatures, which was comparatively small. Temperature fluctuations were a result of the ambient air temperatures outside the environmental room which influenced the temperature within the room despite the air temperature of the environmental room being set to 1823°C. The seasonal variations in outside air temperature were able to impose temperature deviations within the rooms despite best efforts for control. Although a range of temperatures were experienced the temperatures still fell within the range that have been used in laboratory hatching experiments to obtain best results (5-35°C) (Brendonck, 1996). It was not the aim of the study to obtain a temperature best suited to hatching, and such temperatures are usually those of the environment from which sediment is obtained (Brendonck, 1996). The fluctuating temperatures would serve to accommodate a larger portion of species hatching as different species have different optima and hatching of a single species has shown large variability under standard conditions regardless (Scott and Grigarick, 1979; Brendonck *et al*., 1996; Van Dooren and Brendonck, 1998; Mura and Zarattini, 1999). This implies that the temperatures treatments were exposed to were suitable to provide an accurate account of hatching success and diversity.

The mean pH also differed between pans and provinces. The North West pans had the highest pH values for all treatments with pH falling well into the alkaline range. The Mpumalanga pans had the lowest pH ranges out of all three provinces. It is not unusual to find different pH's among pans in different regions, even within the same region. The Bains vlei pans in the Free State Province, studied by Meintjes *et al*. (1994), had a pH range from 6.8 to 10.6. In the Western Cape, pH of pans can range from 4.6 to 9.1. The acidic pans are a result from weak organic acids leaching from decaying fynbos vegetation (Day *et al*., 2010). Pans in the Lake Chrissie region are known to have pH values of 8 and above (Hutchinson *et al*., 1932; Ferreira, 2010). The pH of the water within a pan is largely determined by sediment chemistry. Each pan has a slightly different concentration and composition of ions present in the sediment, a factor which is predetermined by the geology the area. Vegetation (photosynthesis) and organic matter (decay of organic matter) are also known to have effects on the pH (Meintjes *et al*., 1994; Day *et al*., 2010). Given the differences in the pan environment of the three regions one would expect pH differences i.e.

the non-vegetated clay based sediments of the North West, to the grass pans in the Free State to the perennial semi-vegetated pans in the Mpumalanga Province. In the current study where only 25 g of sediment was used in laboratory experiments per replicate, ion composition and ion concentration of the sediment would more likely be a larger contributor to the pH than organic content or photosynthetic activity. The ion content of sediment in the North West Province had a buffering effect on the pH of the AMD treatments, being the only province where the AMD had a mean pH of above 7. The mean pH of the AMD treatments was acidic in the other two provinces.

The EC readings of the 1500 mg/l treatments were higher than the 1000 mg/l treatments as there were more grams of salt per litre in the 1500 mg/l solution. However, there was still large variation in EC from pan to pan considering the standard salinities of the control solutions. The variation between treatments was most prominent in the North West Province. The variation can once again be attributed to the ionic composition of the sediment. The greater the concentration of ions in the sediment the higher the EC readings of the water as more ions can be dissolved. The North West pans had the highest EC readings while the lowest readings occurred in the Mpumalanga pans. In all provinces the AMD treatments had the highest EC. Acid mine drainage is known to contain high concentrations of mineral salts and in addition, the low pH can dissolve and mobilise other minerals such as aluminium and copper which are present in clay (Robb, 1994). Acid mine drainage can therefore contain high concentrations of heavy metals such as: iron, zinc, cadmium and arsenic to name a few (Gray, 1997). These minerals have high valences and are responsible for the electrical charge of AMD. This added to the natural mineral salts within the sediment of certain pans lead to excessively high EC readings i.e. the AMD of NW pan D had an average EC of 10.70 mS/cm which was the highest EC measured across all the pans in the experiment.

4.4.2 Hatching experiments and timing of hatch

The hatching abundance of crustaceans was relatively high overall given the small amount of sediment used per replica (25 g), and that only a single set of hatching conditions were used throughout. Abundances obtained here are comparable to other studies assessing the hatching success of zooplankton communities (Vanderkerkhove *et al.*, 2004; 2005). The North West and Free State provinces in particularly had high cumulative abundances. The Mpumalanga pans had the lowest abundance of hatchlings emerging from the sediment. This was not an unusual result as pans sampled in the North West and Free State provinces were largely ephemeral in nature, while those sampled in the Mpumalanga Province were of a perennial nature. Branchiopod crustaceans are unique to temporary wetlands (Wiggins *et al*., 1980) and are adapted to the ephemerality of such wetlands through drought resistant eggs (Wiggins *et al*., 1980; Brendonck, 1996; Williams, 1997; Lahr, 1997). The dry phase is thus an integral part of the lifecycle of such organisms. As these organisms are well adapted to the dry phase, the dry phase may be a necessity l for the existence of these crustaceans, implying that these organisms (with special reference to the large branchiopoda) can only occur in temporary wetlands.

On the contrary, Ferreira *et al*. (2011) found that the invertebrate community of perennial pans can be representative and comparable to those in ephemeral pans upon drying. The pans sampled by Ferreira *et al.* (2011) were from the Mpumalanga Province and many of these pans were sampled in this current study. It is then reasonable to assume that the low abundances found in the Mpumalanga pans, which are perennial in nature, can be attributed to the hatching conditions. The sediment collected from the Mpumalanga pans was wet sediment and may have required a longer period of desiccation, compared to the sediment collected from the North West and Free State pans which was already dry. Many studies have looked at desiccation periods and hatching rates (Brendonck, 1996) and generally with longer desiccation periods, a greater proportion of hatchlings are seen. A study by Mitchell (1990) found that longer periods of desiccation of up to 65 days lead to a greater hatching success in *Streptocephalus macrourus*. Mura (2005) found that oven dried sediments yielded more hatchlings than naturally dried sediments for *Chirocephalus ruffoi* eggs. Optimising the desiccation period and determining the extent to which it influences hatching needs to be further investigated.

There were significant differences in hatchling abundance between the various pans. This was an expected result as successful hatching is a function of conditions of exposure, the species present and the fraction of quiescent and diapausing eggs. Non-parametric tests were unable to locate where the differences were situated. Hatching of individuals is also known to vary under identical conditions and only a fraction hatch during the inundation period (Brendonck *et al*., 1996; Vanderkerkhove *et al*., 2004). Therefore looking instead at the community composition of pans would provide more insightful information on whether hatching was successful and a pan is thriving or declining rather than relying on overall abundance to hatch. It is possible that in the pans where abundances were low the diapausing deactivating stimuli for these cysts were not met. Another cause for low abundances could be the inundation period not being long enough, and eggs within the sediment may have already lost their viability. The above mentioned issues would require further analysis to rule out, but despite this the majority of pans in this study had reasonable hatchling abundances compared to each other. The only pans where the abundances values were an issue of concern were the AMD treatments as no hatching occurred.

In the experiments more pans had a greater hatching abundance in the 1000 mg/l treatment in all provinces. Statistically there were no significant differences in abundance between the 1000 mg/l and 1500 mg/l control treatments except in: NW pan C, FS pan B, FS pan C and FS pan E. Overall, both control treatments proved to be suitable incubation mediums. The two control treatments may have had an effect on hatching rate rather than abundance as has been noted previously (Ketley, 2007). Factors affecting the rate of hatching, such as salinity, temperature and pH were not evaluated in this study as they were deemed irrelevant in determining the effect of AMD on hatching success. Hatching success as used in this study refers to the abundance and diversity rather than rate of development.

The rate of hatching was assessed though in very general terms, with the time at which hatching started and the time at which hatching peaked monitored for the control treatments separately. In the North West pans hatching generally started early with hatching peaking in the 4-16 day interval (NW pan A, B, E, F, G and H). In a few pans hatching started later and only peaked towards the 24 day interval (NW pan C, D and I). In the Free State pans hatching generally started early in the inundation period, from the 4-12 day interval. However in the majority of the Free State pans hatching peaked later (FS pan A, B, D, E, F, G, H, I and J) during the inundation period compared to the majority of North West pans where hatching peaked early. Hatching only peaked in these pans in the 16-28 day interval. The Mpumalanga pans showed the same pattern of hatching as the Free State pans where hatching started early but peaked later (MP pans A, B, C, G, H and J). Hatching peaked around the 20 day interval for Mpumalanga pans. The only pan in Mpumalanga where hatching peaked early, was at MP pan D where the hatching peaked at day 4.

From the overlap between the provinces two distinct periods of peak hatching can be drawn; hatching peaking in the 4-16 day interval, and hatching peaking in the 16-28 day interval. An observation made during the course of laboratory experiments was that the Anostracan and Notostracans were the first group of crustaceans to be identified. The next group of crustaceans to be identified over the exposure period where the Cladocera followed on shortly by the Ostracoda. It was also noted that those pans that had a peak in hatching within the 4-16 day interval had an abundance of Anostraca hatching. While those pans where hatching peaked in the 16-18 day interval had high numbers of Cladocera and Ostracoda hatching. These observations are discussed further in section 4.3 below.

4.4.3 Hatching diversity

In the North West pans Anostraca were the most prevalent taxon, occurring in: NW pan B, D, E, F, G and H. All of these listed pans demonstrated a peak in hatching in the 4-16 day interval. Ostracods were also a prevalent taxon but were less abundant than the Anostraca. In the Free State pans Chydoridae and Ostracoda were common taxa with high abundances relative to the Anostraca which were less prevalent. These two taxa were present in: FS pan A, B, C, D, E, F, G, H, I, and J. In the Free State pans hatching peaked in the 16-28 day interval in: FS pan A, B, D, E, F, G, H, I, and J. The Mpumalanga pans demonstrated a similar trend to the Free State pans where the Chydoridae and Ostracoda were most prevalent, and occurred in higher abundances than any other taxa. Although Anostracans did hatch in a few pans (MP pan A, B, C, D) their abundances did not compare to those that hatched in the North West Province. Mpumalanga pan A, B, C, D, G and J had a prevalence of either Chydorids or Ostracods or both. The Mpumalanga pans demonstrated peak hatching trends in the 16-28 day interval in pans A, B, C, G and J. It is therefore fair to state for all provinces that the hatching of Anostracans were responsible for hatching peaks early in the inundation period and that the hatching of Cladocerans and Ostracods were responsible for peaks later in the inundation period. Notostraca and Conchostraca were the rarest of the branchiopoda taxa hatching in the experiments.

Different crustaceans hatch at different times and grow at different rates (Day *et al*., 2010), a natural process in pan succession. Succession refers to the patterns of change in community structure and composition over a period of time. Pan succession is largely driven by the life history traits of organisms present which are adjusted to biological and physical characteristics of the pan i.e. the availability of resources and the length of inundation (Lahr, 1997; Brendonck *et al*., 2008). The patterns of hatching found here, throughout all three provinces, were found to be similar to *in situ* patterns of pan succession observed by Meintjes (1996). With regards to crustacean taxa only, Meintjes (1996) found that that the Notostraca and Anostraca were the first group to appear within 48 hours after inundation. The Ostracoda, Conchostraca and Cladocera followed in turn. This pattern differed slightly from pan to pan and no common pattern could be distinguished with certainty. The hatching experiments of Day *et al.* (2010) revealed that Anostracans were the first group of crustaceans to be identified after inundation followed by the Cladocera. The Ostracoda and Conchostraca were the last group to be identified. It is not unusual to find a single representative of Branchiopoda in a pan.

The hatching patterns found in the current study were in greater alignment with those reported by Day *et al.* (2010). The lack of Conchostraca found in this study could be related to the exposure period (length of inundation). A 28 day exposure period may not have been long enough to allow the hatching of these crustaceans given the conditions used, as they are the last group known to hatch. The lack of Notostraca identified in the experiments was unusual, as this taxon is prevalent in many of the pans sampled during the same period that the sediment samples were collected for this study (unpublished data). It could be that conditions were not optimal for the hatching of this taxon. Scott and Grigarick (1979) discovered that the optimum hatching temperature for *Triops* was 22-25°C. The temperature used in the current experiments (18°C) may have been too low. Another factor could be that Notostraca did hatch but were unable to survive the conditions used, and thus died before any positive identification could be made. Mortalities were found to be quite high especially during the early stages of inundation (day 4-16), which is when Anostraca and Notostraca are known to hatch (Meintjes, 1996; Day *et al*., 2010).

Many individuals that hatched in these experiments died before they could be positively identified. Mortalities are not uncommon for hatching experiments and have been encountered in previous studies (Vanderkerkhove *et al*., 2004, 2005). Mortalities have been attributed to the limitations of the culture conditions such as food source and the use of static systems. However because branchiopods are r-selected (Lahr, 1997, 1999) natural mortalities are expected. Environmental conditions that promote hatching do not necessarily promote growth and survival as physico-chemical variables are continuously fluctuating (Bonis *et al*., 1993). Also, many of the individuals that did survive were too small and immature to be identified to genus or species level with accuracy, although hatchlings could be identified to order or family level. The keys for identification are based on adult specimens rather than the nauplius larvae which have to develop some of the distinguishing features that would allow the differentiation between species (Day *et al*., 1999). Identification is based mainly on the mouthparts, often requiring dissection. This procedure is difficult for juvenile specimens and can destroy the whole specimen.

Despite this a few individuals were able to be identified to genus level such as the *Streptocephalus* (NW pan F) and *Triops* (NW pan A, FS pan A, FS pan G), as well as species level such as the *Daphnia barbata* (FS pan B), the *Daphnia carinata* (FS pan H) and the *Moinia belli* (NW pan H). Due to the order and family level identifications the diversity of many pans sampled here are likely to be underestimates of pan diversity. The diversity of pans given the underestimates of taxa is reasonable as the majority of pans had the occurrence of four taxa of which half were always branchiopod crustaceans (Anostraca, Notostraca, Conchostraca and Cladocera). There are 46 known Anostracan species from Southern Africa, belonging to six described genera and at least one undescribed genus (Hamer and Martens, 1998). The Anostraca is of particular importance as many of them are known to be listed on the International Union for Conservation of Nature (IUCN) list for endangered species. This includes two species that are listed as critically endangered, four endangered and three vulnerable species. The list also includes thirteen data deficient species (Day *et al*., 1999). Literature suggests that individuals of the genus *Triops* are restricted to temporary wetlands that are usually less than a hectare in size (Davies and Day, 1998; Day *et al.*, 1999). Ferreira *et al.* (2011), however, has reported the occurrence of *Triops granarius* from more permanent systems in the Mpumalanga Highveld. *Triops* species are usually benthic and feed on organic matter. Like most Branchiopod crustaceans *Triops* can survive conditions of low oxygen availability due to the ability to produce haemoglobin (Guadagnoli *et al*., 2005). It is not unusual to find a single representative of Branchiopoda in a pan.

Field studies at most have only found the coexistence of 10 species (Thiéry, 1991; Hancock and Timms, 2002). Waterkeyn *et al*. (2009) found that wetlands in the Camargue on average have 2.8 species per wetland. Hamer and Appleton (1991) found that in the Zululand (KwaZulu-Natal Province, South Africa) temporary pools the number of branchiopods inhabiting these pools were between 2 and 9 species. In view of the current results the Free State pans (FS pan G, A, D and I) were the most diverse considering just taxonomic richness having six, five, four and four taxa occurring respectively, whereas the greatest taxonomic richness in the North West (NW pan G, F, D and H) was four, four, three and three taxa respectively. The most diverse pans in Mpumalanga (MP pan A, C, D and B) had the occurrence of four, four, four and three taxa respectively. These results fall within the range found by Hamer and Appleton (1991). Based on these findings, given the short exposure period and artificial conditions used along with the underestimates of species diversity, those pans that had four or more taxa had a relatively good diversity. Overall, this diversity data offers baseline reference conditions for future studies.

The individuals that hatched from the family Chydoridae could not be accurately identified. Results indicate that they may possibly be from the genus *Pleuroxus* or *Pseudochydorus*. This family is taxonomically confusing and numerous species from this family is yet to be described (Day *et al.*, 1999). Like the larger Branchiopoda, Cladocerans have a highly adapted lifecycle that ensure efficient dispersal and survival during unfavourable conditions. In addition to producing drought resistant eggs, most cladocerans reproduce parthenogenetically (fertilisation of eggs is not required) hence a single individual can found a population (Havel and Shurin, 2004). Individuals from the family Chydoridae has been successfully hatched from experiments in other studies (Gleason *et al.*, 2003). Studies on the egg banks of Cladocera have proved to be important for biodiversity studies (Vandekerkhove *et al*., 2005; Santangelo *et al.*, 2011). As with most of the larger

Branchiopoda, resting eggs need to undergo a dormant phase before hatching takes place (Santangelo *et al.*, 2011).

The individuals that were sampled from the class Ostracoda were also not identified to genus level. Identification of ostracods is notoriously difficult to identify and requires full dissection. Most of the individuals that hatched were still juveniles when fixed and the available keys are mostly based on adults. The individuals that were preserved will be kept and can be sent to experts for identification if required.

4.4.4 Spatial variation

The multivariate results indicated that there were differences in the taxonomic composition of the different pans and that all three regions had differing compositions, with very little overlap. Hamer and Appleton's (1991) *in situ* study of phyllopod ecology found that there is a large overlap in phyllopod distribution between temporary pools in the KwaZulu-Natal Province. These pools however had a narrow range of physico-chemical variables but species richness varied, it was thus concluded that the physico-chemical variables do not seem to influence phyllopod diversity or distribution. The range of physico-chemical variables measured in our study was also in a narrow range and thus it was expected that the diversity and distribution would be more similar among pans, particularly from pans in the same region. Only a few pans shared close similarities, but pans from a single region when grouped together where dissimilar. The three regions were largely dissimilar in addition.

The dissimilarity can be attributed to local climatic differences between the three regions as well as hydrological differences, both of which are known affect the occurrence of branchiopods (Defaye *et al*., 1998). Precipitation within South Africa increases from west to east which explains the varying hydrology and the perennial pans of the Mpumalanga Province, receiving greater annual rainfall (Hulme *et al*., 1996). All three regions had variations in geology and vegetation. The North West Province had open clay pans, while the Free State Province had grass pans with clay based sediment and the Mpumalanga Province had open water pans with submerged macrophytes, and a mixture of clay and sandy soils. Vegetation will influence habitat availability and organic content, while the underlying geology will influence the salinity (Bond, 1946; Day, 1993). With hydroperiod and salinity said to be key factors influencing the diversity and structure of invertebrate communities in temporary wetlands (Spencer *et al*., 1999; Nielsen *et al*., 2003; Waterkeyn *et al*., 2008), it is not surprising to see differences in taxonomic composition between the three regions.

It has been said that hatching experiments in the laboratory can provide a good representation of the natural crustacean assemblage, and that a dry season assessment can provide a low level surrogate for wet season assessments of biodiversity (Day *et al*., 2010). Given this knowledge it is reasonable to assume that the assemblages found here are representative enough of those found in the field. Even at the most basic level of identification differences between pans could be depicted. Since there were differences in taxonomic composition, with emphasis between regions, the determination of the effects pollutants have on diversity and taxonomic composition has greater bearing. Information of this kind will have important implications on management and conservation strategies for threatened wetlands, as the loss of one wetland could result in a substantial loss in regional diversity.

4.4.5 Conclusion

The use of hatching experiments as a monitoring tool for the assessment of pan diversity proved to be a successful method. Branchiopod diversity and successional patterns could be determined from small amounts of sediment. These data will prove very useful in future studies and monitoring of these pans. Reference conditions of pan communities can effectively be obtained through egg bank analysis. The diversity of pan communities was different between pans and between regions for the larger part of the pans studied. From this it seems that pans have their own unique communities, and each one contributes towards the regional diversity. It would then be erroneous to apply it to Angeler and Garcia’s (2005) concept which states that the community structure of non-impacted pans should differ from impacted pans and therefore present reliable indicators of anthropogenic stress. It was found that the community structures of pans differ in any case, so unless the history of the pan is well known making a diagnosis via this approach could be inaccurate. Pans therefore need to be considered as separate entities and conservation efforts need to preserve each one, as a loss of a single pan can seriously compromise the regional diversity.

5 EFFECT OF ACID MINE DRAINAGE ON HATCHING SUCCESS

5.1 Introduction

The water contained in endorheic wetlands (pans) is not often used for domestic, agricultural or industrial purposes. The water quality of these systems is, however, affected by various land use activities throughout South Africa. Pans are increasingly under threat from coal mining activities and the extensive agricultural activities (especially in the Mpumalanga Province) which is fuelled by South Africa's ever increasing population growth and need for energy resources (Ziramba, 2008; Amusa *et al*., 2009). In recent years the threat of Acid Mine Drainage (AMD) has become an important concern in water resource management throughout the world and particularly in South Africa. In September 2002 the threat that AMD held for water resources became a reality when AMD started decanting from an abandoned mine shaft near Mogale City/Randfontein area of the Western Basin (DWAF, 2010). The Western Basin refers to one of the mining basins within the Witwatersrand Basin a geological formation in the Witwatersrand that has been mined since 1885 (Kirk *et al*., 2003). The Basin has produced most of the gold that has been mined in the world. In addition to the Western Basin (Krugersdorp area), the areas that are of particular concern include the Central Basin (Roodepoort to Boksburg) and the Eastern Basin (Brakpan, Springs and Nigel area) (McCarthy, 2011).

AMD occurs due to the flooding of the mine voids from disused mines. The waters that flood the mines are both from surface and groundwater origins (DWAF, 2010). The formation of AMD is well understood and has been extensively studied in many countries (DWAF, 2010.) Probably the most important mineral involved in the generation of AMD is the presence of Iron disulphide (FeS^2), or pyrite (Gray, 1997). In the presence of oxygen and water the pyrite undergoes oxidation in a two-stage process as indicated below (Singer and Strumm, 1970). Apart from pyrite, there are numerous other metal sulphides that can be involved in the process. Regardless of the compound, the end product is always a highly acidic, sulphate rich drainage.

$$FeS_2 + {}^{7}/_{2}O_2 + H_2O \longrightarrow Fe^{2+} + 2SO_4^{2+} + 2H^+$$

$$Fe^{2+} + {}^{1}/_{4}O_2 + H^+ \longrightarrow Fe^{3+} + {}^{1}/_{2}H_2O$$

$$Fe^{3+} + 3H_2O \longrightarrow Fe(OH)_2 + 3H^+$$

$$FeS_2 + 14Fe^{3+} + \; + H_2O \longrightarrow 12Fe^{2+} + 2SO_4^{2+} + 16H^+$$

A

B

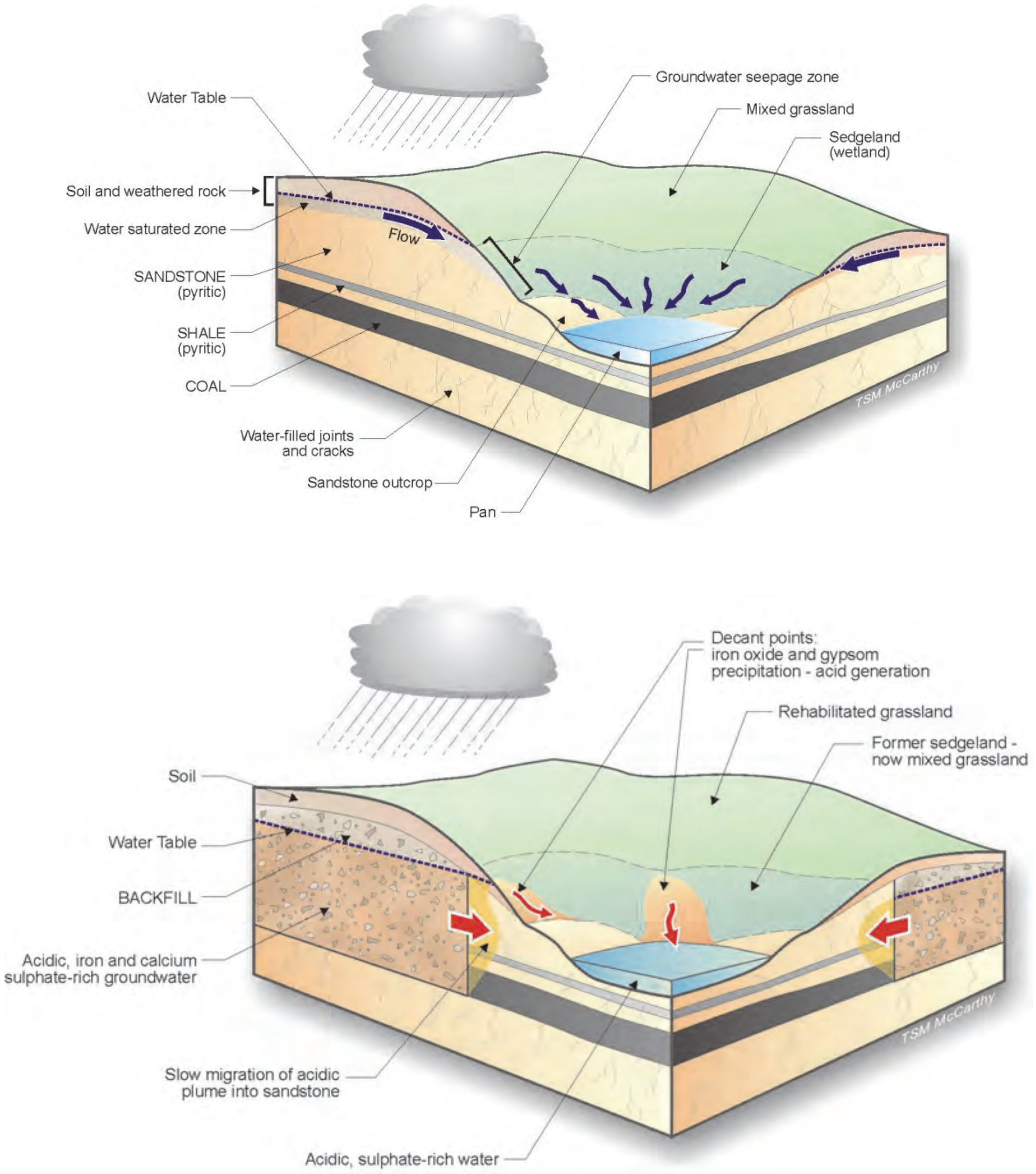


Figure 5-1: Diagram illustrating the geology and hydrology of a typical pan in the Lake Chrissie area (A) and the hydrological functioning of a typical pan after mining and rehabilitation have been completed (B) (From McCarthy *et al*., 2007).

Although the Witwatersrand Basin (and specifically the Central and Western Basin) has received a lot of attention with regards to research and monitoring, AMD has been reported from a number of other areas within South Africa, including the Mpumalanga and KwaZulu-Natal Coal Fields, Klerksdorp/Orkney/Stilfontein/Hartebeesfontein (KOSH), Free State, Far West Rand, Evander gold mining areas and the O'Kiep Copper District. The formation of AMD appears to be related mostly to gold and coal mining activities. The diamond, iron, manganese, chrome, vanadium and even platinum mines (to some extent) generally do not produce waste that is acidic in nature (McCarthy, 2011). The process of AMD formation can also be natural and drainage from deposits containing metal sulphides does occur. This process is, however, very slow and is naturally neutralised. Figure 5.1 provides information on the functioning of pans in Lake Chrissie as well as how mining and rehabilitation would affect the pan hydrology.

5.2 Materials and methods

The materials and methods used to complete the hatching success study are indicated in sections 3.4 and 3.5.

5.3 Results

5.3.1 Physico-chemical variables

The means of the physico-chemical readings measured every four days over the 28 day exposure period are presented in Table 5.1. The table compares the variables between the AMD recovery treatments and the original AMD treatments. The pH of the original AMD in the North West pans ranged from 6.98 to 7.58, while the pH in the AMD recovery ranged from 7.08 to 7.22. In the Free State pans the pH ranged from 2.70 to 4.31 in the AMD original and from 2.76 to 4.55 in the AMD recovery. In the Mpumalanga pans the pH ranged from 2.45 to 6.15 in the AMD original and from 2.51 to 5.17 in the AMD recovery. The North West pans had the highest range of pH.

The EC in the North West pans ranged between 4.17 and 10.70 in the AMD original while the EC in the AMD recovery ranged between 3.43 and 8.46. In the Free State pans the EC ranged between 5.4 and 8.46 in the AMD original and between 4.84 and 8.47 in the AMD recovery. In the Mpumalanga pans EC ranged between 3.03 and 5.20 in the AMD original and between 4.57 and 5.21 in the AMD recovery. The North West pans had more variation in EC of both the AMD original and the recovery compared to the Free State and Mpumalanga pans. Overall the physico-chemical variables of the AMD recovery treatments did not vary much from the original AMD treatments based on a replacement of the AMD with distilled water.

Table 5-1: The averages of the physico-chemical variables taken during the AMD recover exposures compared to those taken during the original AMD exposures. The EC was measured in mS/cm, TDS in mg/l, O2 CONC. in mg/l and O2 SAT as %.

Pans	Treatment	TEMP	pH	EC	TDS	O_2 CONC.	O_2 SAT
NW pan A	AMD Original	17.00	7.39	6.17	4.30	4.74	51.08
NW pan A	AMD Recovery	18.93	7.08	5.88	3.11	5.96	65.37
NW pan B	AMD Original	18.19	7.40	8.79	6.10	6.67	43.08
NW pan B	AMD Recovery	18.79	7.14	8.85	4.57	5.86	64.55
NW pan C	AMD Original	18.39	7.58	5.36	3.51	4.02	44.32
NW pan C	AMD Recovery	18.73	7.20	5.32	2.80	5.93	65.39
NW pan D	AMD Original	14.65	6.95	10.70	7.56	5.17	54.07
NW pan D	AMD Recovery	18.69	7.10	9.67	5.23	5.87	64.74
NW pan E	AMD Original	15.74	7.04	6.94	4.80	4.94	52.20
NW pan E	AMD Recovery	18.67	7.14	6.63	3.52	5.79	63.82
NW pan F	AMD Original	18.67	7.25	4.60	2.59	5.92	63.03
NW pan F	AMD Recovery	18.67	7.25	3.83	1.99	5.83	64.36
NW pan G	AMD Original	18.51	7.22	5.66	3.23	5.78	61.46
NW pan G	AMD Recovery	18.71	7.22	4.90	2.57	5.77	63.70
NW pan H	AMD Original	18.47	7.04	7.99	4.67	5.77	60.98
NW pan H	AMD Recovery	18.72	7.12	7.69	4.11	5.77	63.64
NW pan I	AMD Original	18.43	6.98	4.17	2.33	5.85	62.18
NW pan I	AMD Recovery	18.93	7.15	3.43	1.77	5.82	64.22
FS pan A	AMD Original	19.56	3.80	8.46	4.23	4.80	64.05
FS pan A	AMD Recovery	18.83	3.72	8.47	4.54	5.93	65.25
FS pan B	AMD Original	19.93	3.82	5.55	2.78	4.89	66.16
FS pan B	AMD Recovery	18.92	3.95	5.35	2.93	5.60	63.82
FS pan C	AMD Original	20.14	4.21	5.55	2.76	4.89	66.10
FS pan C	AMD Recovery	18.86	4.25	5.35	2.81	5.81	63.95
FS pan D	AMD Original	18.74	3.86	6.34	3.14	5.11	66.81
FS pan D	AMD Recovery	18.80	3.72	6.37	3.38	5.91	64.97
FS pan E	AMD Original	19.25	3.95	6.17	3.08	4.64	63.38
FS pan E	AMD Recovery	18.81	4.13	5.81	3.07	5.83	63.97
FS pan F	AMD Original	20.35	4.31	6.61	3.75	5.21	58.88
FS pan F	AMD Recovery	18.77	4.55	5.46	2.88	5.92	64.91
FS pan G	AMD Original	20.29	3.08	5.54	3.11	5.13	58.20
FS pan G	AMD Recovery	18.82	3.31	4.92	2.58	5.91	65.12
FS pan H	AMD Original	20.42	3.24	5.91	3.29	5.29	60.48
FS pan H	AMD Recovery	17.94	3.39	5.22	2.73	5.82	63.87
FS pan I	AMD Original	21.57	2.99	5.58	3.25	5.21	60.51
FS pan I	AMD Recovery	19.01	3.21	4.84	2.53	5.92	64.53
FS pan J	AMD Original	20.26	2.70	6.92	3.91	5.37	60.70
FS pan J	AMD Recovery	18.98	2.76	6.41	3.40	5.88	64.26

Table 5.1: Physico-chemical variables (continued).

Pans	Treatment	TEMP	pH	EC	TDS	O_2 CONC.	O_2 SAT
MP pan A	**AMD Original**	21.21	6.15	3.76	2.26	6.06	48.52
MP pan A	**AMD Recovery**	18.64	5.08	5.04	2.65	5.74	62.83
MP pan B	**AMD Original**	21.98	5.63	3.03	1.80	3.91	44.62
MP pan B	**AMD Recovery**	18.29	5.17	5.06	2.65	5.58	62.37
MP pan C	**AMD Original**	22.08	2.94	3.47	2.10	4.25	48.85
MP pan C	**AMD Recovery**	18.22	2.77	5.06	2.65	5.84	63.37
MP pan D	**AMD Original**	22.45	2.77	3.70	2.21	4.41	50.50
MP pan D	**AMD Recovery**	18.25	2.57	5.21	2.82	5.78	62.84
MP pan E	**AMD Original**	21.27	2.58	4.49	2.89	4.37	49.33
MP pan E	**AMD Recovery**	18.26	2.51	5.34	2.81	5.80	63.20
MP pan F	**AMD Original**	19.47	2.51	4.88	2.95	6.01	66.00
MP pan F	**AMD Recovery**	18.30	2.58	4.73	2.48	5.78	63.00
MP pan G	**AMD Original**	19.17	2.47	5.20	3.06	8.46	62.28
MP pan G	**AMD Recovery**	18.31	2.60	4.57	2.37	5.74	62.57
MP pan H	**AMD Original**	19.14	2.47	5.18	3.05	5.83	63.74
MP pan H	**AMD Recovery**	18.31	2.57	5.19	2.72	5.74	62.52
MP pan I	**AMD Original**	19.09	2.72	5.20	3.06	5.90	64.72
MP pan I	**AMD Recovery**	18.33	2.59	5.05	2.64	5.78	63.01
MP pan J	**AMD Original**	19.05	2.45	5.07	2.97	5.86	64.18
MP pan J	**AMD Recovery**	18.35	2.57	5.12	2.69	5.88	63.72

5.3.2 Hatching experiments

Overall there was a negative outcome in the sediment treated with AMD. Hatchlings were absent from the sediment treated with AMD in all pans in all three provinces. In the recovery experiments, in which the AMD was allowed to evaporate and then replaced with distilled water, only a minimal number of eggs hatched compared to numbers hatching in the controls. Intact ephippium were observed on top of the sediment in some of the pans. Only 5 out of the 29 pans tested had hatchlings in the recovery experiments. The five pans that showed recovery were all from the North West Province and included NW pan A, B, F, G and H. Cumulative hatching graphs (Figure 5.2) illustrate how the mean hatchling abundances in the recovery experiments compare to those in the 1000 mg/l and 1500 mg/l control treatments. Overall the cumulative abundances were lower in the AMD recovery treatments than those in both control treatments.

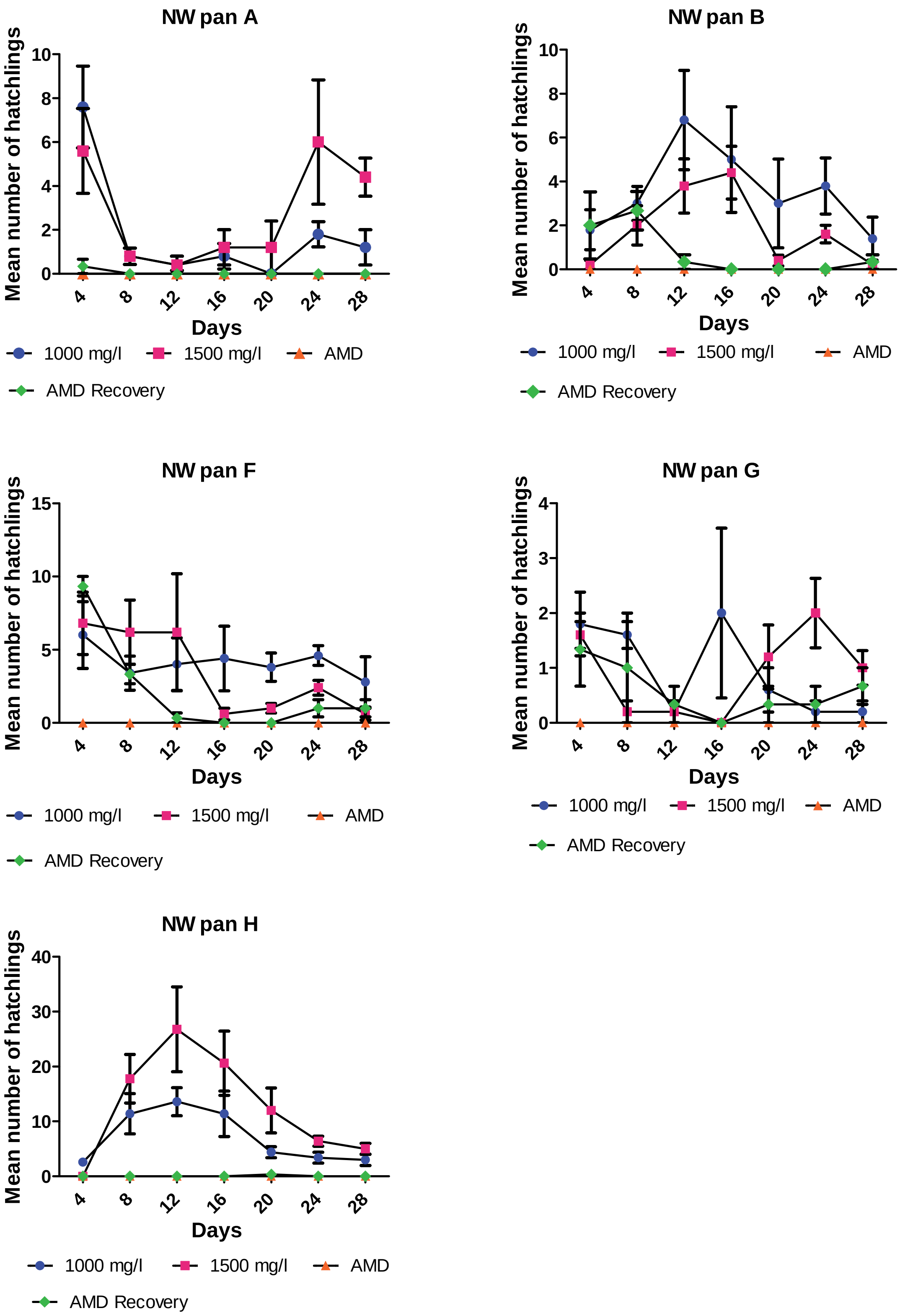


Figure 5-2: Cumulative hatching graphs showing the cumulative hatchling abundance to occur in all treatments including the AMD recovery exposures. Means and standard deviations included.

Figure 5.3 represents the total number of hatchlings in the control treatments compared to the AMD treatment and the AMD recovery treatment. The total abundances of the controls are higher than the total abundances in the AMD recovery treatments for all pans that showed recovery.

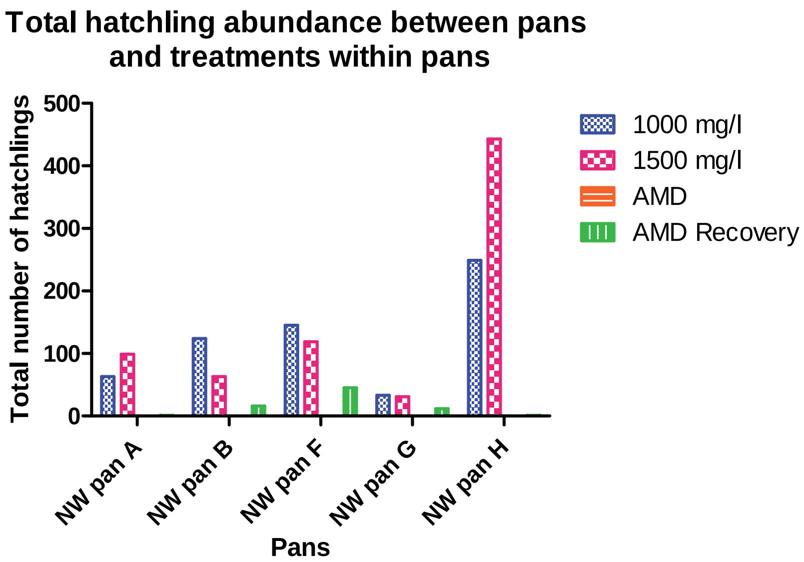


Figure 5-3: Graph indicating the total hatchling abundance in the AMD recovery in relation to the 1000 mg/l and 1500 mg/l control treatments. Means and standard deviations are not indicated.

Table 5.2 shows the species composition of hatchlings in the AMD recovery treatments compared to the composition in the controls. The recovery treatment in NW pan A had only one *Triops* species compared to 7 in the 1000 mg/l control and 3 in the 1500 mg/l control. Both the control treatments had Ostracoda present which the recovery treatments did not have. In NW pan B the AMD recovery treatments had 16 Anostraca which was greater than the abundance in the 1500 mg/l treatment but less than half the abundance in the 1000 mg/l control treatment. NW pan F had four taxa in the 1000 mg/l and three taxa in the 1500 mg/l control treatments, and only two taxa in the AMD recovery treatments. Conchostraca and Copepoda were present in the controls but were not present in the AMD recovery. Only a third of the Anostracans present in the 1000 mg/l control hatched in the AMD recovery treatments. The AMD recovery treatments in NW pan G had the same richness of taxa as both the control treatments and the same occurrence of taxa but the abundances were lower in the AMD recovery. In NW pan H there was only one individual to hatch of the order Anostraca, the Moinidae and Ostracoda that were present in the controls were absent, and the abundance of Anostraca was lower than that of the controls. The controls of NW pan H had the highest abundances of Anostraca hatching originally out of all the pans sampled the North West.

The Margalef's species richness (Figure 5.4) indicates that the species richness in the AMD recovery treatments was lower than that of the controls, except in NW pan G where the AMD recovery had a higher richness. The species richness index takes into account the total abundance of the assemblage, in which case the AMD recovery treatment appeared to be more species rich than the two controls. The number of taxa between the controls and the recovery treatments in NW pan G were the same. There were more individuals in the controls though, making the ratio of individuals to taxa appear smaller than it was. Pielou's evenness index (Figure 5.5) shows that compared to the controls there was a dominance of taxa in the recovery treatments. This is because the number of taxa to recover in the recovery treatments was less than those in the control treatments for all pans except NW pan G where the numbers of taxa were equal. The Shannon's diversity index (Figure 5.6) confirms the above mentioned trends but is skewed in NW pan G because of the Margalef's and Pielou's indices. The diversity in all treatments that were able to recover from AMD exposure was lower than the diversity in comparison to the respective controls.

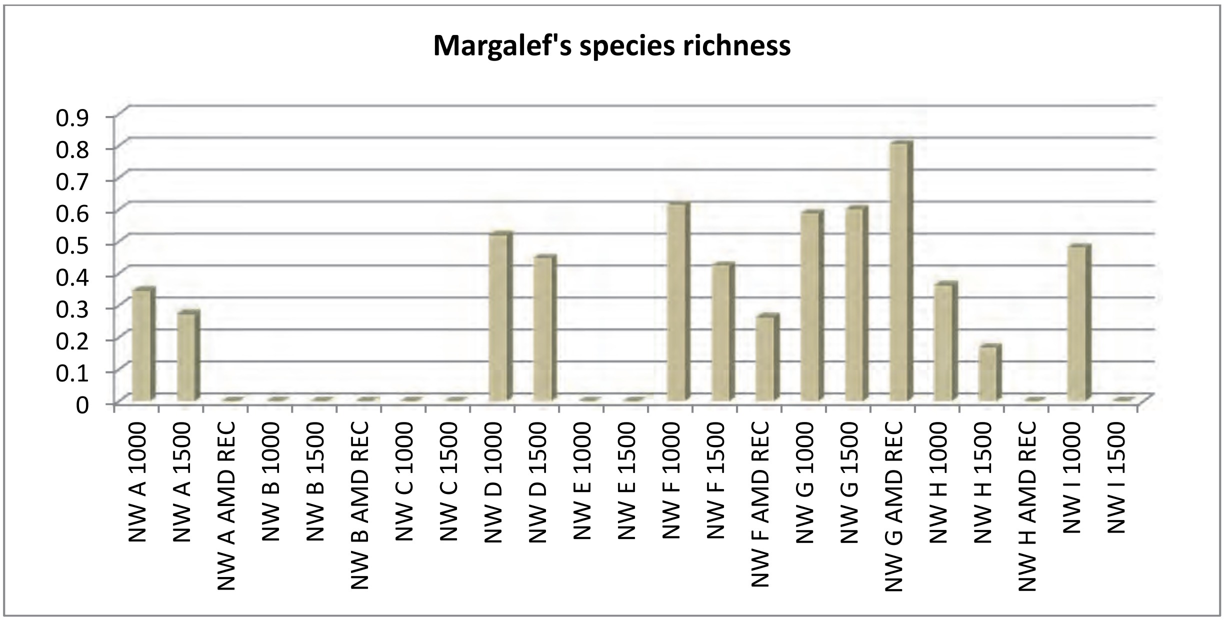


Figure 5-4: Margalef's species richness index comparing the diversity of hatchlings from the control treatments separately with the recovered hatchlings from the AMD.

Table 5-2: Species diversity and abundances in the control treatments and the AMD after the addition of distilled water.

	NW pan A			NW pan B			NW pan F		
	1000 mg/l	1500 mg/l	AMD Rec	1000 mg/l	1500 mg/l	AMD Rec	1000 mg/l	1500 mg/l	AMD Rec
Anostraca	0	0	0	46	15	16	117	106	39
Triops	7	3	1	0	0	0	0	0	0
Conchostraca	0	0	0	0	0	0	3	0	0
Chydoridae	0	0	0	0	0	0	0	0	0
Daphniidae	0	0	0	0	0	0	0	0	0
Moinidae	0	0	0	0	0	0	0	0	0
Copepoda	0	0	0	0	0	0	2	2	0
Ostracoda	11	36	0	0	0	0	9	3	6

Table 5-3: Continued

	NW pan G			NW pan H		
	1000 mg/l	1500 mg/l	AMD Rec	1000 mg/l	1500 mg/l	AMD Rec
Anostraca	27	12	8	246	376	1
Triops	0	0	0	0	0	0
Conchostraca	0	2	0	0	0	0
Chydoridae	0	0	0	0	0	0
Daphniidae	0	0	1	0	0	0
Moinidae	0	0	0	1	23	0
Copepoda	1	0	0	0	0	0
Ostracoda	2	14	3	2	0	0

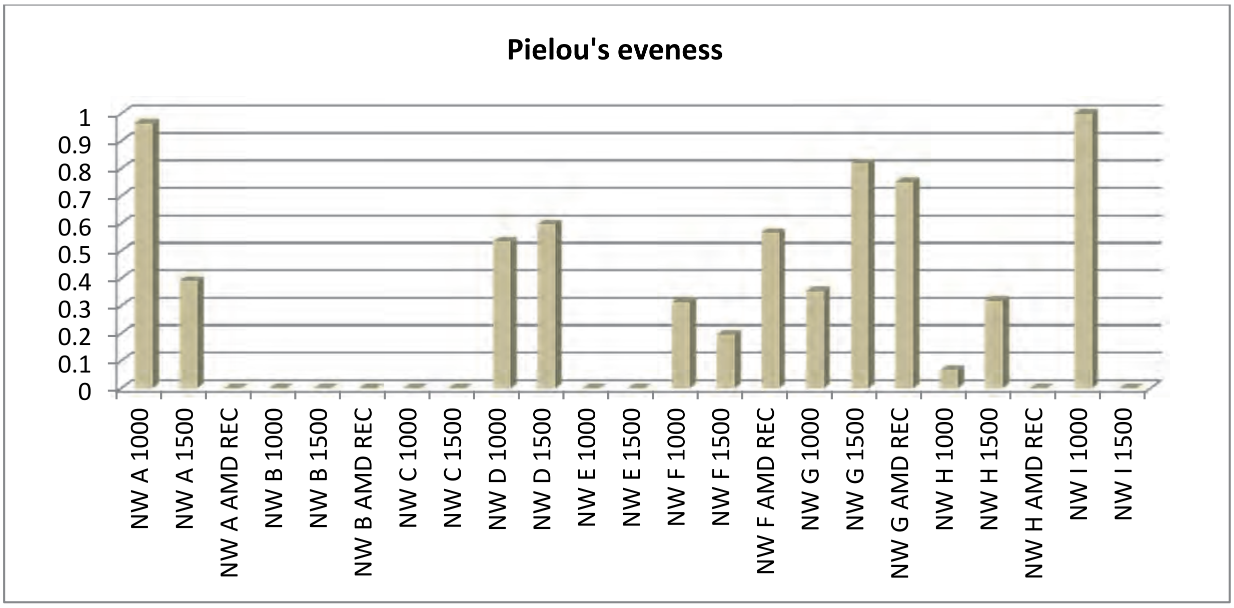


Figure 5-5: Pielou's evenness index comparing the diversity of hatchlings from the control treatments separately with the recovered hatchlings from the AMD.

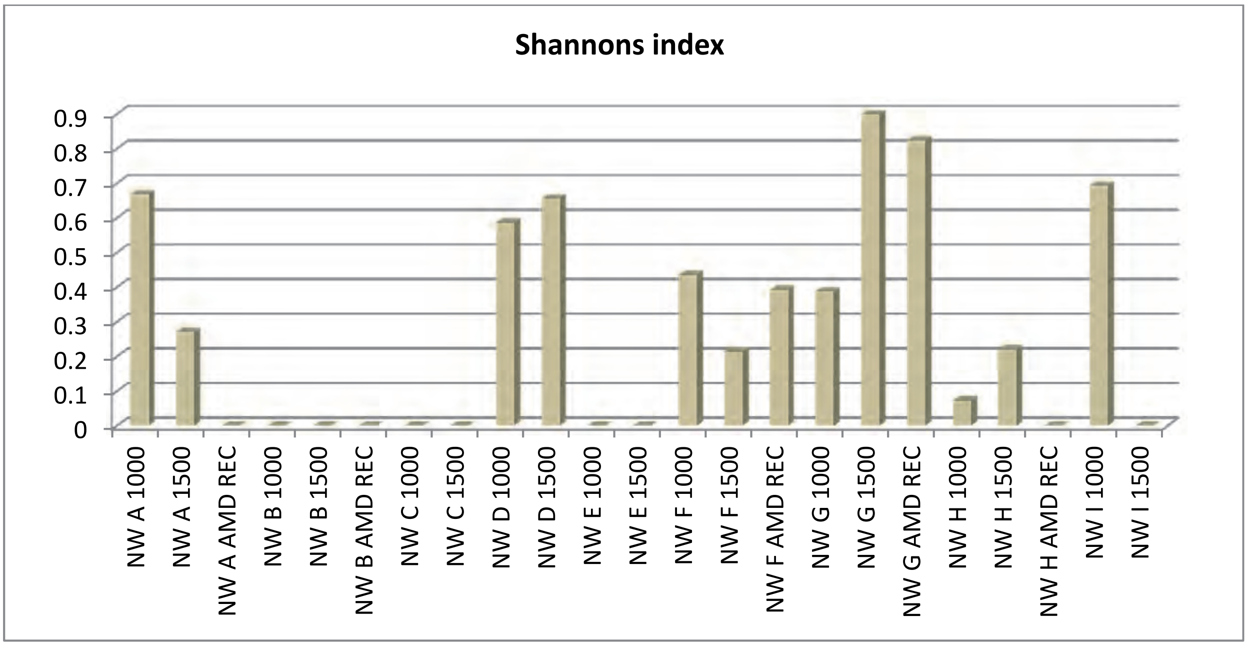


Figure 5-6: Shannon's diversity index comparing the diversity of hatchlings from the control treatments separately with the recovered hatchlings from the AMD.

The PCA bi-plot (Figure 5.7) gives a spatial comparison of how the AMD recovery treatments differed from the control treatments in the North West only, as only the North West pans displayed any recovery from the AMD exposure. The PCA shows that the AMD recovery treatments were dissimilar to their respective control treatments. The hatchlings recovered from the AMD treatments in NW pan A (NW A AMD) along with those recovering in NW pan H (NW H AMD) were situated closer to controls of NW pan C and NW pan I. The hatchlings recovered from the AMD in NW pan B (NW B AMD) were more similar to the composition of hatchlings found in NW pan E. While the hatchlings recovered from the AMD in NW pan F (NW F AMD) and NW pan G (NW G AMD) were dissimilar to the taxonomic compostions of their respective controls, but not dissimilar enough to group with other North West pans, as is the case with NW A AMD, NW H AMD and NW B AMD. The latter are still dissimilar non the less to the assemblages present in their respective control treatments.

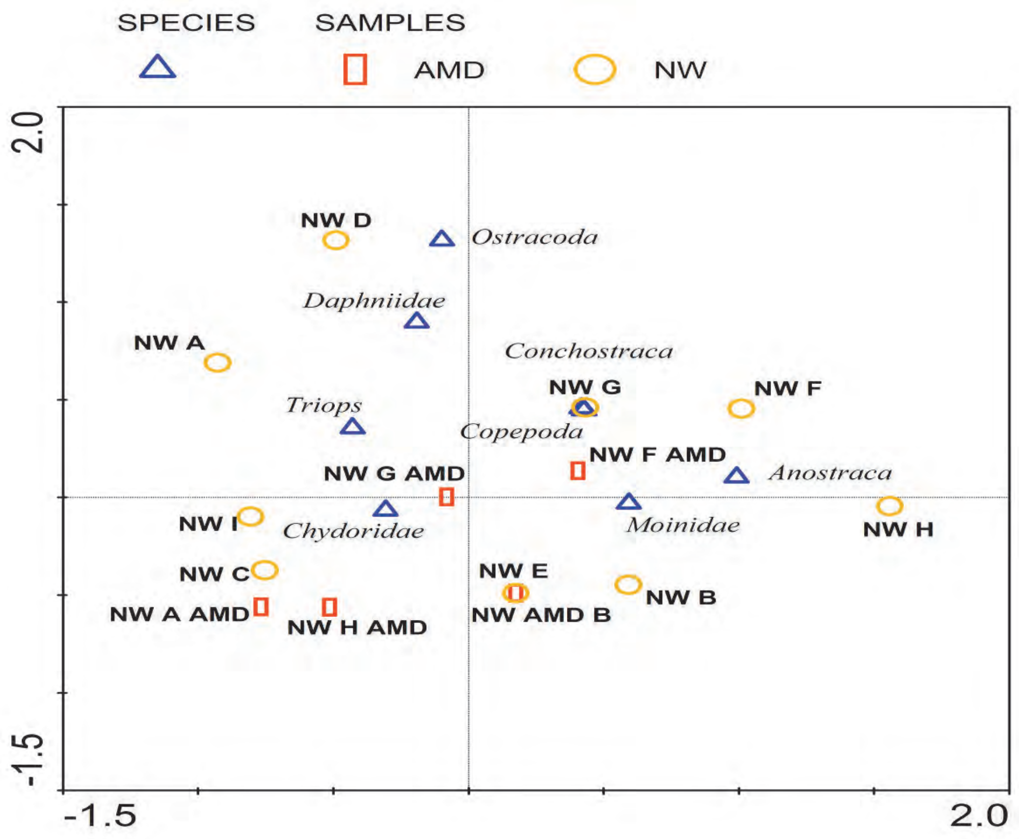


Figure 5-7: PCA bi-plot comparing the taxonomic composition of hatchlings between the AMD recovery and the control treatments in the North West Province. The PCA describes 81.4 % of the total variation, with 53.4 % of the variation being described on the first axis and 28 % of the variation described by the second axis.

5.4 Discussion

5.4.1 Physico-chemical variables

The comparison between the physico-chemical variables of the AMD and the AMD recovery show that there was very little change between the two treatments. The water quality did not improve much upon replacing the AMD with distilled water. Ferric hydoxides of the AMD tend to precipitate out of solution forming a smothering blanket on the sediment surface (Warner, 1971), which was clearly visible as a yellowish crystalline layer on the sediment after the evaporation of the AMD. Therefore as soon as the sediment is re-inundated with water the mineral salts are mobilised and go back into solution, resulting in the same concentration of solutes and pollutants being present in the water column as before. This scenario is identical to that which would occur naturally in the pan environment. It also demonstrates how difficult it would be to dilute the concentration of contaminants, as to increase the pH by one unit using dilution alone, requires a 1:10 dilution (Baldwin and Fraser, 2009). The probability of this happening is low, as for dilution of this kind to occur naturally would require a flooding event, which are rare events in the arid regions where pans are predominantly situated. Anthropogenic inputs could alternatively assist in alleviating this problem. However, using dilution in an effort to remediate impacted pans is risky due the large volumes of water necessary, which could permanently alter the hydrological regime. The sudden onset of a prolonged hydroperiod can have consequences for dormant eggs which require a desiccation period as a prerequisite to hatching. Prolonged inundation also affects the temperature, turbidity and nutrient content of the water. This alteration of pre-established hatching cues could substantially lower the diversity of the water column should such eggs not be able to hatch. The pans at greatest risk of this scenario are those in arid regions.

Another factor of concern with regards to acidification and dilution thereof in wetlands is that AMD is high in iron sulphates (Gray, 1998). Baldwin and Fraser (2009) gave a comprehensive account of the rehabilitation options for inland waterways impacted by sulfidic sediments. Wetlands containing sulfidic sediments through a complex series of reactions can produce acid, and undergo acidification. In addition such wetlands can also experience a build-up of metals in the water column which were mobilised through the acidic conditions. It has been said that to minimise the formation of sulfidic sediments conditions which favour sulphate reduction need to be prevented along with the prevention of sulfidic sediments accumulating in large volumes. The natural hydrological cycle of pans includes a wet and a dry phase. This will prevent the build-up of reduced inorganic sulphur such wetlands (Baldwin *et al*., 2006). Sulfidic sediments pose the biggest risk in those wetlands that have not undergone a periodic drying cycle. However if large amounts of sulfidic minerals have built up in the sediment, as would happen if a wetland is exposed to AMD, then adding large volumes of water to increase the dilution capacity has the potential to increase the oxidation process leading to acidification, as well as cause the further build-up of sulfidic sediments resulting in further acidification (Baldwin and Fraser, 2009).

An alternate remediation option include could involve neutralisation of affected wetlands through the use of ameliorants such as lime or ash. The problem with using ameliorants is that they are expensive and large volumes are also required. The ameliorants also need to be applied to the sediment when pans are dry, which can disrupt the egg banks within and cause further damage. Neutralisation of the water column on the other hand has the disadvantage of causing precipitation of metals present in the water column which again has consequences for the egg banks (Baldwin and Fraser, 2009). Rehabilitation of impacted wetlands thus can be problematic as there are lots of factors to consider for any option. The impact on the invertebrate community should always be a first priority in wetland management for reasons to follow.

5.4.2 Hatching experiments

The addition of AMD had a negative effect on the hatching success of branchiopod crustaceans from their egg banks. Eggs were unable to hatch in the presence of AMD. The AMD did not, however, have permanent negative effects on all the eggs, as some eggs were cable of hatching when the AMD was removed. Therefore it is likely that AMD only blocked hatching in these eggs. Although recovery did take place, recovery was very low. The only pans to show any recovery were those in the North West Province, and based on total abundances only 5 out of the 29 pans studied in this project demonstrated a recovery capacity.

Aquatic organisms respond in different ways to various pollutants, with their responses depending on the chemical and physical factors of the pollutant and the physiological and behavioural responses of the organisms exposed (Warner, 1971). From the recovery experiments it appears that the two most resilient taxa which were capable of surviving initial AMD exposure were the Anostraca and Ostracoda. Ostracods have been found to be the most tolerant group of crustaceans to a broad range of salinities (De Deckker, 1983; Nielsen and Brock, 2009; Waterkeyn *et al*., 2010). Cladoceran and Copepod species were found to be less tolerant as they were virtually absent in the recovery treatments. The diversity of hatchlings occurring in the recovery treatments was lower than the hatchlings occurring in the respective control treatments. In addition to this the multivariate data revealed that the taxonomic composition in the recovery treatments did not compare to those in the controls. Thus the AMD altered the community composition of these pans. The study by Ning *et al*. (2011) had similar findings with regards to zooplankton recovering from wetlands affected by sulfidic sediments. Zooplankton recovering from sulfidic wetlands had altered community compositions and was less diverse than those in non-impacted wetlands.

A possible factor that could have played a role in the survival of eggs is the type of dormancy. Diapausing eggs may be more tolerant to stressors than quiescent eggs as they rely on internal conditions to hatch regardless of external conditions being favourable. The diapausing eggs of copepods have been found to be less sensitive to metal pollution due to the thick chorion membrane surrounding it (Marcus, 1984; Marcus and Lutz, 1998). Quiescent eggs relying directly on external conditions could possibly be stimulated to hatch by favourable light and temperature conditions, but conditions such as pH and conductivity which were likely unfavourable could counteract this and inhibit hatching. In this regard diapausing eggs can lie dormant for longer without external factors interfering in the hatching process. In the North West where recovery took place it is speculated that only the diapausing fraction hatched as the quiescent fraction were adversely affected by "partial" hatching. Partial hatching is a term given here to describe the situation in which metanauplii were triggered to hatch by some favourable condition, but depleted their energy reserves in the process of hatching. Partial hatching would explain why abundances of hatchlings were so low in the recovery treatments compared to the respective control treatments'. This would require further investigation though as the different egg types were not assessed in the current study. In the future, using hypochlorite in a decapsulation technique, which is known to assist in activating diapausing eggs (Lavens and Sorgeloos, 1987; Drinkwater and Clegg, 1991; Brendonck *et al*., 1996), will aid in determining if diapause did have an influence on the fraction of cysts to hatch after AMD exposure.

An explanation for eggs not hatching in the presence of acid mine drainage is that AMD has a high concentration of mineral salts (consisting of toxic metals) and a low pH (Kelly, 1991; Gray, 1998; DeNicola and Stapleton, 2002). The high concentration of mineral salts increases the osmotic pressure of the water (Parsons, 1957). Should enough water pass through the tertiary membrane into the egg metabolic processes within the metanauplius will be activated. Should the metabolism be activated glycerol will start building up inside the egg, creating an osmotic gradient for more water to pass into the cyst. This water build up creates an osmotic pressure inside the egg which results in the bursting of the outer membrane enabling hatching. However because of the increased osmotic pressure of the water outside the egg, more glycerol has to be produced by the metanauplius to enable the movement of sufficient quantities of water into the egg to burst the outer membrane (Lavens and Sorgeloos, 1987; Van Stappen, 1996). The metanauplius lands up depleting its energy reserves in the process before this osmotic pressure can be established (partial hatching), rendering the egg non-viable. Such eggs will be incapable of future hatching. Salinity has consequently been said to be the most important factor explaining the distribution of branchiopods, adversely affecting hatching and survival and resulting in lower species richness (Waterkeyn *et al*., 2009).

The effect that low pH has on hatching has to do with the optimal functioning of the hatching enzyme. The enzyme is secreted by the metanauplii allowing it to break free of the inner membrane, the final membrane that has to be broken through allowing the release of the free-swimming nauplii (Van Stappen, 1996). The low pH of the AMD may have denatured this enzyme which would prevent hatching even if the metanauplii were successful in bursting through the outer membrane. This explanation provides an additional reason for the recovery seen in North West Province. The pH of the AMD treatments in the North West pans was neutral, whereas the pH was acidic in both the Free State and Mpumalanga AMD treatments. Thus the hatching enzyme was still functional in eggs within the North West pans, although not optimal. *Artemia* species for instance require a pH above 8 for optimal functioning of the hatching enzyme (Van Stappen, 1996). This adds further reason for recovery being so low, other than the type of dormancy. Apart from the physical effect of a low pH it should also be considered that the low pH as present in AMD may further effect the availability of selected metals. It is well known that acidification can affect speciation, mobility and bioavailability of certain toxicants (Lopes *et al*., 1999).

It is possible that the lower pH from the AMD influences the availability of metals in the sediments, whilst AMD also contains high concentrations of metals (Robb, 1994). This is important as the effect of metals on the hatching success of branchiopoda and crustaceans in general has been well documented (Brix *et al*., 2006; Jiang *et al.,* 2007). The high concentrations of sulphates found in AMD may also be responsible for the decreased hatching success. In addition to the pH of the surrounding medium, salinity may also have an important influence. The natural salinity of the pans that were included may also be an important factor. Only pans from the North West should recovery after exposure to AMD. Pans in the North West had naturally high salinities which species are tolerant to. Salinity tolerances are determined by physical and chemical properties of the egg which are species specific (Gray, 1988). The salts in the North West probably had a buffering effect on the pH of the AMD. Salinity effect on eggs is not always permanent and that they can recover (Day *et al*., 2010). The combination of salinity and pH however may have totally different consequences for the organisms involved.

However, it is important to note that the eggs that hatched were able to do so because those particular eggs was unaffected by the AMD exposure. That is not to say that survival of those nauplii was unaffected by AMD exposure. This was not tested here as nauplii were transferred to honey jars containing ADaM medium after hatching to encourage growth to a stage at which identification could take place. The nauplii, should they have been left in the recovery treatments, may not be able to survive long enough to carry out their lifecycle. Acid mine drainage is also known to alter benthic algae communities (Verb and Vis, 2000), thus affecting an essential food source for many species. Acidic waters also cause the leaching of metals from sediments (Snoeyink and Jenkins, 1980). The effect of metal exposure was not investigated here, but a few studies have investigated species sensitivities to metal contaminants, particularly in marine *Artemia* species. Nickel and vanadium metal pollution has been found to be toxic to *Artemia urmiana* and *Artemia fransicana* affecting species lifespan and growth rate (Asadpour *et al*., 2013). The sensitivities of other orders of branchiopoda have also been investigated i.e. *Streptochephalus*, which have been found to be more sensitive to metal pollution than the standard test species *Daphnia magna* (Crisinei *et al.,* 1994). Given the sensitivity of these branchiopods, the effects of AMD are two-fold with effects on the pan community involving both the primary (egg bank) and secondary stages (adult species) of the community. The egg banks however are still the most important component of the community as the egg banks recolonize the pan community.

5.4.3 Conclusion

The hypothesis that AMD will have a negative effect on the hatching success of branchiopod egg banks is not rejected. The hatching of branchiopod crustaceans is thus inhibited by the presence of AMD, most likely due to its high conductivities and low pH.

It was also demonstrated that the recovery of these aquatic invertebrates after AMD exposure was low. When compared to the diversity of aquatic invertebrates obtained from the controls it could clearly be seen that AMD altered the community structure of the branchiopods which recovered. The diversity of individuals was much lower as a result of the AMD. This shows how poorly the community will respond to the removal of this stressor. Even though recovery did take place in a few pans, the number of individuals may be too low to replace the number of eggs affected by the AMD. The buffering capacity of the egg bank will be lost, and the egg bank will eventually deplete itself in inundations to come. Species extinctions as a result are inevitable, which raises the concern that wetlands impacted to such an extent by such a stressor may be beyond rehabilitation. Also rehabilitation efforts are very difficult because of the large efforts and costs involved (dilution, neutralisation), as well as the continual variability these environments experience. An array of variables need considering i.e. hydrology and salinity. The alteration of one can have effects on the others, all of which play an essential role in maintaining the unique biodiversity of these wetlands.

Branchiopod crustaceans are endorheic wetland specialists and make up the largest biotic component of these wetlands next to phytoplankton. They are also the primary consumers of these wetlands. The loss of this functional component has further consequences, as it will have an impact on the secondary consumers which can extend to migratory waterfowl. It can also impact on the phytoplankton community, resulting in blooms that can cause eutrophication during longer inundation periods.

6 WATER QUALITY CLASSIFICATION OF ENDORHEIC PANS

6.1 Introduction

Nearly all studies that have been completed on pans in Southern Africa indicate that there is a large variation in the physico-chemical characteristics of these ecosystems. This variation has been observed not only between different pans, but during different time periods within the same pan. Most studies dealing with physico-chemical characteristics of pans and the influence on the biotic communities that inhabit them deal with temporary pans or large lakes (Weir, 1966; 1969; Seaman *et al.*, 1991; Moss, 1994; Meintjes, 1996; Drago and Quiros, 1996). Hutchinson *et al.* (1932) and Seaman *et al*. (1991) have probably completed the most detailed studies on perennial pans. According to Allen *et al.* (1995) there are a number of variables that has an influence on the physico-chemical characteristics of a pan. These can include: desiccation, chemical variation, high temperatures, and low oxygen concentrations. The water chemistry of pans is also affected by the local geochemistry. The dominant ions in most salt lakes worldwide appear to be Na^+ and Cl^-, although the best-known lakes in Africa are dominated by Na^+ and HCO_3^-/CO_3^{2-} (Wood and Talling, 1988; Dallas and Day, 2004).

The physico-chemical characteristics of an aquatic ecosystem are obviously a limiting factor with regards to the biotic communities found in these systems. There is a large body of literature regarding the classification (typing) of inland water throughout the world. Lakes, for example has been classified based on the way in which they have formed or the chemical characteristics of lakes. Over the last few decades the chemical characteristics of inland waters has become increasingly important due to the influence thereof on the biological communities within these ecosystems and the impact of human activities on the water quality. As a result a large number of terms have been introduced to express the general biological conditions of lakes in particular. Davies and Day (1998) indicate that the lakes in Southern Africa can be classified as:

- Oligotrophic lakes: these lakes usually have nutrients in very low concentrations. As a result plant growth and productivity in this system is generally low. Certain organisms do occur in these lakes, but the abundance of a particular species is usually low. These lakes tend to be deep, but always have enough oxygen available in the lower regions and water clarity is usually very good.
- Eutrophic lakes: lakes are rich in nutrients and other inorganic material that are necessary for plant growth. These lakes are often shallow, are able to support a large variety of biota, and are rich in organic matter.

- Dystrophic lakes: usually hold a limited number of organisms. These lakes are rich in organic material and the water is often brown in colour. Light penetration is thus minimal and the decomposition of organic material leads to a lack of oxygen, especially in the deeper sections.

In addition to the trophic state indicated above, the Organization for Economic Cooperation and Development (OECD, 1982) also includes mesotrophic and hypertrophic lakes as part of the classification of lakes based on trophic state. These lakes are classified as:

- Mesotrophic lakes: These lakes are intermediates of oligotrophic and eutrophic lakes. These lakes generally have moderate concentrations of nutrients with organic sediment accumulating and some loss of oxygen in the lower waters. Submerged aquatic vegetation can often be found in these systems and there is also an intermediate level of plankton production.
- Hypertophic lakes: These lakes are very fertile and are supersaturated in phosphorus and nitrogen. These lakes are highly productive lakes, and support large amounts of plants and animals. Excessive phytoplankton growth is common in these lakes as a result of the availability of nutrients. This contributes to poor water clarity and large variability in oxygen availability.

There are few natural large lakes in South Africa. Most of the lakes that do occur in the country are artificial in nature or coastal lakes. Pans also function as lakes and in general pose an interesting problem with regards to classification. Apart from the large variation in physico-chemical variables, the water depth in pans is generally very shallow. As a result, stratification is often absent in these ecosystems and nutrient and oxygen concentrations are similar throughout the pan. These shallow systems are also exposed to continuous wind action and are largely polymictic. Due to the endorheic nature of pans and the way pans are formed these ecosystems often vary in trophic state when compared to larger lakes. Hutchinson *et al.* (1932) suggested that pans in the Lake Chrissie area can be classified based on the trophic state. The classification that was suggested is a variation of the lake classification suggested above. These variations are known as alkaline dystrophic types and saline eutrophic. Alkaline dystrophic waters are shallow and are formed by wind erosion, are often dark in colour (grey or brown) with very little light penetration, the pH is usually above 8, they are rich in suspended organic material from allochthonous origin and support very little organisms. Saline eutrophic waters are also shallow and occupy basins close to estuaries or those that have been formed by wind erosion. The water is turbid, with little light penetration and a pH above 7. The water is rich in organic material from both allochthonous and autochthonous origin. These lakes often support a variety of plants and animals in large numbers.

It is obvious that the trophic state of a pan has a major influence on the abundance and diversity of biotic communities within a pan. The trophic state of a particular pan can thus become important for the selection of relevant reference conditions. Some pans may naturally have a low diversity and reference conditions should be selected accordingly. Water resource monitoring and assessment in South Africa depends on the selection of reference conditions. Reference conditions are important in determining the degree of deviation of degradation from the natural, unimpacted characteristics of the resource that is being studied (Dallas, 2000). The selection of reference conditions is becoming increasingly important as there has been an increase in the number of studies concerning pans, especially in the Mpumalanga Highveld. This increase in the number of studies is as a direct result of the increase threat to the ecological integrity of pans in South Africa through mining activities and extensive agricultural activities. This in turn is fuelled by South Africa's ever increasing population growth and need for energy resources (Ziramba, 2008; Amusa *et al.*, 2009).

6.2 Materials and Methods

Water sampling

The samples were taken directly below the water surface with a clean scoop bucket, transferred to a set of bottles, and transported to the laboratory in a cooler box. Two litres of water were taken for general analysis in pre-washed plastic bottles. All water quality analyses were carried out by Chemtech Laboratories (a SANAS accredited laboratory). The water analysis included nutrients, salts and metals. The *in situ* physico-chemical variables that were sampled during the current survey included temperature, pH, dissolved oxygen concentration ([DO]) and saturation (DO%), total dissolved solids (TDS) and electrical conductivity (EC). The *in situ* analysis was undertaken using a pre-calibrated WTW 340i multi-parameter hand-held water quality meter.

Statistical analysis

Multivariate statistical analyses were carried out to determine whether any spatial or temporal differences were evident in the water quality results. The statistical analysis was performed on Primer Version 6 and Canoco Version 4.5. Primer Version 6 was used for the hierarchical clustering and non-metric dimensional scaling (NMDS) using Bray Curtis similarity coefficient. Canoco Version 4.5 was used for the Principal Component Analysis (PCA) to determine groupings and dominant water quality variables.

The Bray-Curtis similarity coefficient was used to determine similarities between sites as it is found to be reliable (Clarke and Warwick, 1994) as well as that joint absences have no effect (Cyrus *et al.*, 2000). Hierarchical clustering with group average linking, which is based on the similarity matrix, was applied. The similarity ranks between samples was used to construct non-multidimensional scaling (NMDS) ordination plots as it makes few assumptions about the nature and quality of the sample data. Both the hierarchical clustering and NMDS techniques are constructed form triangular similarity matrices between each pair of samples (Clarke and Warwick, 1994) and were applied to identify spatial differences between pans. The difficulty of compressing the relationships between samples into two dimensions was calculated as a measure of the reliability of NMDS technique and is reflected as a stress value. Should the stress be below 0.1, the two dimensional plot is an accurate representation of the sample patterns (Cyrus *et al.*, 2000). Significance testing of *a priori* selected groups for province and sampling survey was tested using the ANOSIM procedure.

Another multivariate technique implemented was ordination which determines differences, if any, in composition of various sites or samples (van den Brink *et al.,* 2003). The best fit values, derived with multiple linear regressions between each variable in turn, are used in the analysis together with environmental data as a second matrix instead of the original data (Shaw, 2003). The assumption is also made that one of the sets of environmental variables can be considered “independent” while the other set is considered “dependent” (Ter Braak and Smilauer, 2002). PCA is an ordination method which can determine differences in water quality composition at various sites. A PCA is a weighted summation method that models the absolute data and presents a linear modelled response (Van den Brink *et al.,* 2003). The data were log transformed prior to analyses. All the data was log transformed.

The salt ion concentrations were used to construct Maucha diagrams of each pan and for each survey in Mpumalanga, Free State and North West. Maucha (1932) developed a method to construct a radial ionic diagram of the relative ionic concentrations so that it provides a compact summary of the raw data (Silberbauer and King, 1991). All the variables are transformed to milli-equivalents per litre so that samples can easily be compared. The dotted circle in the background illustrates the theoretical levels should all the ions have the same concentrations. Broch and Yake (1969) modified the diagram so that the whole star is scaled in proportion to the total dissolved salts. Silberbauer and King (1991) added a log scale that is used when there is widely differing salinities in the samples. A theoretical Maucha diagram with the representation of salt ratio’s for freshwater is presented in Figure 6.1.

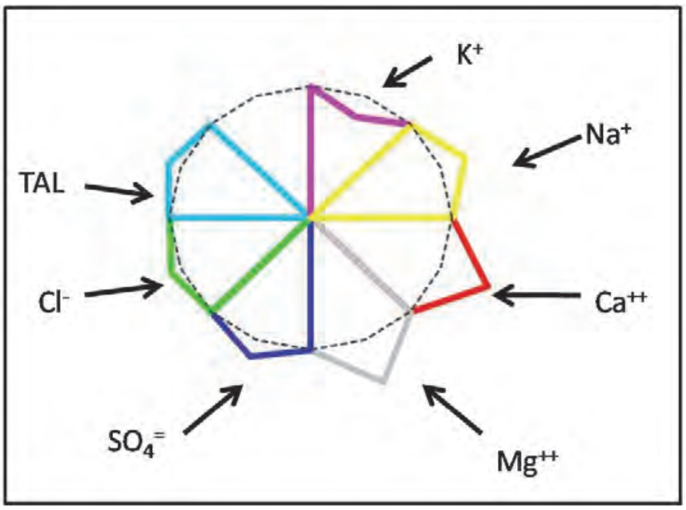


Figure 6-1: Maucha diagram representing the salt concentrations found in the freshwater environment.

6.3 Results

The water quality analysis results were summarised in Table 6.1 due to the large dataset that were generated through the project. The full results for all of the parameters are presented in Appendix A. The results in Table 6.1 provide the number of samples, the median, maximum and minimum concentrations as well as the 95th and 5th percentiles. The percentiles were included in the summary as many variables indicated outliers in the data. The total number of samples analysed during the project totalled 61 pan samples from the various provinces over the three sampling surveys. The dissolved metal concentrations presented in Table 6.1 indicate that the majority of the metals analysed did not occur in high concentrations and where below detection limits fairly often. The salt parameters did indicate a large variation in concentrations for each of the various ions especially when looking at the maximum and minimum concentrations. The nutrient concentrations were also generally low with outliers in the data often present. It was noted that the phosphate concentrations were generally higher than the nitrate concentrations in the sampled pans.

Table 6-1: Summary water quality data from all three sampling surveys from 2012 to 2013.

Parameter	Unit	Count	Median	Maximum	Minimum	95th Percentile	5th Percentile
Oxygen content	mg/l	61	6.97	21.57	1.52	9.82	4.26
TDS	mg/l	61	2.47	76.9	0.00418	27.3	0.1224
pH	-	61	9.04	10.35	7.11	9.79	7.93
EC	ms/cm	61	3.36	151.6	0.1704	53.8	0.212
Temperature	°C	61	21.9	38.5	10	33.2	10.7
Alkalinity	-	61	429	6400	18	1681.14	45.02
Ammonium	mg/l	61	0.01	44.4	0.01	1.27	0.01

Parameter	Unit	Count	Median	Maximum	Minimum	95th Percentile	5th Percentile
TH		61	90	20000	18	2650	23
Fluoride	mg/l	61	0.53	2.01	0.037	1.31	0.037
Chloride	mg/l	61	612	92800	12.38	11350	21.98
Nitrite	mg/l	61	0.07	5.8	0.07	2.95	0.07
Nitrate	mg/l	61	0.17	23.03	0.07	4.5	0.07
Phosphate	mg/l	61	0.62	63.2	0.065	10.1	0.065
Sulphate	mg/l	61	105.6	22620	1.03	4109	4.04
Ag	mg/l	61	0.001	0.376	0.001	0.004	0.001
Al	mg/l	61	0.003	1.224	0.003	0.621	0.003
As	mg/l	61	0.005	5.842	0.001	4.058	0.001
B	mg/l	61	0.074	11.843	0.003	3.129	0.004
Ba	mg/l	61	0.219	0.729	0.031	0.617	0.055
Ca	mg/l	61	15.886	2410.06	1.712	131.433	4.193
Cd	mg/l	61	0.001	0.005	0.001	0.005	0.001
Co	mg/l	61	0.001	0.005	0.001	0.005	0.001
Cr	mg/l	61	0.005	0.022	0.005	0.006	0.005
Cu	mg/l	61	0.005	0.06	0.005	0.03	0.005
Fe	mg/l	61	0.135	6.838	0.004	4.555	0.008
K	mg/l	61	33.755	7508.61	2.245	1106.704	5.869
Mg	mg/l	61	7.611	4161.21	0.355	294.149	1.825
Mn	mg/l	61	0.001	0.796	0.001	0.259	0.001
Mo	mg/l	61	0.001	0.099	0.001	0.009	0.001
Na	mg/l	61	655.894	187631.8	7.175	11574.77	13.809
Ni	mg/l	61	0.001	0.042	0.001	0.026	0.001
Pb	mg/l	61	0.001	0.01	0.001	0.01	0.001
Se	mg/l	61	0.001	0.01	0.001	0.01	0.001
Si	mg/l	61	0.219	4.063	0.001	2.782	0.002
Sr	mg/l	61	0.219	65.186	0.012	5.952	0.055
Ti	mg/l	61	0.007	0.072	0.001	0.046	0.007
U	mg/l	61	0.004	0.004	0.004	0.004	0.004
V	mg/l	61	0.021	0.457	0.003	0.158	0.003
Zn	mg/l	61	0.01	0.333	0.001	0.154	0.001

The Maucha ionic diagrams were used to compare pans from different sampling surveys as well as between different provinces. The diagrams are presented in Table 6.2 for Mpumalanga, Table 6.3 for North West and Table 6.4 for the Free State. The general trend in Figure 6.2 indicates that sodium and chloride are the dominant ions in the Mpumalanga systems. However, some exceptions were MP pan A during the May 2012 survey that indicated a high alkalinity together with the sodium and chloride dominance. This trend was not evident during the next two surveys of the pan.

Table 6-2: Maucha diagrams for the Mpumalanga pans from May 2012 to March 2013.

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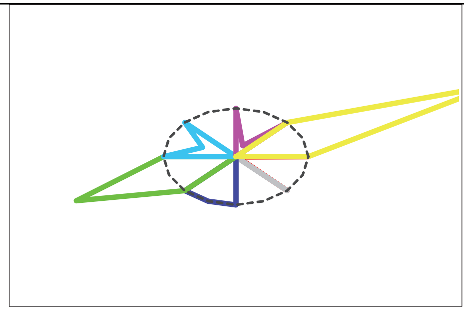
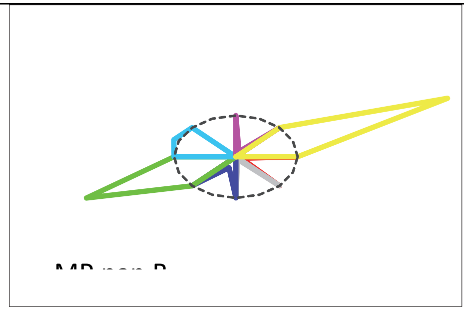
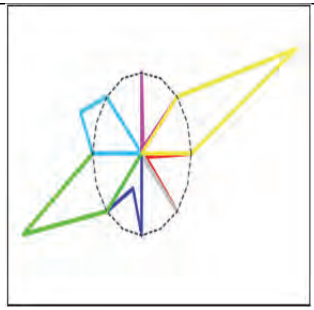
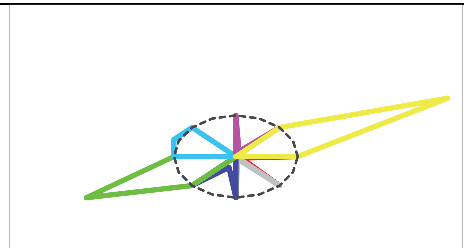
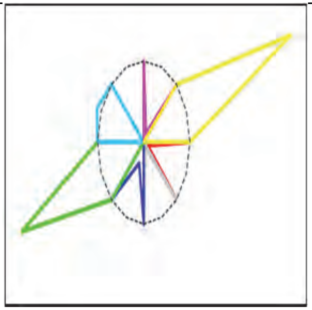
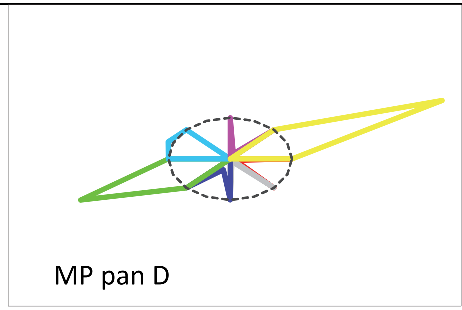
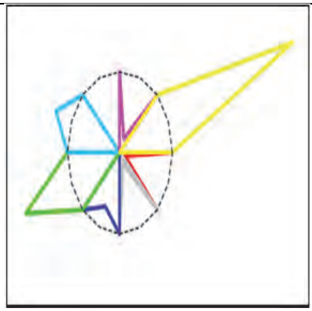
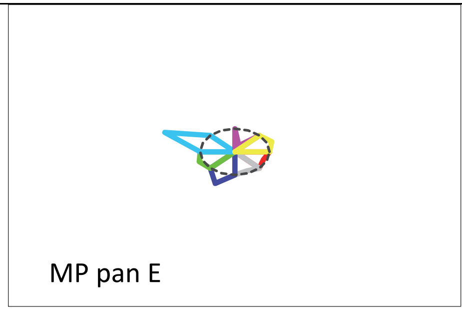
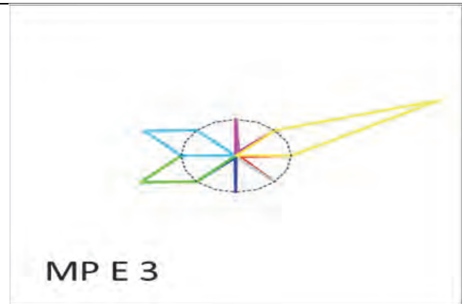
		
MP B – May 2012	**MP B – December 2012**	**MP B – March 2013**
		
MP C – May 2012	**MP C – December 2012**	**MP C – March 2013**
		

MP D – May 2012	MP D – December 2012	MP D – March 2013
		
MP E – May 2012	MP E – December 2012	MP E – March 2013
		
MP F – May 2012		
		

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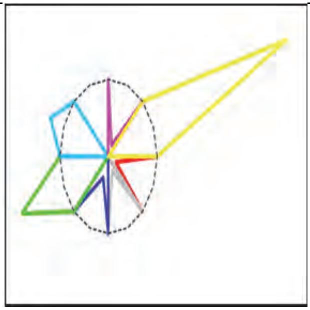
<tr><td></td><td></td><td></td></tr>
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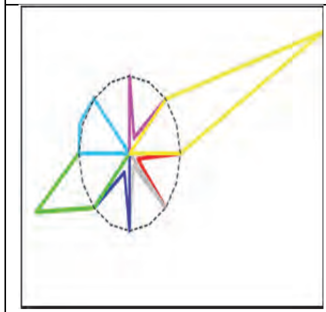
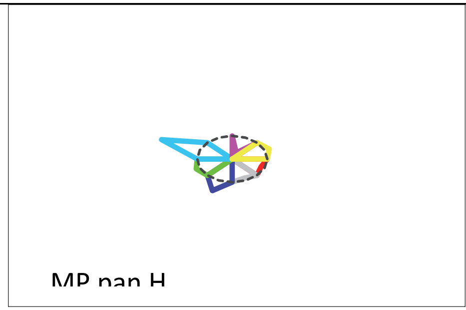
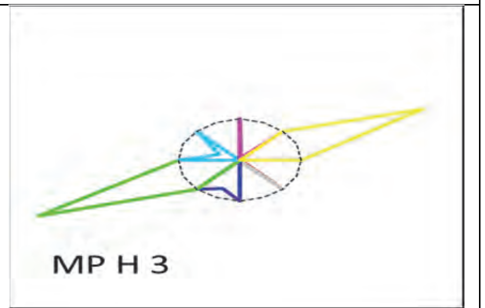
<tr><td></td><td></td><td></td></tr>
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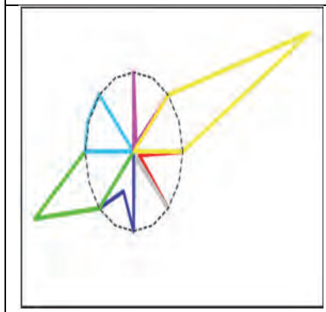
<tr><td></td><td></td><td></td></tr>
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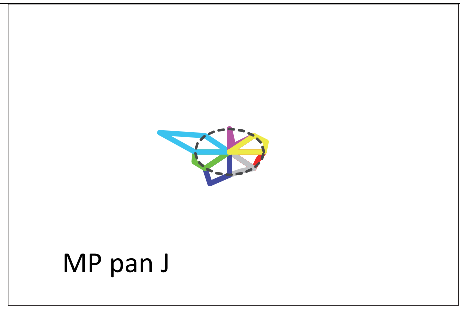
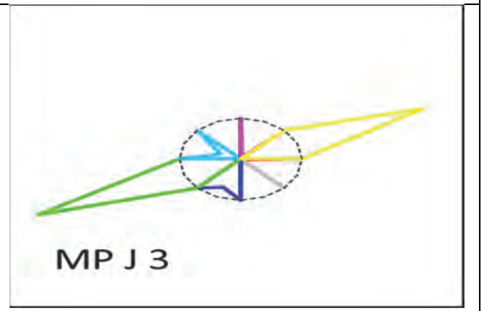
<tr><td></td><td></td><td></td></tr>
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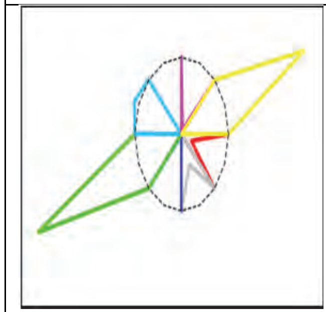
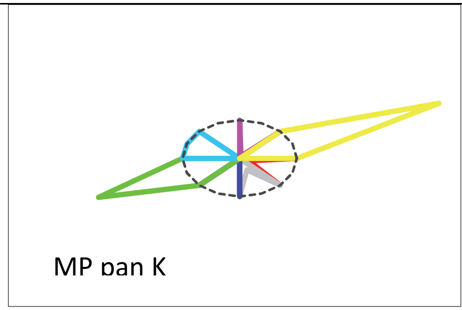
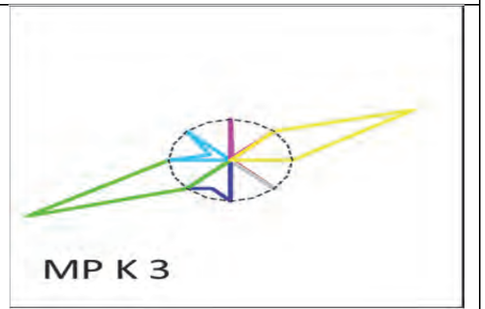
<tr><td></td><td></td><td></td></tr>
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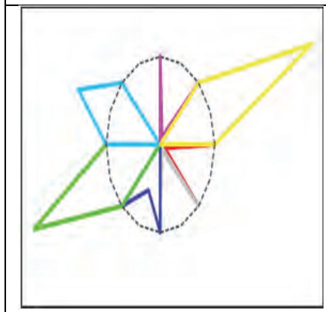
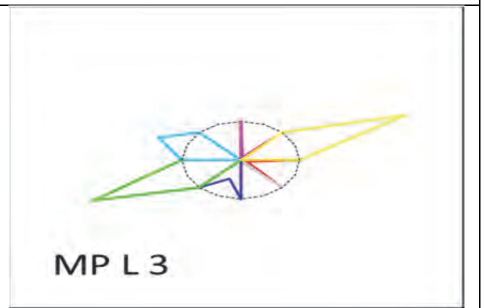
<tr><td></td><td></td><td></td></tr>
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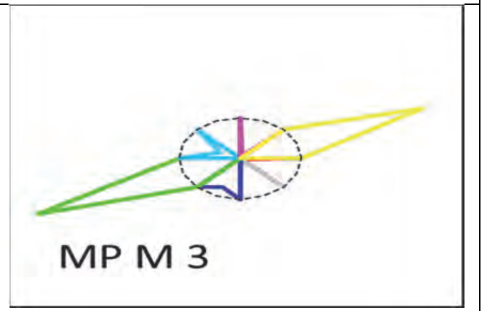
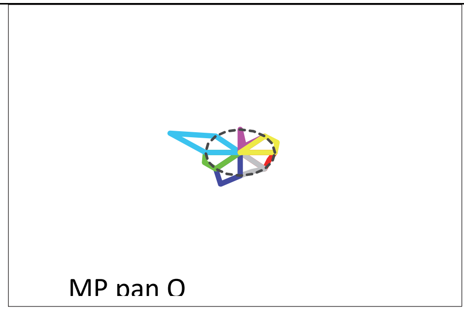
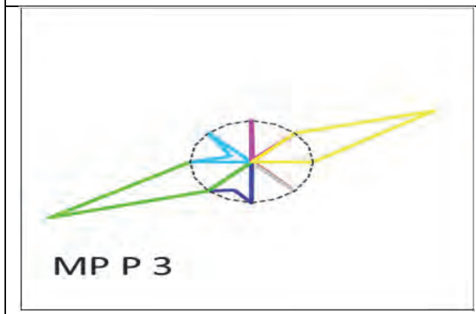
</table>

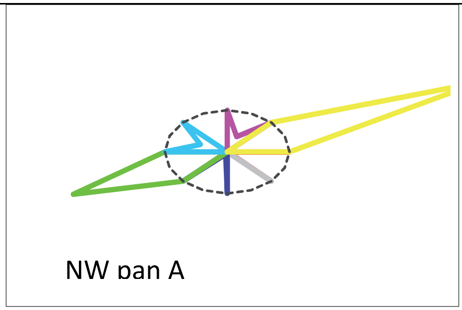
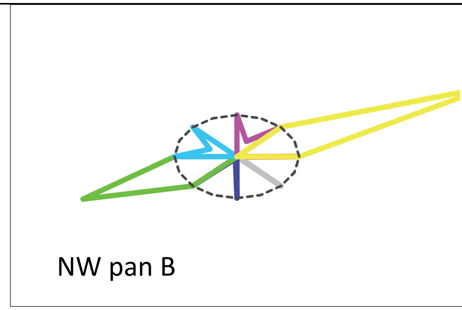
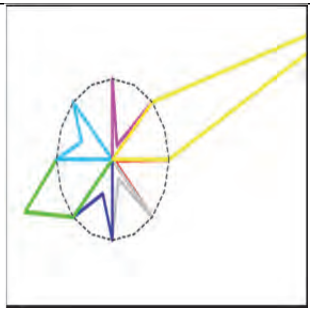
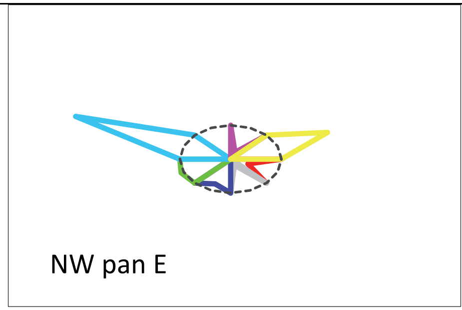
MP M – May 2012		MP M – March 2013
		
MP O – May 2012	MP O – December 2012	MP O – March 2013
		
MP P – March 2013	MP Q – March 2013	MP R – March 2013
		

Another exception was MP pan E during the December 2012 survey where the alkalinity was higher than previous sampling trips while the dominant anion was the sulphate instead of the chloride. This trend was also seen at MP pan G during the December survey with the dominance of the sulphate anions.

The Maucha diagram for MP pan J during the May 2012 survey also indicated a higher alkalinity as compared to other pans in the area. The final exception was MP pan O that consistently indicated that sulphates are the dominant anion from May 2012 to March 2013. The alkalinity also tended to be dominant in this pan during all three sampling trips. In Figure 6.4 the North West Province Maucha diagrams are presented for comparison with the Mpumalanga pans. The results indicate more variation than seen in Mpumalanga and only NW pan I reflected similar Maucha diagrams to the Mpumalanga pans over the project duration. The dissimilarity between the systems was due to the higher alkalinity present in NW pan I. The North West pans in general also indicates that sodium and chloride are dominant but at lower concentrations than seen in Mpumalanga. However, the majority of pans from North West had high total alkalinity scores.

The Free State pans were limited in the number of samples taken due to the majority of pans being dry throughout the sampling duration of the project. The Maucha diagrams for the Free State pan (Figure 6.4) indicates that sodium is the dominant cation while the dominant anions was chloride. However, no sample from the Free State indicated any form of total alkalinity in the results. The sodium and chloride ions were higher than seen in the majority of Mpumalanga pans that were sampled during the project.

Table 6-3: Maucha diagrams for the North West pans from May 2012 to March 2013.

NW A – December 2012	NW A – March 2013	NW B – December 2012
		
NW E – May 2012	NW E – December 2012	
		

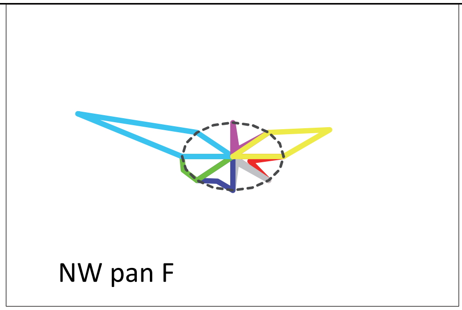
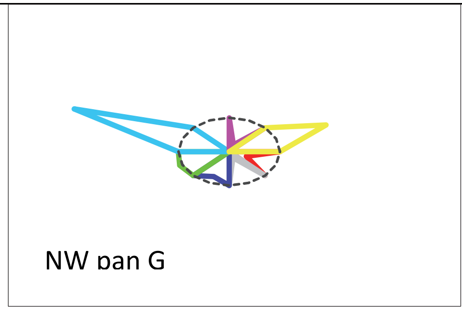
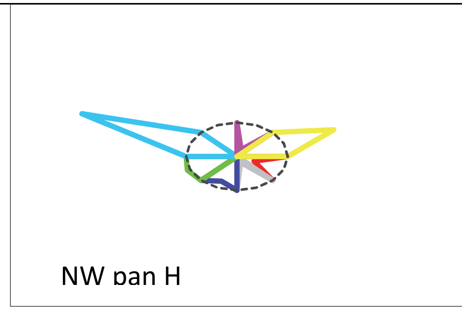
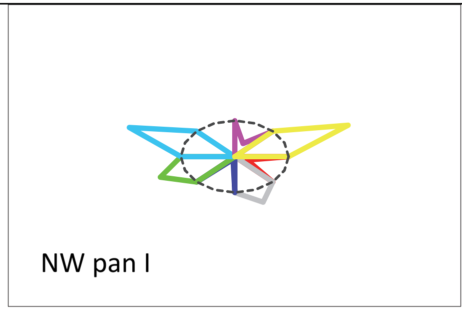
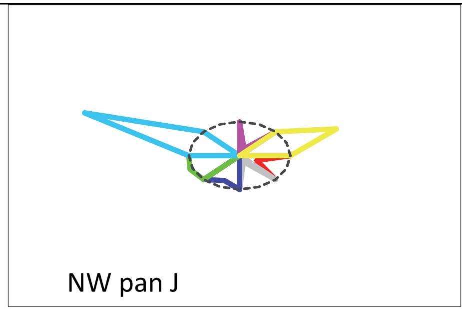
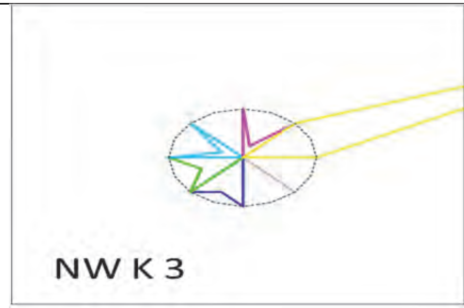
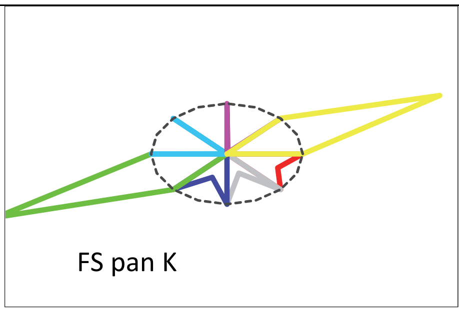
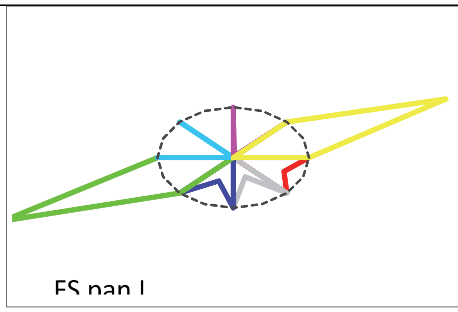
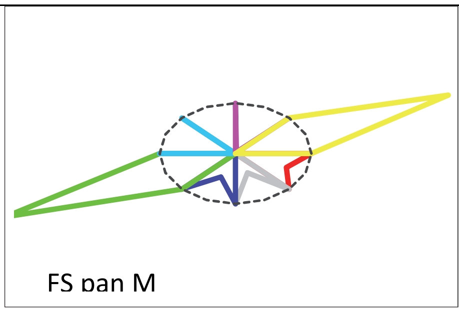
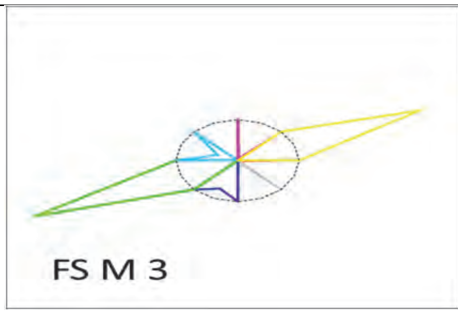
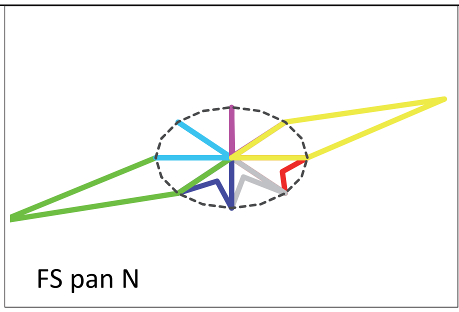
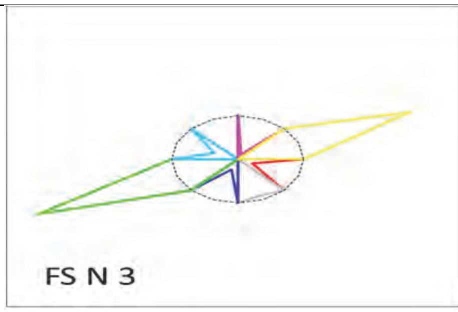
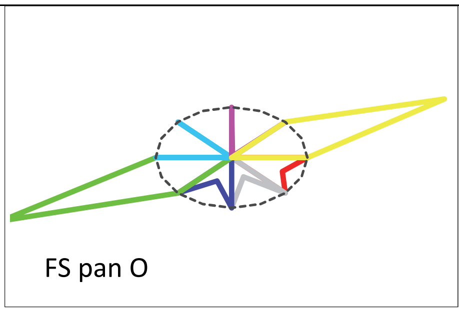
NW F – December 2012	NW G – December 2012	NW H – December 2012
		
NW I – May 2012	NW I – December 2012	NW I – March 2013
		
	NW J – December 2012	NW K – March 2012
		

Table 6-4: Maucha diagrams for the Free State pans from May 2012 to March 2013.

FS K – December 2012	FS L – December 2012
	
FS M – December 2012	**FS M – March 2013**
	
FS N – December 2012	**FS N – March 2013**
	
FS O – December 2012	
	

Multivariate Statistical analysis

The results of the multivariate statistical analysis is provided in the following section for the water quality results of pans from Mpumalanga, Free State and North West for samples collected from May 2012 to April 2013. The multivariate analysis were firstly completed using all of the available data from the water analysis and secondly with only the nutrient and salt variables. The data that was excluded from the second analysis were mostly metal concentrations within the water phase which was deemed not important to determine water quality conditions in pans in South Africa. Most of the pans sampled during the survey had land use comprising of mostly livestock grazing. Therefore, metal concentrations determined within the water phase are probably background concentrations rather than due to anthropogenic impacts in the systems.

The PCA biplot in Figure 6.2 indicates the ordination of samples from all of the pans sampled during the project in Mpumalanga, North West and Free State for all of the available water quality data. Each different coloured symbol represents a different province but differences between the various sampling surveys are not indicated. It is evident from this figure that the pans from Mpumalanga and North West are grouping towards the middle of the figure while the Free State pans are generally grouping towards the top right quadrant. Some outliers from North West are evident in the bottom right quadrant of the biplot. Mpumalanga pans do not seem to have any outliers with the exception of a few pans that group towards the top left quadrant. Metal concentrations of strontium (Sr) and to a lesser degree molybdenum (Mo) and manganese (Mn) where found to be the highest contributors to the Free State pan grouping. In selected pans from the North West vanadium (V), copper (Cu) and silver (Ag) where found to be contributing to the grouping. Iron, cadmium, cobalt, lead and aluminium indicated grouping towards pans within Mpumalanga.

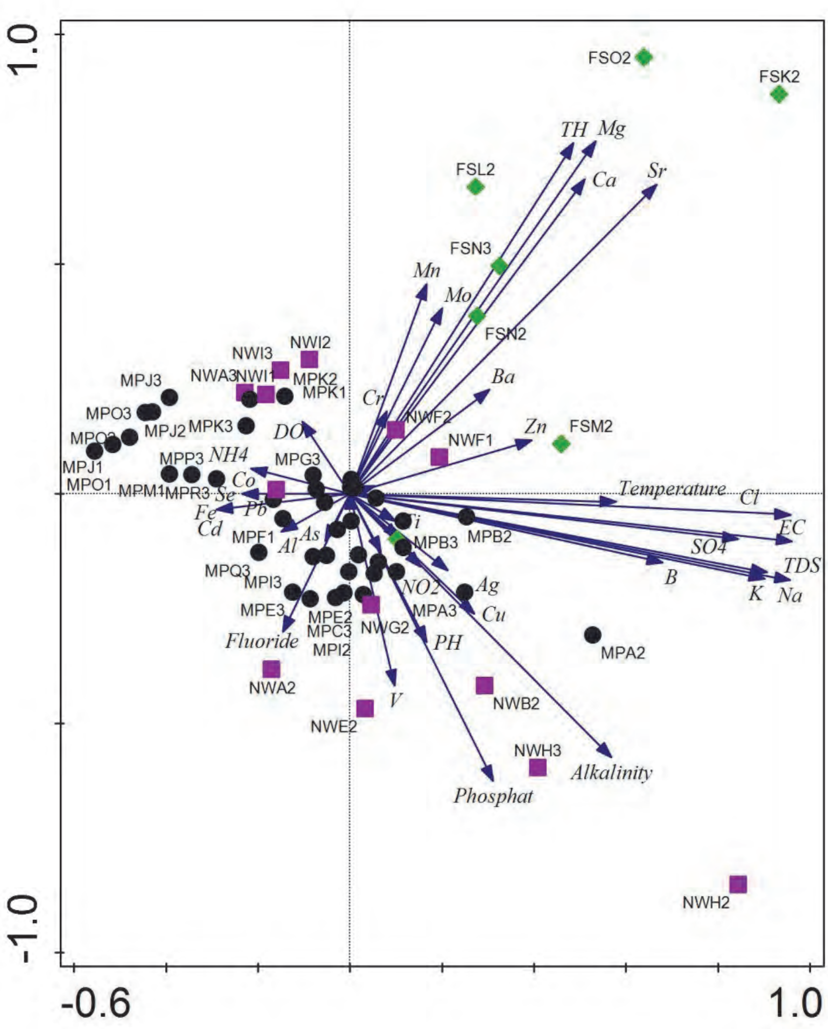


Figure 6-2: PCA biplot of water quality data from pans in Mpumalanga (black circle), North West (purple block) and Free State (green diamond) from 2012-2013. The biplot represents 75.8% of the variance with 58.5% of the variance represented on the first axis and 17.3% on the second axis.

The following PCA biplot only made use of the nutrient and salt parameters that were analysed during the project for all of the pans in Mpumalanga, North West and Free State. The groupings in Figure 6.3 are exactly the same as seen in Figure 6.2 thereby indicating that the metal concentrations are not affecting the variation between the various pans sampled during the project. The salt and nutrient parameters are therefore the driving variables that determine the groupings between the sampled pan systems in this study. The NMDS statistical analysis using Primer Version 6 was completed on the water quality data from all of the sampling surveys. However, as the groupings of the samples were similar to the biplots in Figure 6.2 and Figure 6.3 it was decided to not include the graph in the text. The Bray-Curtis similarity matrix used for the NMDS plot were also used to determine the significance of the groupings for province and sampling survey that were selected a priori.

The ANOSIM significance test were used for the analysis and it indicated that no significant difference were present for either province or sampling survey. A hierarchical cluster analysis based on the Bray-Curtis similarity matrix was also used to select groupings based on the similarity between the different sites. However, at a similarity percentage of 60%, 70% and 80% no significant differences were seen. Another group were constructed of sites that were 90% similar and the ANOSIM analysis indicated that the grouping were significant (Global R = 0.978). However, this grouping resulted in 33 different groupings based on the Bray-Curtis similarity matrix which indicates many of the pan water quality are unique.

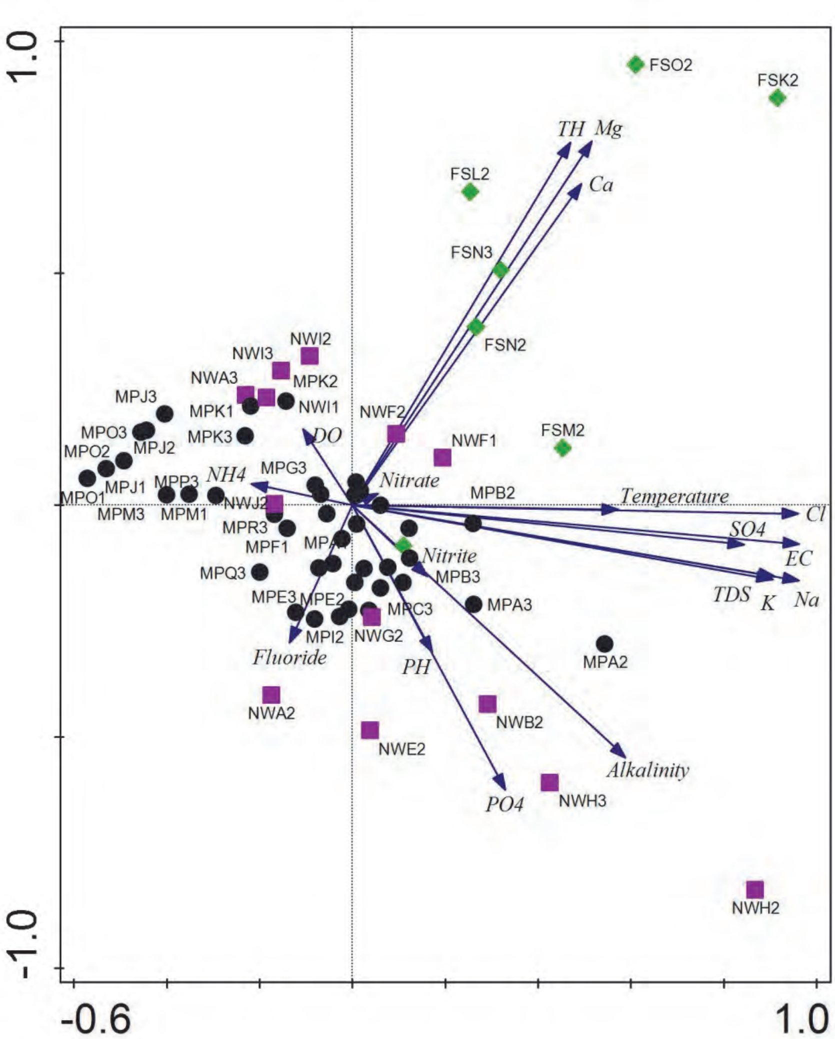


Figure 6-3: PCA biplot of nutrient and salt water quality data from pans in Mpumalanga, North West and Free State from 2012-2013. The biplot represents 78.4% of the variance with 61.1% of the variance represented on the first axis and 17.3% on the second axis.

The nutrient and salt variables that were driving the groupings in Figure 6.3 were mostly the phosphate and alkalinity that were the highest at selected pans in the North West, specifically NW pan H during the second and third surveys. Total hardness, calcium and magnesium were the highest water quality parameters contributing to the Free State pan grouping in the upper left quadrant of the biplot. The chloride, sulphate, EC, TDS, sodium and potassium concentrations were all similar at the majority of the pans in the study. The Free State grouping and the few North West pans that grouped in the bottom left quadrant had the highest concentrations for these variables.

The PCA biplot in Figure 6.4 presents the nutrient and salt water quality data from only the Mpumalanga pans from the project. The aim was to determine if there was any spatial variation within the pans of the province and which water quality parameters caused the variation. The majority of the pans grouped around the centre of the biplot indicating the pans have fairly similar water qualities. There are four pans that grouped away from the rest of the Mpumalanga pans. These where MP pan J, MP pan O, MP pan P and MP pan K. Each of the three sampling surveys at these pans grouped together indicating no temporal variation is present with the exception of MP pan P where only a once off sample was taken. MP pan J and MP pan O grouped separately due to the lowest EC, TDS and other salt variables measured at the pans. There is also a slight indication that the ammonium ions were higher at these pans compared to the other pans in the area. MP pan K during all three surveys also grouped separately at the top of the biplot in Figure 6.4. The magnesium ion was slightly higher at this site and it could have resulted in the separate grouping.

The North West and Free State provinces were combined and analysed to determine if there were any differences between pans from the same province or between provinces. The PCA biplot in Figure 6.5 indicated two groupings while NW pan H from the second survey grouped separately. The first grouping comprised mostly pans from the NW Province and can be seen in the top left quadrant. This grouping occurred due to the pH, fluoride, and phosphate concentrations being high at the site. The second grouping can be found in the bottom right corner in Figure 6.5 where some pans from the Free State Province grouped. This grouping was due to the calcium, magnesium and total Hardness being relatively higher than the other variables. The variables that can be seen in the top right corner indicated that these parameters were similar at most of the pans.

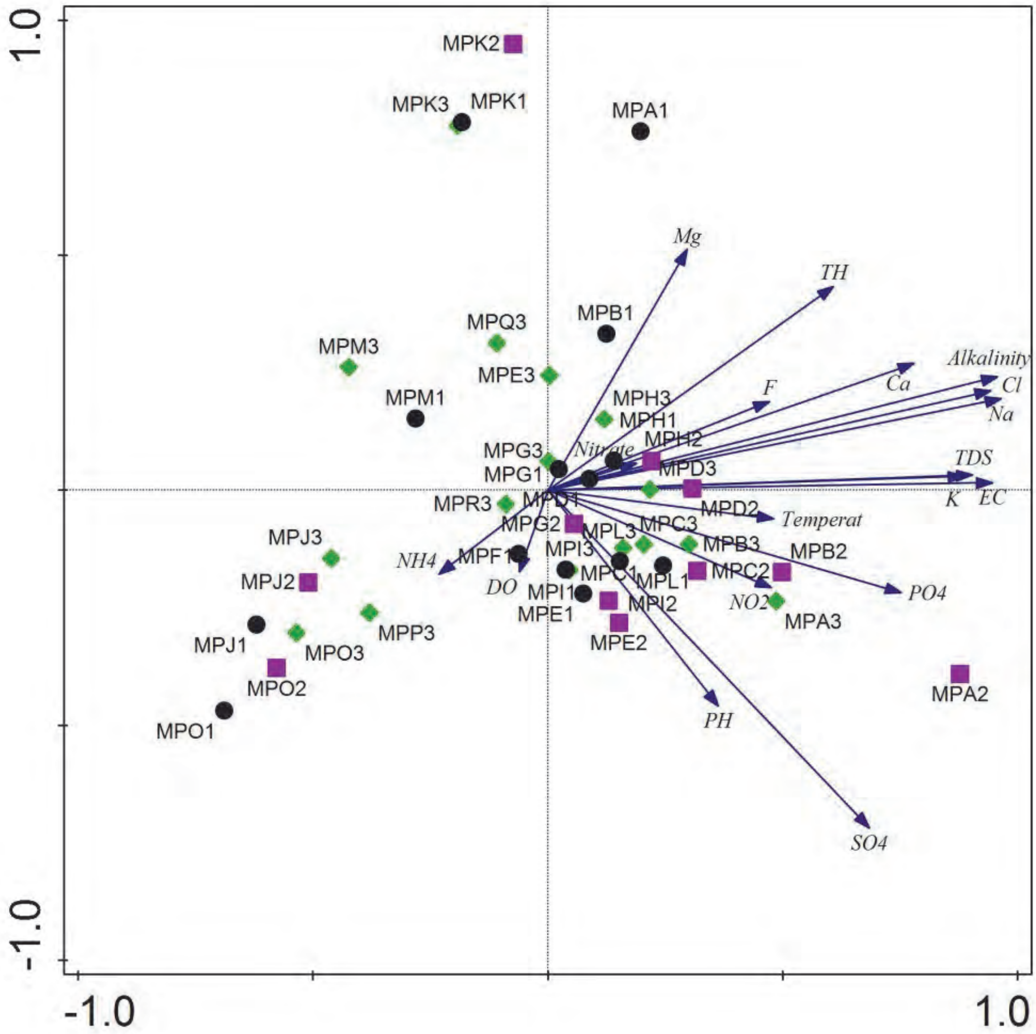


Figure 6-4: PCA biplot of nutrient and salt water quality data from pans in Mpumalanga from 2012-2013. The biplot represents 81.7% of the variance with 68.2% of the variance represented on the first axis and 13.5% on the second axis.

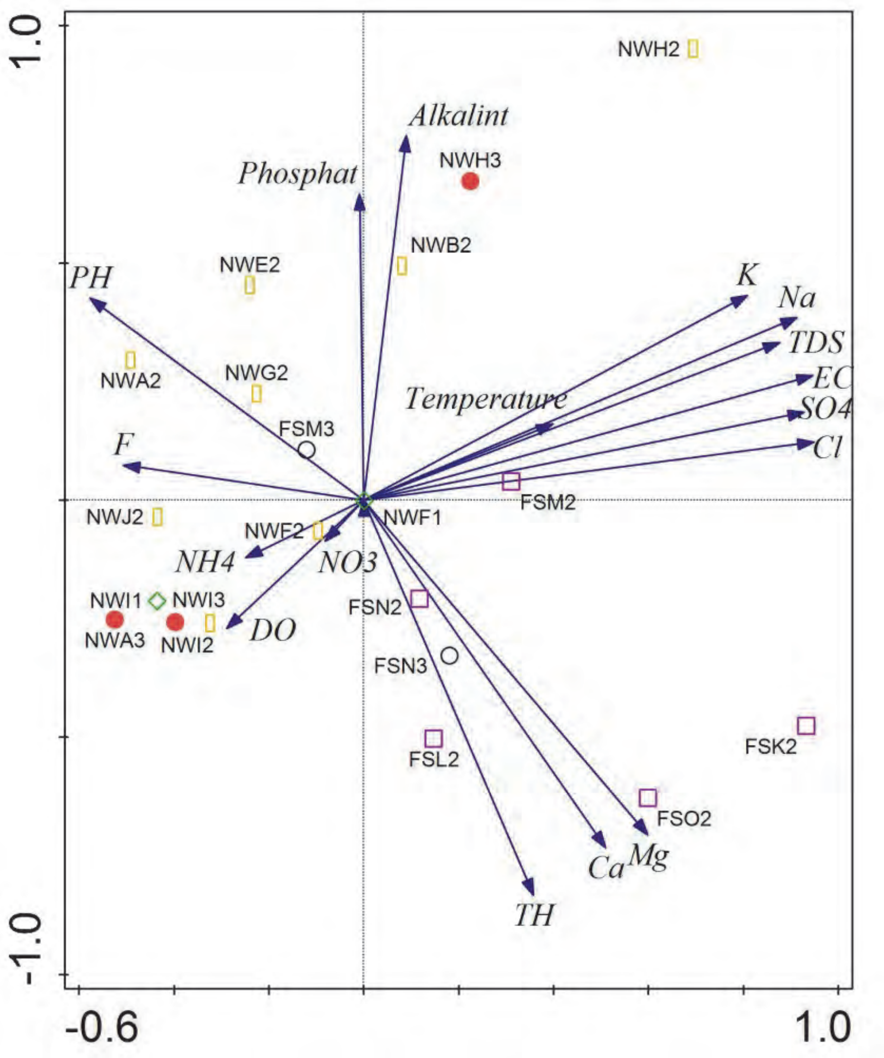


Figure 6-5: PCA biplot of nutrient and salt water quality data from pans in North West and Free State from 2012-2013. The biplot represents 82.8% of the variance with 53.3% of the variance represented on the first axis and 29.5% on the second axis.

The 5th and 95th percentile values for all of the pans sampled during the project for nutrient and salinity variables were used to determine possible ranges for these variables (Table 6.5). The Mpumalanga, North West and Free State provinces were also separated to determine the differences in the ranges for the various provinces (Table 6.6 and Table 6.7). The low range for the pH is similar between all the provinces and the Mpumalanga pans while the North West and Free State pans are a full pH value higher. The high range between the various pans and the combined results are similar with the Free State and North West pans only slightly higher. The total inorganic nitrogen and phosphate concentrations indicated that the low ranges were the same concentrations but the high concentrations showed increasing concentrations from all the pans to Mpumalanga to the

North West and Free State pans. This trend was evident for both the total inorganic nitrogen and the phosphate concentrations.

Table 6-5: Low and high ranges for nutrient and salinity / salt variables of pans in Mpumalanga, North West and Free State.

	Unit	Count	Low	High
pH	-	61	7.93	9.79
TN	mg/L	61	0.15	8.72
Phosphate	mg/L	61	0.07	10.10
TDS	mg/L	61	0.12	27.30
EC	Ms/cm	61	0.21	53.80
Sulphate	mg/L	61	4.04	4109.00
Ca	mg/L	61	4.19:	131.43
K	mg/L	61	5.87	1106.70
Mg	mg/L	61	1.83	294.15
Na	mg/L	61	13.81	11574.77
TH	mg/L	61	23.00	2650.00
Fluoride	mg/L	61	0.04	1.31
Chloride	mg/L	61	21.98	11350.00
Alkalinity	mg/L	61	45.02	1681.14

The salinity and salt variables listed in the tables below indicate one major trend for the various provinces. The trend indicates that all of the variables (Table 6.5) from the Free State and North West provinces' pans have similar low and high range values. The Mpumalanga pan ranges are all lower than the ranges for all of the pans combined. The only exception is the fluoride and alkalinity variables which indicated that the ranges are similar across all of the provinces.

6.4 Discussion

Pan ecosystems are some of the most variable systems in South Africa. Their endorheic nature and the resultant variability make comparison to other wetlands and even other pans problematic. The Target Water Quality Requirements from the Department of Water Affairs (DWAF, 1996a, 1996b, 1996c) does not include requirements for pans and therefore the requirements were not used. The variability in the physico-chemical characteristics of pans is often related to the duration of inundation. The duration in inundation in turn is affected by a variety of physical and climatic conditions. These may include the volume of water that was present prior to filling, the permeability of the soil, and the variability in meteorological conditions (Crawford, 1981, Meintjes *et al.*, 1994 McCulloch *et al.*, 2008). The water quality in this section will be discussed based on the nutrients, salinity or salts and the future management of these systems.

Table 6-6: Low and high ranges for nutrient and salinity / salt variables of pans in Mpumalanga.

	Unit	Count	Low	High
pH	-	41	7.89	9.45
TN	mg/L	41	0.15	10.36
Phosphate	mg/L	41	0.07	10.10
TDS	mg/L	41	0.12	9.02
EC	Ms/cm	41	0.19	9.06
Sulphate	mg/L	41	1.56	667.00
Ca	mg/L	41	6.62	38.13
K	mg/L	41	5.02	168.18
Mg	mg/L	41	3.14	22.38
Na	mg/L	41	11.58	1502.11
TH	mg/L	41	37.00	170.00
Fluoride	mg/L	41	0.14	1.32
Chloride	mg/L	41	17.28	1940.00
Alkalinity	mg/L	41	45.02	1100.00

Table 6-7: Low and high ranges for nutrient and salinity / salt variables of pans in North West and Free State.

	Unit	Count	Low	High
pH	-	20	8.79	10.09
TN	mg/L	20	0.15	16.09
Phosphate	mg/L	20	0.07	12.67
TDS	mg/L	20	0.53	55.72
EC	Ms/cm	20	0.81	110.56
Sulphate	mg/L	20	19.05	11828.00
Ca	mg/L	20	2.69	1252.97
K	mg/L	20	20.77	2222.78
Mg	mg/L	20	0.58	997.70
Na	mg/L	20	120.10	68804.72
TH	mg/L	20	18.00	9958.50
Fluoride	mg/L	20	0.04	1.24
Chloride	mg/L	20	123.18	55655.00
Alkalinity	mg/L	20	51.20	1917.08

Nutrients

The concentrations of nitrates (NO_3^-), nitrites (NO_2^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) ions are important as these are the major nutrients that are involved in eutrophication of aquatic resources. The biggest concern, regarding pans, is the diffuse source of nitrogen and phosphate. The diffusion of nutrients is often seasonal as it linked to agricultural activities. In general the nutrients were fairly low in the sampled pans. However, in some cases the nutrient concentrations were measured as eutrophic with very high concentrations. The general trend in the pans was that the phosphate concentrations were higher than the total inorganic nitrogen measured at the sites.

Nitrate concentrations were fairly high during certain surveys at some pans for example at NW pan E. Dallas and Day (2004) does mention that nitrates are not often found in high concentrations in natural systems (< 0.1 mg/l), and is the result of fertilizers, agricultural runoff, etc. entering aquatic ecosystems. Therefore it is a possibility that the phytoplankton bloom seen during May 2012 was a result of excess nitrates being available. The combination of high salinity and high nutrients in NW pan E during the May 2012 survey created an environment favourable for the high growth rates and algal biomass.

The nutrient analysis of the January 2013 survey indicated that no nitrates, nitrites or phosphates were above the detection limits. This indicates these pans are potentially nutrient poor, which indicates that diversity within the pans could potentially be poor. These pans are situated in the Bultfontein area where large scale crop farming is present. Many of the other pans in the area are heavily impacted by these farming activities as the fields encroach on the pan boundary. However, the March 2013 survey indicated that some of the pans in the Free State that did have water also indicated much higher nutrient concentrations. Nitrate concentrations at FS pan N were found to be very high. However, an abundance of flamingos were present at the site as this was the only water source in the area. The diffusion of nutrients is often seasonal as it can be linked to either agricultural activities or a concentration of nutrients due to the declining water levels. As the pans that were selected in the province were selected based on a lack of human activities, it could be assumed that the nutrient concentrations observed are natural. Phosphate concentrations were found to be the highest at many of the pans. Due to the endorheic nature of the pan, the nutrients that enters the system does get trapped and unlike riverine ecosystems, pans do not get “flushed”. Phosphates are surface-active, i.e. they tend to be observed onto surface particles of clay particles or other particles such as Al and Ca ions (Webster *et al*., 2001).

In Mpumalanga, the nitrite concentrations were fairly similar in most of the pans sampled in the Mpumalanga Province with the exception of MP J, MP K and MP O which had low concentrations. Nitrite is an intermediated compound during the conversion of ammonia to nitrate. Both ammonia and nitrite can be toxic to aquatic biota, especially at an increased pH and temperature (Dallas and Day, 2004). All the Mpumalanga pans had higher nitrite concentrations than present in the North West and Free State pans. This could probably be related to the presence of water being more abundant in Mpumalanga than in the other provinces. Nitrate concentrations were found to be higher than the nitrite concentrations. Apart from these nutrients, phosphate also plays a major role in eutrophication (Dallas and Day, 2004). The highest phosphate concentrations were observed at MP pan A, MP pan B and MP pan C where phosphate concentration ranged from 7 to 17 mg/L. It could be expected that most phosphate concentrations in most of the selected pans would be low. Phosphates bind to most soils and sediment and as a result, will not be present in high concentrations within the water column (Dallas and Day, 2004). Apart from phosphate concentrations, the concentrations of most of the nutrients were much higher at NW pan A. There are little to know anthropogenic activities within the watershed of this pan and it should be assumed that the high concentration that was observed is a natural occurrence. The high values of phosphate observed corroborates with the observations made by Hutchinson *et al.* (1932). This occurrence is probably due to large quantities of phosphate that is accumulated in the pans, but not used due to the low abundance of especially phytoplankton and vegetation.

Nitrogen and phosphate concentrations are important variables in the management of inland waters. Both these ions can contribute to eutrophication of aquatic ecosystems when concentrations of these nutrients are excessively high (Carpenter *et al.*, 1998) and promotes excessive plant growth (Dallas and Day, 2004). The biggest concern, regarding pans, is the diffuse source diffusion of nitrogen and phosphate. Diffuse pollution is often seasonal in nature as it is linked to agriculture.

Salts / Salinity

The pans from Mpumalanga are generally more perennial in nature when compared to pans in the North West and Free State. This was evident in this study as most of the pans in Mpumalanga had water during both surveys while only on the second survey water was present in North West. No water was present in the Free State although in previous seasons water was present throughout a whole year indicating the importance of rainfall in the pan catchment for filling of the pans. The more perennial a pan is the more stable the physico-chemical variables will be and vice versa for pans that are more ephemeral. The endorheic pans and the associated physico-chemical variables are determined by the climate and pan geology as well as the source of the water. A study completed by Russel (2008) indicated that the water in the pans around the Lake Chrissie area in Mpumalanga is a mixture of perched and rain water although it has been indicated that certain pans may be linked to deeper aquifers. This was seen in this study where MP J and MP O were very fresh pans with low salt concentrations, pointing to a suspected groundwater influence in the pan.

The Free State and North West pans were mostly more ephemeral and even though water was present it was evident that the inundation period would not have been much longer as all of the pans were had extremely shallow water depths. If rainfall had occurred in the few weeks following the second field survey the pans would have kept water for a longer period of time allowing the physico-chemical variables to stabilise. The measurements of the in situ variables during the second field survey indicated high salinities due to the rapidly dropping water level and concentration of salts. This was especially evident in the Free State pans where hypersaline conditions occurred.

The physical chemical water quality changes are linked to physical characteristics of the pan and climatologically induced changes. The source of water in a pan may also contribute to the physico-chemical characteristics of a pan. The pans in the Free State and North West are generally more reliant on rainfall as a source of surface water to the pans. A rainfall event during the March 2013 survey indicated that the surface water of a pan can quickly increase following a rainfall event but that the current dry state of the pans results in the water quickly seeping into the sediment of the pan.

Not much water quality information is available from the North West Province for comparison; however, Seaman *et al.* (1991) discussed some of the chemical and biological results obtained for pans in the then western Transvaal (North West) and the Northern Cape, but the discussion is focussed on selected ions (Na, K, Cl, Mg, Cl, SO_4, HCO_3). The Maucha diagrams indicated that NW pan A and NW pan B are dominated by Na and Cl ions while the other pans are dominated by HCO_3 and Na. The majority of the Mpumalanga pans were also dominated by the Na and Cl ions with some pans indicating a slightly higher alkalinity.

The sulphate concentration at NW pan I during the December 2012 survey indicated high concentrations and sulphate is very important for living organisms as it is an essential compound of proteins. The sulphate concentrations were similar during the March 2013 survey. In natural aquatic ecosystems, sulphates are often found in lower concentrations than bicarbonates (Dallas and Day, 2004). Although this was the case at NW pan I the concentration was still higher than the previous survey in May 2012. In May 2012 the sulphate concentrations at NW pan E were much higher when compared to bicarbonate concentrations.

The PCA biplots indicated that based on the ions there is some spatial variation between the North West pans with especially NW pan H grouping separately due to extremely high Cl and Na concentrations. These concentrations could potentially have arisen from the Delareyville town as this pan is in close proximity to the town. This pan was the only one in the North West that could potentially have a source of effluent from the WWTW. The data does show increased phosphates, sulphates, chlorides and sodium that could have originated from sewage water. All of the other pans in North West grouped similarly with only small changes in the water quality. This is interesting as NW pan I is Barberspan which is not a classic pan ecosystem as it does have an outflow. Expected results would have shown this system to group separately but during this survey it was not the case.

The water quality of the Free State pans was similar with the exception of FS K that had very high ion concentrations of especially Cl and Na. In natural aquatic ecosystems, sulphates are often found in lower concentrations than bicarbonates (Dallas and Day, 2004).The Maucha diagram indicated that all of the pans were dominated by Cl and Na ions which are similar to the majority of pans in Mpumalanga and some pans in the North West Statistically, the Free State pans grouped together with the North West and Mpumalanga surveys but some spatial variation was evident in the PCA biplot as the pans did not group close together. All of these pans tended to be very shallow with only one pan indicating the presence of marginal vegetation.

Seaman *et al.* (1991) and Dallas and Day (2004) both indicate that $Na^{+} > Mg^{2+} > Ca^{2+} > K^{+}$ and $Cl^{-} > SO_4^{-} > HCO_3^{-}$ predominates the cation and anion composition of saline water in Southern Africa. The composition did differ in the pans in Mpumalanga and North West. In the pans selected in Mpumalanga it was observed that $Na^{+} > K^{+} > Ca^{2+} > Mg^{2+}$ although the anion composition remained similar. While in the pans selected in the North West Province $K^{+} > Ca^{2+}$ and $HCO_3^{-} > SO_4^{-}$. Seaman *et al.* (1991) does indicate that a variety of patterns in ion dominance, although there are no obvious regional differences amongst cation dominance patterns. The variety observed in cation dominance does decrease with an increased salinity. It has been indicated that Cl^{-} and SO_4^{-} are the most dominant anions. As with cations, there is no evidence of regional patterns in anion dominance and the variety observed in anion dominance also decreases with an increased salinity (Seaman *et al.*, 1991). Seasonal variation can often occur as the sampling surveys are generally a snap shot of what is occurring within the pan. The source of these salts in pans is probably the underlying and surrounding geology.

In the Mpumalanga Province the pans are mostly underlain by the Karoo Supergroup. This supergroup is regarded as immature and will weather easily, leaching Na^{2+} and Ca^{2+} into the surrounding environment (Russell, 2008). The source of the Cl^- in these systems is predominantly the marine shales within the Vryheid Formation (Russell, 2008). From the Maucha diagrams it was also evident that all the alkalinity at MP E, MP G, MP J and MP O was higher during the surveys. The results of this study have shown that the alkalinity is predominantly above 200 mg/l. Alkalinity in this study has been measured as the total of the anions (OH^-, CO_3^{2-}, HCO_3^-) of weak acids, hydroxyl ions and bicarbonates. The alkalinity of a system is often seen as the buffer capacity of a system. The higher the buffer capacity of water, the smaller the change will be in pH with the addition of an acid or alkali (Dallas and Day, 2004). The buffer capacity of pans may be an important factor when considering the impact of AMD.

The high conductivity observed at many of the pans is directly related to the salts concentrations within the systems. The salts that form on the surface of the substrate after desiccation are often removed through wind action (Russell, 2008). The salt concentrations within a given system will change after inundation. This explains the variation seen between the different sampling surveys at the specific pans in the PCA biplots. However, some pans remained similar in their salt concentrations. This was especially true for the Mpumalanga pans that did not dry out.

Nutrient and Salinity Variable Ranges

The classification of pans based on their water quality variables were first attempted by Hutchinson *et al*. in 1932 on various pans in the Lake Chrissie area. Since then a few studies (Ferreira, 2010) have attempted to classify pans based on their water quality as well as their biological communities. However, no one method has really been successful. This is due to the inherent variability within these pan systems. The current study did initially attempt to classify the pans from Mpumalanga, Free State and North West based on their salinity and nutrient data based on the Hutchinson *et al.* (1932). However, as was seen in the statistical analysis no real classification or different groups were identified based on the water quality. Oberholster *et al.* (2014) identified a proposed methodology for wetland classification and risk assessment for people that are not wetland specialists. In this work they also identified a range for specifically EC for pans based on the work of De Klerk (2009; 2012) on reed pans in Mpumalanga. The ranges identified for their classification scheme was similar with this study in terms of the lower concentrations of EC but as Oberholster *et al.* (2014) did not include pans from North West and Free State the upper limits to their classification is lower than found during the current surveys. Classification can be very useful in the management of pans and wetlands but without an accurate method for the classification no real progress will be made. Therefore a way needed to be found to provide useful information to facilitate management of pans in South Africa. Management of pans in South Africa is important as many of the pans in Mpumalanga, Free State and North West are under threat from various anthropogenic activities.

The way forward was then decided to be ranges for the salinity and nutrient variables of pans in the three selected provinces. The ranges were identified for all of the provinces initially but after making a comparison between the Mpumalanga and North West and Free State provinces it was decided to recommend ranges for each of the provinces. The aim of the ranges will be to provide a relative range within which pans should fall for the majority of the time. However, it is acknowledged that pans that dry out will normally exceed the salinity variables as the ions are concentrated when the water evaporates.

6.5 Conclusion

The water quality of the various pans sampled during the Free State, North West and Mpumalanga provinces was assessed during three surveys from May 2012 to April 2013. Various *in situ*, nutrient, salt and metal variables were analysed by a SANAS accredited laboratory. The full water quality results are presented in the Appendix A while the summarised data were presented in the chapter. The results indicated that the water quality data varied between all the different pans within the various provinces as well as between the different provinces. The salt variables were summarised with Maucha diagrams and that indicated that the majority of pans are dominated by the sodium and chloride ions. This trend was seen in the North West and in the Free State but the concentrations of the ions was lower when compared to the Mpumalanga Province. This was the major trend but exceptions at specific pans and sampling surveys were evident during the duration of the project. The statistical analysis indicated that the metal concentrations did not have an effect on the various groupings that were identified. The major contributors to the groupings were the salt and nutrient variables. However, statistical significance was not found for groups of pans from the various provinces as well as between the sampling survey times. This project has increased the amount of available water quality from pans in South Africa and the ranges that have been identified will be useful in the management of pans in South Africa in the future.

7 INVERTEBRATE ASSESSMENTS

7.1 Introduction

The National Water Act (NWA) of 1998 describes a wetland as “a land in transition between terrestrial and aquatic systems, where the water table is at or near the surface or the land is periodically covered by shallow water and which in normal circumstances supports or would support vegetation typically adapted to life in saturated soil. A pan is a type of wetland, defined as a natural shallow depression in the surface of the earth which floods during the rainy season, has no outlets and usually dries up seasonally due to evaporation (Geldenhuys, 1982). These systems have unique characteristics that are not found in palustrine and lacustrine systems and thus are not classified as such. In order for a system to be classified as endorheic it must adhere to the following characteristics as set out by Duthie (1999):

- circular to oval in shape, in some cases kidney shaped or lobed;
- possess a flat basin floor;
- less than 3 m deep when fully inundated; and
- have closed drainage (i.e. lacking any outlet).

Pans act as sinks for drainage of substances and for this reason they are threatened by the drainage and filtering of anthropogenic effects such as pollution, eutrophication, introduction of exotic species, cultivation, road construction, mineral extraction and ground water pumping (Rodriguez-Rodriguez, 2007). Although many studies have been conducted on wetlands, there has been limited focus on pans and even less of that focus is on pans in South Africa. Recently much interest has been shown in the Mpumalanga Lake District (MLD) as coal mining companies are interested in opencast mining within in this region. Mining would have a serious negative impact upon the natural ecology of these areas as acid mine drainage (AMD) is a major hazard associated with mining and impacts water sources severely.

Consequently, research has recently started on pans, not only in the MLD but other areas where pans occur, such as the North West, Northern Cape and upper parts of the Free State. For instance, Russell (2008) studied pans in the MLD in order to further understand how the hydrology and chemistry of pans are influenced by various factors. De Klerk (2009) conducted an ecological assessment of reed pans in the MLD focusing on the use of aquatic invertebrates to determine the ecological integrity of pans. More recently, Ferreira (2010) conducted a study on perennial pans with the intent of creating a standardised method to assess the ecological integrity of pans in the MLD. Very little attention has been given to the effect that land use has on wetlands and how this in turn will then affect society as most wetlands are not used for much more than pasture production (Kotze *et al*., 2000).

The amount of information about perennial pans in South Africa is rather scarce although much research has been done on these type of pans in North America and Australia. Many of the pans found in these countries are comparable to those found in South Africa, especially those that occur in the drier regions (Ferreira, 2010). Wetlands, especially smaller and less obvious wetlands such as pans, are overlooked with no policy or strategy to protect them even though they perform many important functions. These functions include improvement of water quality, and acting as a habitat for wetland dependant plants and animals, some of which are rare, endangered or so specialized that they do not occur anywhere else (Kotze, *et al*., 1995). Pans are also seen as less important ecosystems since these water bodies do not perform the functions that other wetlands normally do (Ferreira, 2010). This is problematic as de Klerk (2009) found that endorheic wetlands such as the pans in the MLD are unique and support an abundant biodiversity as well as functioning as important regions for water storage, filtering of impurities and carbon fixation. Wetlands play an integral role in the hydrological cycle and biodiversity (Ferreira, 2010). Pans are important habitats to waterfowl, especially migratory birds but are however not usually habitats of fish, reptiles or amphibians (Allan, 1987). Wetland areas are also deemed of ecological importance to much wildlife according to Marius Wheeler of the Avian Demography Unit at the University of Cape Town who says that the MLD qualifies as a Global Important Bird Area (McCarthy *et al.*, 2007).

The term water quality is used as a general term to indicate the concentration of dissolved solutes and of particulate sediment. Solutes can either be nutrients or compounds that have a toxic effect (Macfarlane *et al.*, 2006). The nature and level of solutes that are found in wetlands varies from one to another. These solutes can also vary within a single water body over time, especially in areas that have a wide variety of climatic changes. This difference means that a generalised standard for determining the health of a wetland in terms of water quality is difficult if not impossible (Macfarlane *et al.*, 2006). Endorheic pans occur in areas with rainfall of less than 500 mm per annum, while the evaporation loss is more than 1000 mm per annum. For this reason pans lose most of their water through evaporation which also then contributes to the high salinity that is found in most of these systems (Duthie, 1999). Hydrological variability is thus a feature that greatly affects the ecology of pans as the system may start as freshwater at the beginning of the wet season, but as the dry season progresses and there is more evaporation, salinity increases severely. With each inundation there are considerable changes in the physical and chemical properties of pans that take place (Allan *et al.*, 1995). As stated before, endorheic pans are dependent upon rainfall for water.

The duration of water in an endorheic pan depends on the amount of water that enters it at inundation, soil permeability as well as other meteorological conditions i.e. temperature and humidity. These systems are subjected to high temperatures as well as high evaporation rates which causes changes in water characteristics during the span of inundation (Meintjes *et al.*, 1994). This is what makes creating a baseline ecological integrity study on pans so difficult.

Invertebrate diversity in temporary pans are extensive. Most of these individuals have an opportunistic lifestyle owing to the fact that their habitats may dry up many times a year and will not receive water at regular intervals. Aquatic invertebrates are very important for bioassesment due to the fact that they have rapid life cycles and because they are close to the bottom of the food chain they act as a good source of nutrition to many individuals and thus play an integral part in the food chain (Dickens and Graham, 2002). This is especially important in pans because of the integral role they play for waterfowl (Allen, 1987). A wide variety of crustaceans and insects are well represented in temporary wetlands (Day *et al*., 2010). Not all insects permanently reside in these areas and rather travel to them from more permanent water bodies close by (Day *et al.*, 2010). Even though there is information available regarding the fauna that live in and around pans, there is still not a lot of information about the biogeographical distribution of them as a whole. Distribution trends of aquatic invertebrates are, however, known for more well studied areas.

Because of the uncertainty that comes with living in temporary wetlands such as pans, individuals who do live there, such as aquatic invertebrates, have specialised adaptations to survive the dry phases of these water bodies (Day *et al*., 2010). Information on the aquatic invertebrates of inland saline waters in South Africa is limited. Most information arises from studies done by Hutchinson *et al.* (1932) on two pans in the Transvaal region. The salt pans that occur in South Africa have rather low invertebrate species diversity and also no fauna that are confined to saline pans (Seaman *et al.*, 1991).

The hypothesis of this study was that spatial variation in terms of water quality and aquatic invertebrate biodiversity is present between pans in various provinces as well as between seasons in each province. The aim was to determine the spatial and temporal variation in aquatic invertebrate species in endorheic pans from three different provinces over three seasons.

7.2 Materials and Methods

A total of three surveys were undertaken in each of the provinces during the winter (sample trip 1), summer (sample trip 2) and autumn (sample trip 3) seasons from 2012 to 2013. Where possible the sites were sampled from in each of the seasons but as pans are so reliant on water from rainfall, it was not possible to sample each site three times, especially in the drier regions such as the Free State and North West. The Free State sites were sampled twice during summer and autumn (FS N-2 and FS N -3 respectively). In the North West, four sites were sampled only once during summer (NW A-2, NW B-2, NW E-2, NW J-2), one site were sampled twice, once during winter (NW F-1) and once during summer (NW F-2) and only one site was sampled from three times (NW I-1, NW I-2 and NW I-3). In Mpumalanga three sites were sampled from only once, one during summer (MP F-1) and the other two during autumn (MP Q-3 and MP R-3) whilst all the rest of the pans were sampled during every survey.

Water Quality

Water samples were collected at each site from the surface in rinsed 2L polypropylene bottles. One water sample was collected at each site. The sample was stored at -4˚C in a mobile freezer, transported to the laboratory at the University of Johannesburg and kept frozen until analysis was performed. Frozen samples were allowed to defrost in the laboratory and analysed with a Merck Spectroquant Pharo 100 Spectrophotometer. The chemical variables analysed were: nitrates, nitrites, sulphates, ammonium, total hardness, chlorides, and orthophosphates using standard protocols of the test kits Samples were also tested for presence of calcium, potassium, sodium, magnesium and total alkalinity by a SANAS accredited laboratory.

Aquatic invertebrate analysis

One sample of zooplankton was collected at each site using a plankton net (60x60cm) made up of 63 µm mesh. These samples were collected by submerging the net in the water or placing it as deep as possible in cases where the water was not deep enough and then sweeping for approximately 10-12 meters. In pans with deeper water, light traps were also placed in the bottom of pans at dusk and then removed early the next morning. Aquatic invertebrates were collected from each site using the "kick stir sweep" method and a standard sweeping net (30x30cm) with a 500µm mesh size. The net was swept through the water for approximately two meters and through vegetation if present. All invertebrate and zooplankton samples from each survey were placed in polyethylene honey jars and fixed with 5% neutral buffered formalin (NBF) and stained with the vital dye, rose bengal. Samples were then stored and transported back to the laboratories of the University of Johannesburg for further analysis. In the laboratory aquatic invertebrates and zooplankton were removed, washed under flowing tap water and placed in 70% ethanol where they were then identified to the lowest taxon possible and finally counted. Aquatic invertebrates and zooplankton were identified using “Guides to Freshwater Aquatic invertebrates of Southern Africa” (Day *et al.*, 1999).

Statistical analysis

In order to determine whether there was a spatial variation in the invertebrate communities between the sites, a variety of statistical analyses were conducted. Primer version 5 was used to conduct the diversity indices and Multidimensional Scaling (MDS). Margalef’s index was used to determine species richness, Pielou’s evenness index was used to determine evenness and Shannon-Wiener diversity index was used as it integrates both species richness and equitability components (Clarke and Warwick, 1994). These various univariate indices were used in order to explain the species abundance relationships amongst the various invertebrate -communities, -diversity and -evenness of distribution amongst the sampling sites. A Bray-Curtis similarity matrix, constructed from the aquatic invertebrate data of each site, was used to formulate a two dimensional non-metric Multidimensional Scaling (MDS), which in turn was used to determine groupings of sites based upon the invertebrate communities present (Clarke and Warwick, 1994). Canoco version 4.5 was used to create a Redundancy Analysis (RDA) based on invertebrate and water quality data obtained from the various sites

7.3 Results

The results in Figures 7.1 and 7.2 show the number of taxa and number of aquatic invertebrates sampled at each of the pans during the various surveys. The lowest total number of taxa in the Free State was during the summer survey with 10 taxa and 830 aquatic invertebrates sampled (Figure 7.1) and the greatest number of taxa and aquatic invertebrates were sampled during the autumn period with values of 15 and more than 1300 respectively. North West sites generally had the least amount of taxa ranging between 4 taxa sampled during summer (NW J-2) and 19 taxa sampled during autumn (NW I-3). These pans also had the least number of aquatic invertebrates ranging between 400 in NW A-2 and 3700 in NW J-2, both during the summer sampling period (Figure 7.2). The greatest number of aquatic invertebrates found in the North West was during the summer period in NW I-2 which had almost 15 000 aquatic invertebrates. Generally the greatest number of taxa as well as number of individuals were found to occur in the Mpumalanga pans with a total of 26 taxa and almost 35 000 individuals found in MP D-3 and more than 32 000 individuals in MP Q-3 during the autumn survey while the lowest number of taxa and aquatic invertebrates in

Mpumalanga were six and 780 respectively from MP J-2 during the summer sampling period.

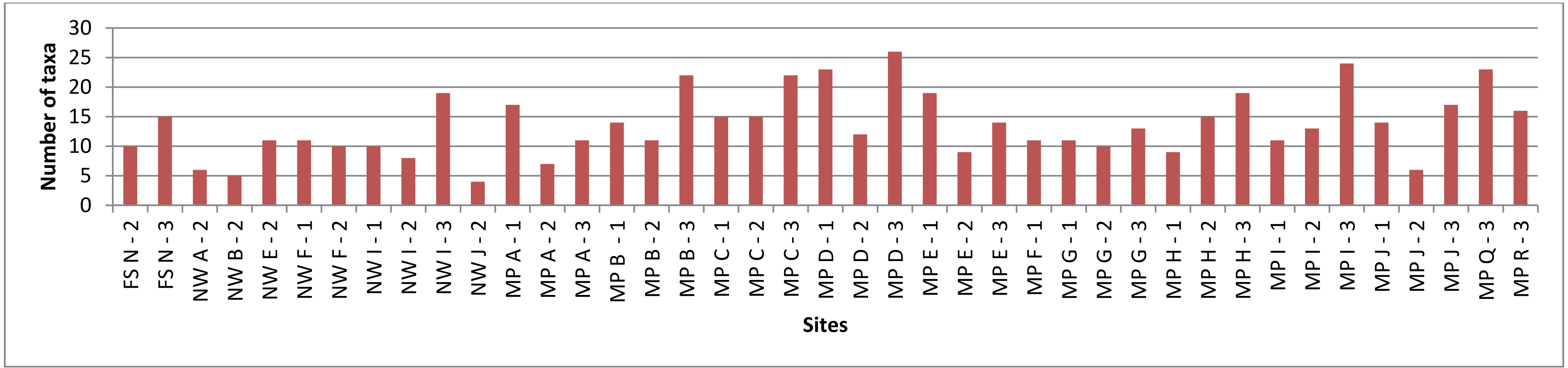


Figure 7-1: The number of taxa sampled at each of the pans during the various surveys over three seasons (1-winter, 2-summer and 3-autumn).

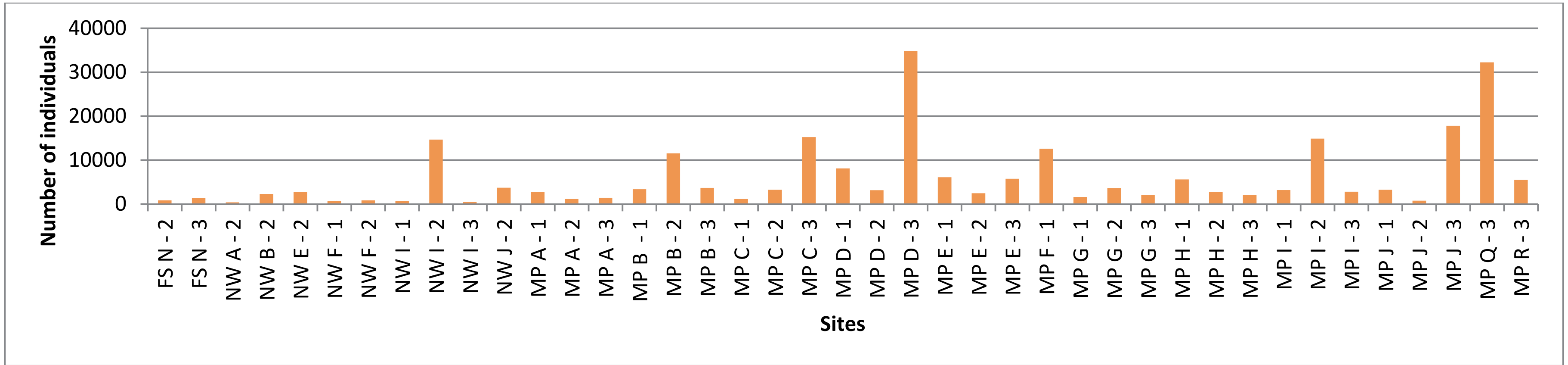


Figure 7-2: The number of individual aquatic invertebrates sampled at each of the pans during the various surveys over three seasons (1-winter, 2-summer and 3-autumn).

Species richness in the various pans across the various provinces and surveys are shown in Figure 7.3. The greatest species richness was found in the North West during autumn (NW I-3) with a score of almost 3 while the least species rich site was also in the North West during summer (NW J-2) with a species richness score of 0.3 (Figure 7.3). The Free State showed a slight difference between the two sampling trips with more species richness occurring during the autumn (FS N-3), with a score of almost 2, than the summer (FS N-2) which had a score of 1.3. Overall Mpumalanga was the most specious province as almost all sites had a score of 1 or more with the highest score occurring during the autumn (MP I-3) with a score of almost 3.

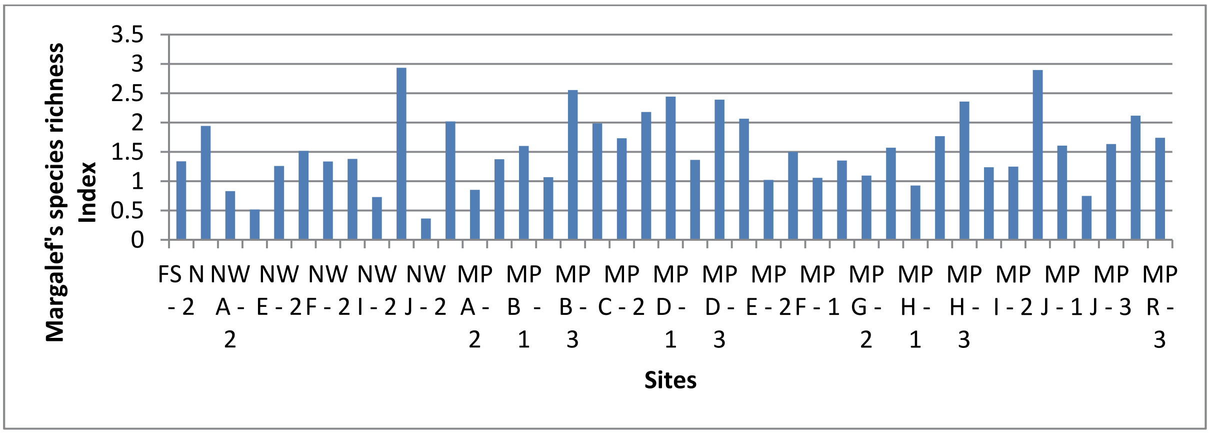


Figure 7-3: Margalef's species richness index for aquatic invertebrates sampled from each site during the various surveys over three seasons (1-winter, 2-summer and 3-autumn).

The Free State had similar evenness scores for both summer (0.52) and autumn (0.58). In the North West the greatest evenness was found during the winter (NW I-1) with a score of 0.5 while the lowest was found during the summer (NW I-2) with an evenness score close to zero. This was the same site that had the largest number of aquatic invertebrates in the North West which indicates that the pan was dominated by certain taxa of aquatic invertebrates. The most even distribution of aquatic invertebrates in Mpumalanga was found during the winter sampling period (MP C-1) with an evenness of 0.67 while the least evenly distributed site in Mpumalanga was MP J-1 with an evenness of 0.15. Mpumalanga was also the province with the most even distribution overall as most of the sites had a value over 0.2 and the most evenly distributed site overall was MP C-1. The Shannon index showed that diversity in the Free State was quite similar between summer and winter (Figure 3.5) with values of 1.2 (FS N-2) and 1.5 (FS N-3) respectively. The least diverse sites were found during the summer in the North West namely NW E-2 and NW J-2 with values of 0.1 and 0.03 respectively and the most diverse site in the North West was NW I-3 during autumn with a value of 1.3. In Mpumalanga the least diverse site was during the winter period

(MP J-1) with a value of 0.4 whilst the most diverse site overall was also in Mpumalanga during the autumn sampling period (MP I-3) with a value of almost 2. Overall the most diverse province in terms of the invertebrate populations in the pans was Mpumalanga and the least diverse province was North West.

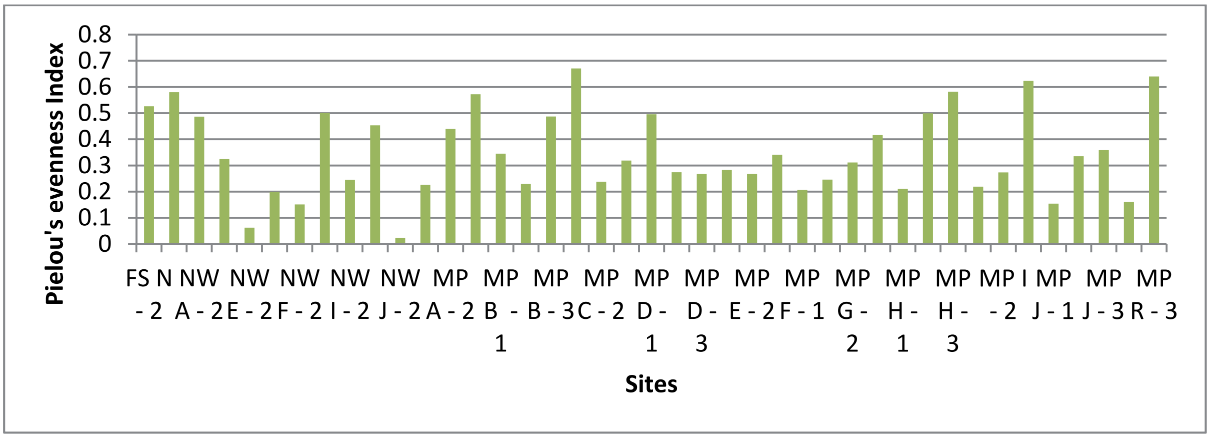


Figure 7-4: Pielou's evenness index for aquatic invertebrates sampled from each site during the various surveys over three seasons (1-winter, 2-summer and 3-autumn).

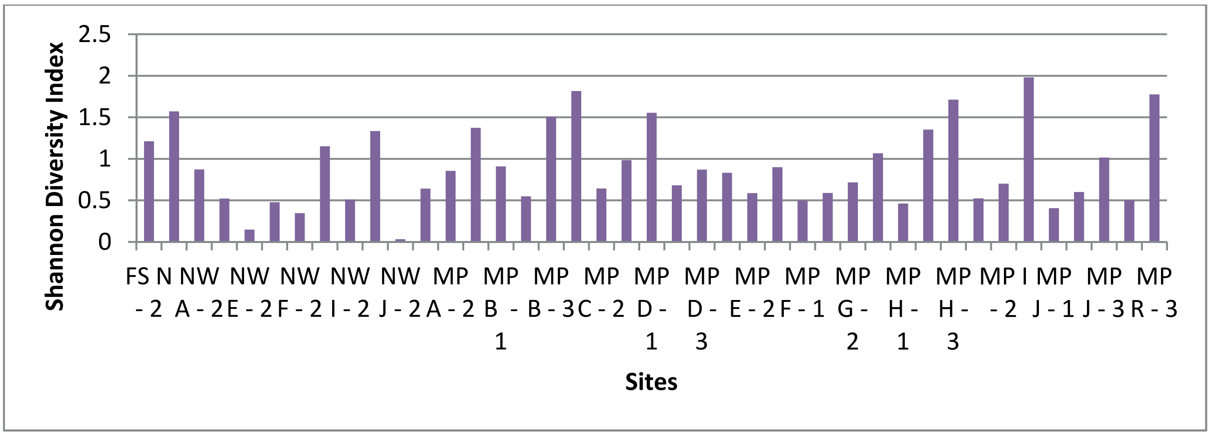


Figure 7-5: Shannon's diversity index for aquatic invertebrates sampled from each site during the various surveys over three seasons (1-winter, 2-summer and 3-autumn).

Results of the Bray-Curtis similarity analyses are shown in Figure 7.7 and 7.8. Three groups have been differentiated at 40% similarity in the hierarchical cluster plots according to province and sampling season (Figure 7.7 and 7.7) and the NMDS plot confirms these groupings at a stress level of 0.16. When the samples are separated based on province in which they were collected there is a clear trend that emerges (Figure 7.7 and 7.8). The greater part of all the sites have separated into three distinct clusters representing the three provinces with the two largest groupings formed by the sites from North West and

Mpumalanga. However, these groupings are formed at a very low similarity of between 20-30%. At a higher similarity percentage all of the sites start to group separately indicating the variation present. The ANOSIM analysis for significance indicated that no significance between provinces were found (Global R = 0.585).

When the samples are separated based on the sampling season in which they were sampled there is once again a clear trend that emerges (Figure 7.7 and 7.8). Three clusters have formed again to represent the three seasons in which samples were collected. However, ANOSIM analyses indicated that the sampling survey is not significantly different (Global R = 0.339). When looking at Figure 7.7 at a similarity of 40% it can be seen that the sampling survey during autumn in Mpumalanga grouped separately while the majority of pans from the North West during the summer survey grouped separately from the other pans. The Mpumalanga, Free State and North West pans during the winter survey grouped together. This grouping at 40% similarity was also not significant (Global R = 0.775) according to the ANOSIM analysis.

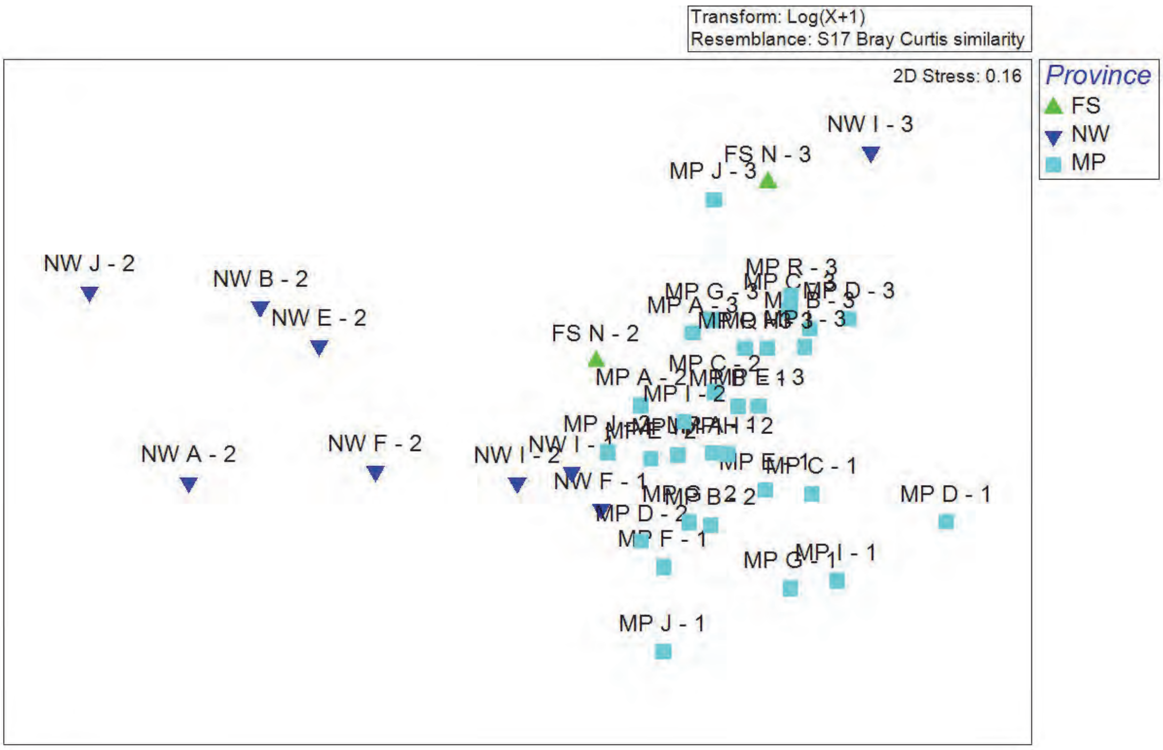


Figure 7-6: Bray-Curtis similarity matrix-based two dimensional representation of the NMDS ordination of the aquatic invertebrates sampled from Free State (FS), North West (NW) and Mpumalanga (MP) provinces.

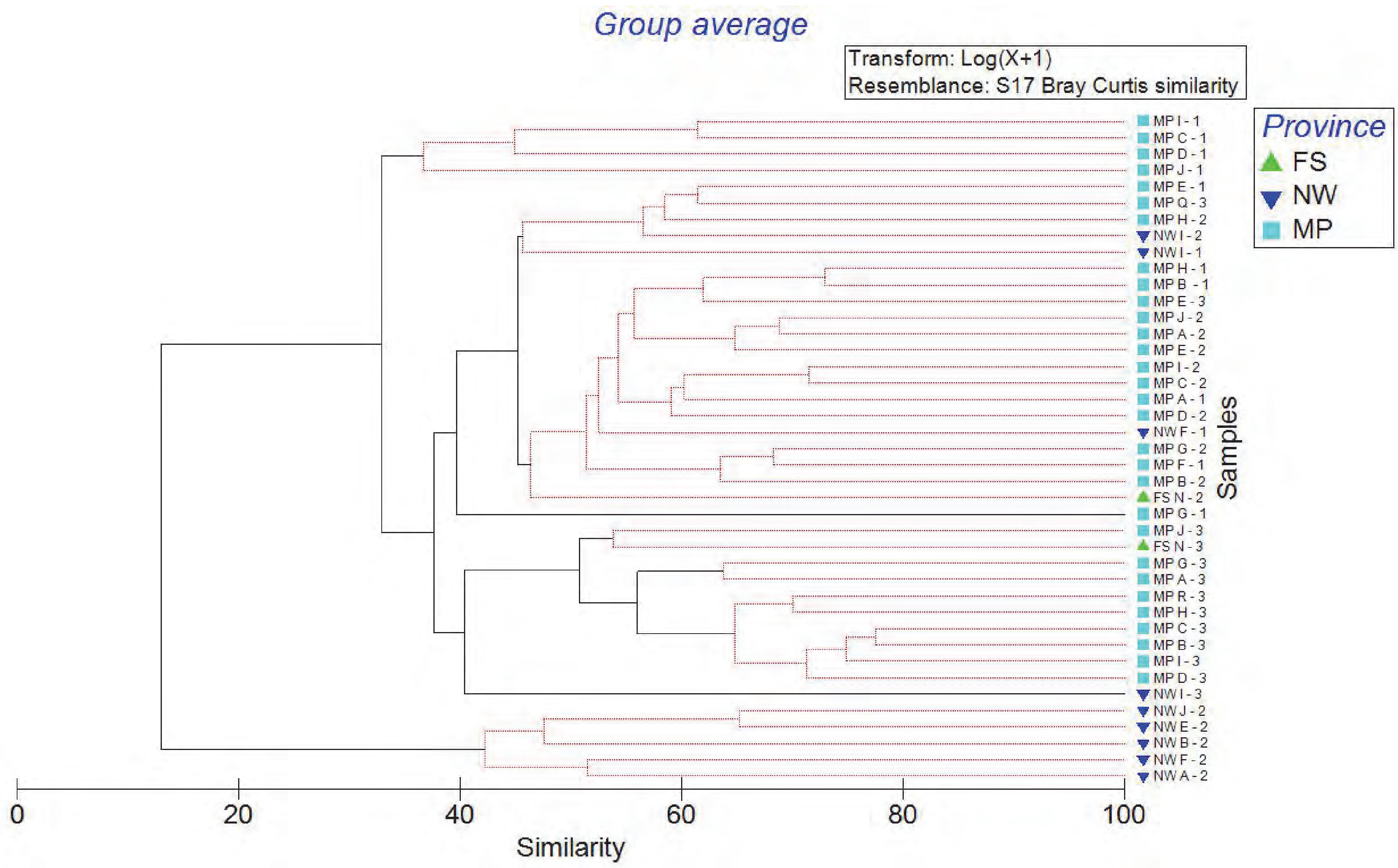


Figure 7-7: Hierarchical cluster based on the Bray Curtis similarity matrix representation of the NMDS ordination of the aquatic invertebrates sampled from Free State (FS), North West (NW) and Mpumalanga (MP) provinces

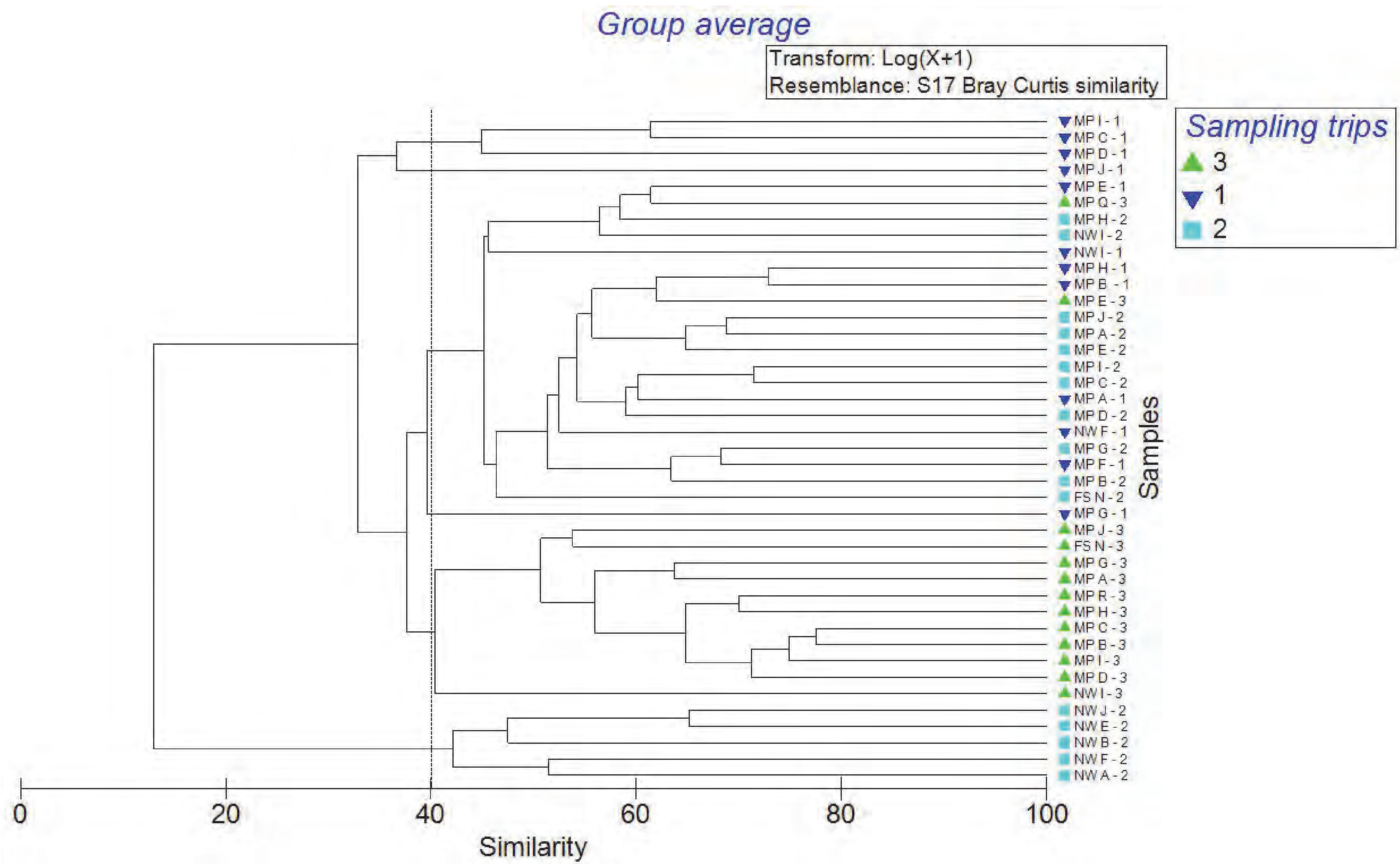


Figure 7-8: Bray-Curtis similarity matrix-based (A) cluster analysis and (B) two dimensional representation of the NMDS ordination of the aquatic invertebrates sampled during winter (sampling trip 1), summer (sampling trip 2) and autumn (sampling trip 3).

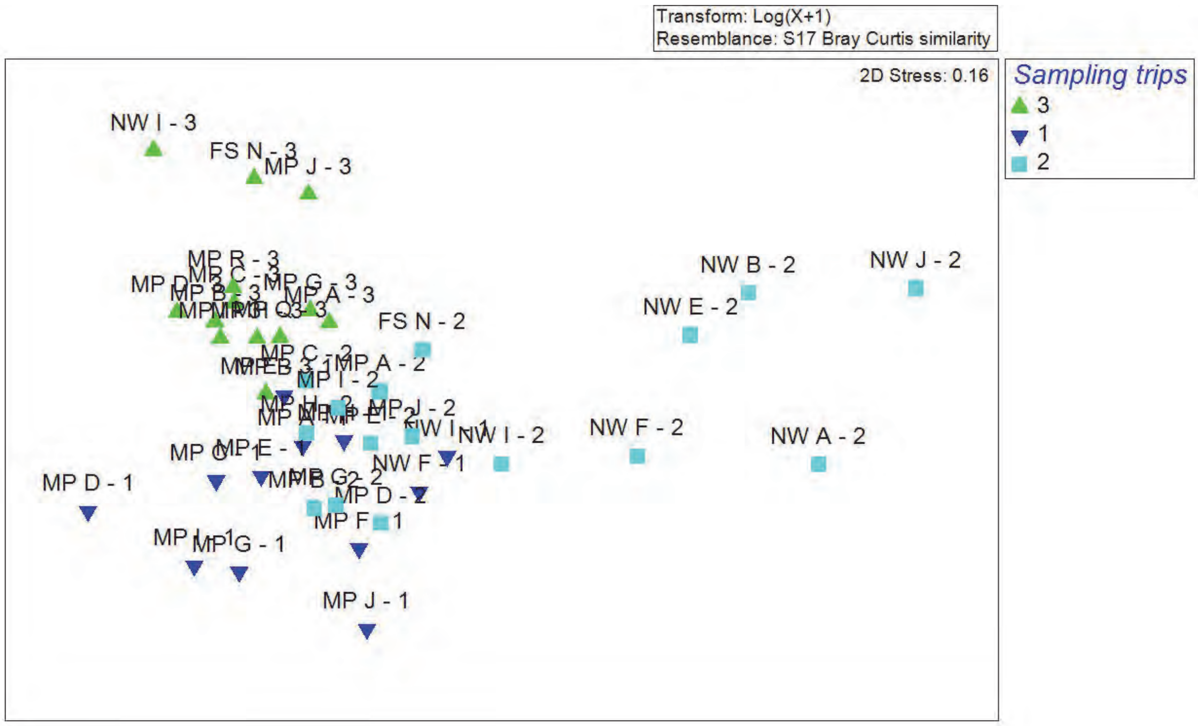


Figure 7-9: Bray-Curtis similarity matrix-based (A) cluster analysis and (B) two dimensional representation of the NMDS ordination of the aquatic invertebrates sampled during winter (sampling trip 1), summer (sampling trip 2) and autumn (sampling trip 3).

The RDA tri-plot in Figure 7.10 describes the similarities and dissimilarities between the various sites, invertebrate communities and water quality parameters. Water quality variables have been overlaid onto the invertebrate groupings. This tri-plot explains 28.18% of all variation with 18.67% explained on the first axis and 9.51% on the second axis. On the first axis sites have separated with water parameters and invertebrate communities as driving factors. Temperature is a major influence in the separation seen here as most of the Mpumalanga sites from the autumn sampling period have grouped together on the right and on the left most of the North West sites sampled during the summer period have clustered together due to three species that were only sampled in this region. Water parameters have in turn caused the separation based on invertebrate structure with more invertebrate diversity and abundance occurring on the right of the axis than on the left. On the second axis separation has occurred based on invertebrate community structure and water parameters. Sites below the second axis have different invertebrate community structures than the sites above the second axis and this is due to different water parameters especially the difference in salt content.

The Mpumalanga sites have separated into two groups based on seasonal differences. The majority of sites clustered in the lower right section of the tri-plot were sampled during while the sites clustered in the upper left section of the tri-plot were sampled during winter and summer. The North West sites have mostly clustered in the lower left section due to similar invertebrate communities as well as temperature and water parameters. These were the only sites in which *Artemiidae*, *Simnocephalus sp.* and *Branchiopodosis sp* were found.

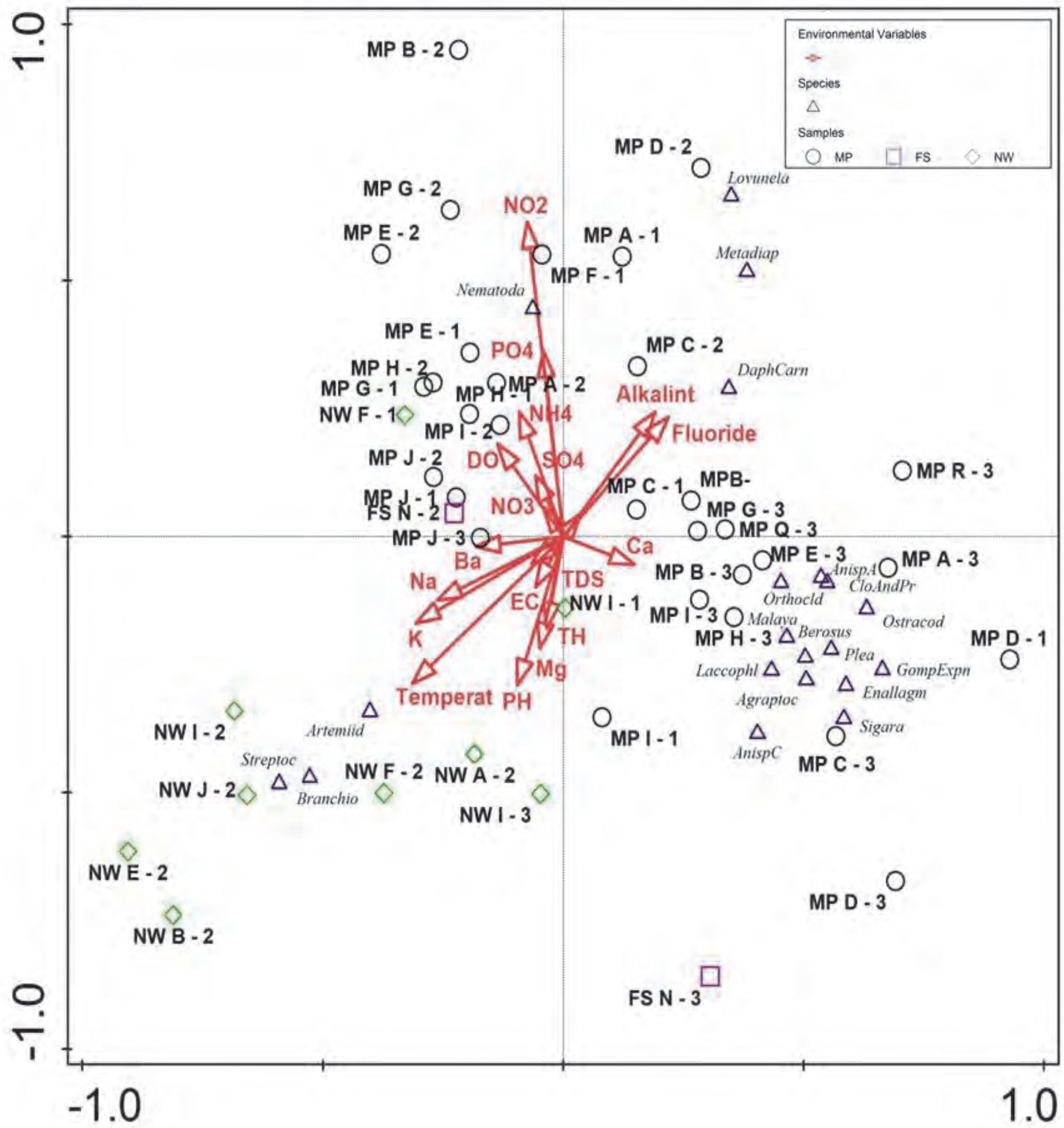


Figure 7-10: RDA tri-plot illustrating relationship between the three sampling trips, aquatic invertebrates and chemical water variables. The tri-plot explains 28.18 % of all variation with 18.67% explained on the first axis and 9.51% on the second axis.

The RDA tri-plot in Figure 7.11 describes the similarities between the Free State and North West sites, the invertebrate communities and water quality parameters. This tri-plot explains 56.53% of the variation seen in the data with 32.56% explained on the first axis and 23.97% on the second axis. Separation on the first axis has occurred based mostly on invertebrate community structure as well as, to a lesser extent, on water composition. Magnesium, calcium and total hardness are major drivers on the first axis and indicate that the sites on the right of the first axis consist of chemically harder water than the sites on the left of the axis. This could be the reason as to why there is such a difference in invertebrate community composition between the right and left of the first axis. The Free State sites have not really separated from the North West sites and the tri-plot indicates this is due to similar water parameters and invertebrate community structures. On the second axis separation has occurred based mostly on season and water composition with temperature and phosphates acting as major driving factors. The North West sites have separated into two groups i.e. the summer sampling period while most of the sites above the axis were sampled during the winter period. This difference in temperature has caused the difference in invertebrate community structure that is seen between the upper and lower sections of the second axis.

The RDA tri-plot in Figure 7.12 indicates the relationship between the three seasons during which sampling took place in Mpumalanga. The tri-plot explains 35.43% of all the variation with 25.52% explained on the first axis and 12.91% explained on the second axis. On the first axis separation has occurred mostly due to invertebrate community structure. Seasonal separation in invertebrate communities is clearly seen on this axis as on the right of the axis most of the sites sampled during the autumn season have clustered together with a different invertebrate assemblage than the sites sampled in the summer and winter periods on the left. These differences in invertebrate assemblages may be due to water parameters as species have different water quality and temperature tolerances and preferences. On the second axis it is clear that separation has occurred based mostly on water parameters with temperature acting as a major driving factor. Most of the sites above the axis were sampled during the winter season (MP D-1, MP C-1, MP I-1, MP J-1, MP E-1 and MP H-1) while the rest of the sites were sampled during either autumn or summer when the water temperature would be much warmer.

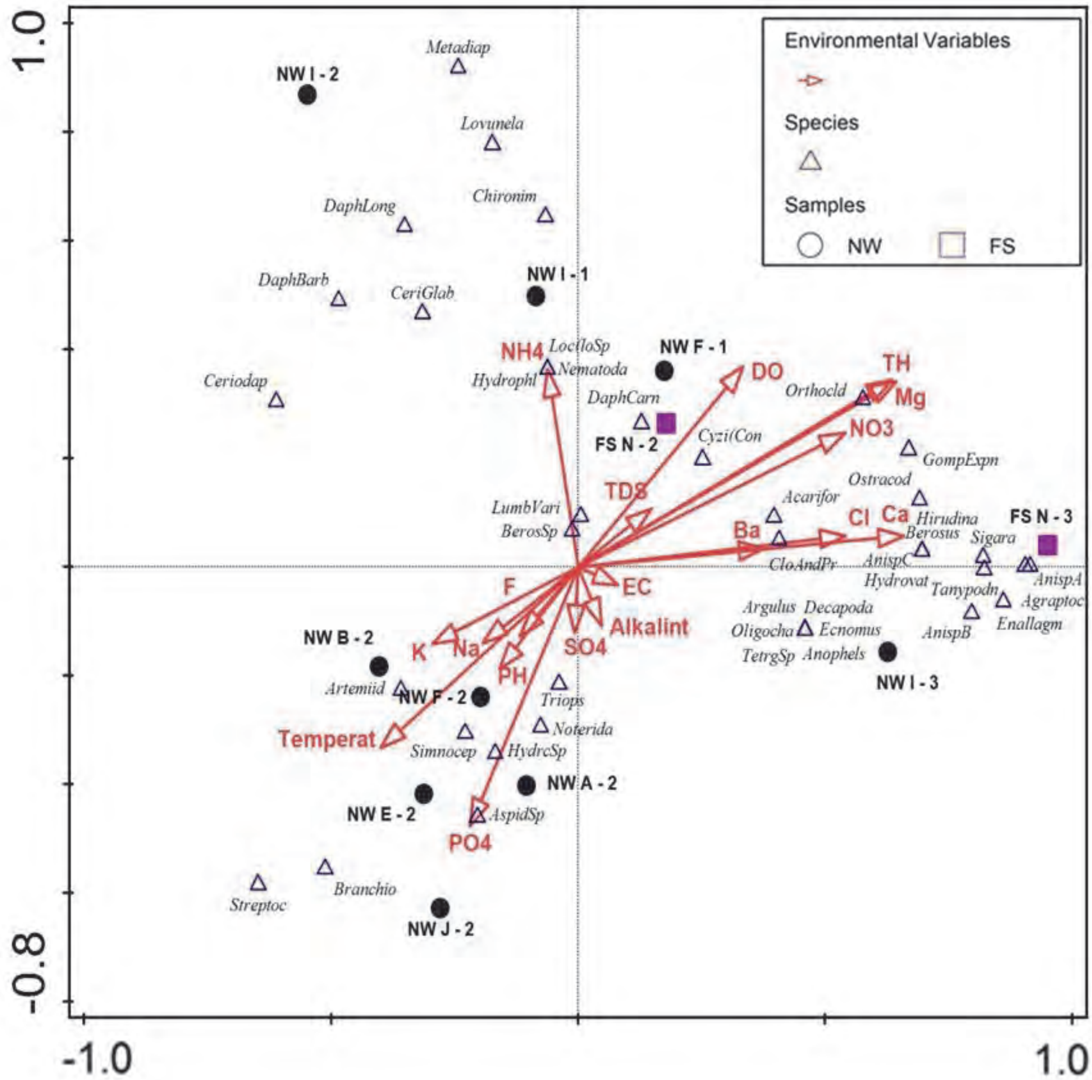


Figure 7-11: RDA tri-plot illustrating similarities between the Free State and North West, aquatic invertebrates and chemical water variables. The tri-plot explains 56.53 % of all variation with 32.56% explained on the first axis and 23.97% on the second axis.

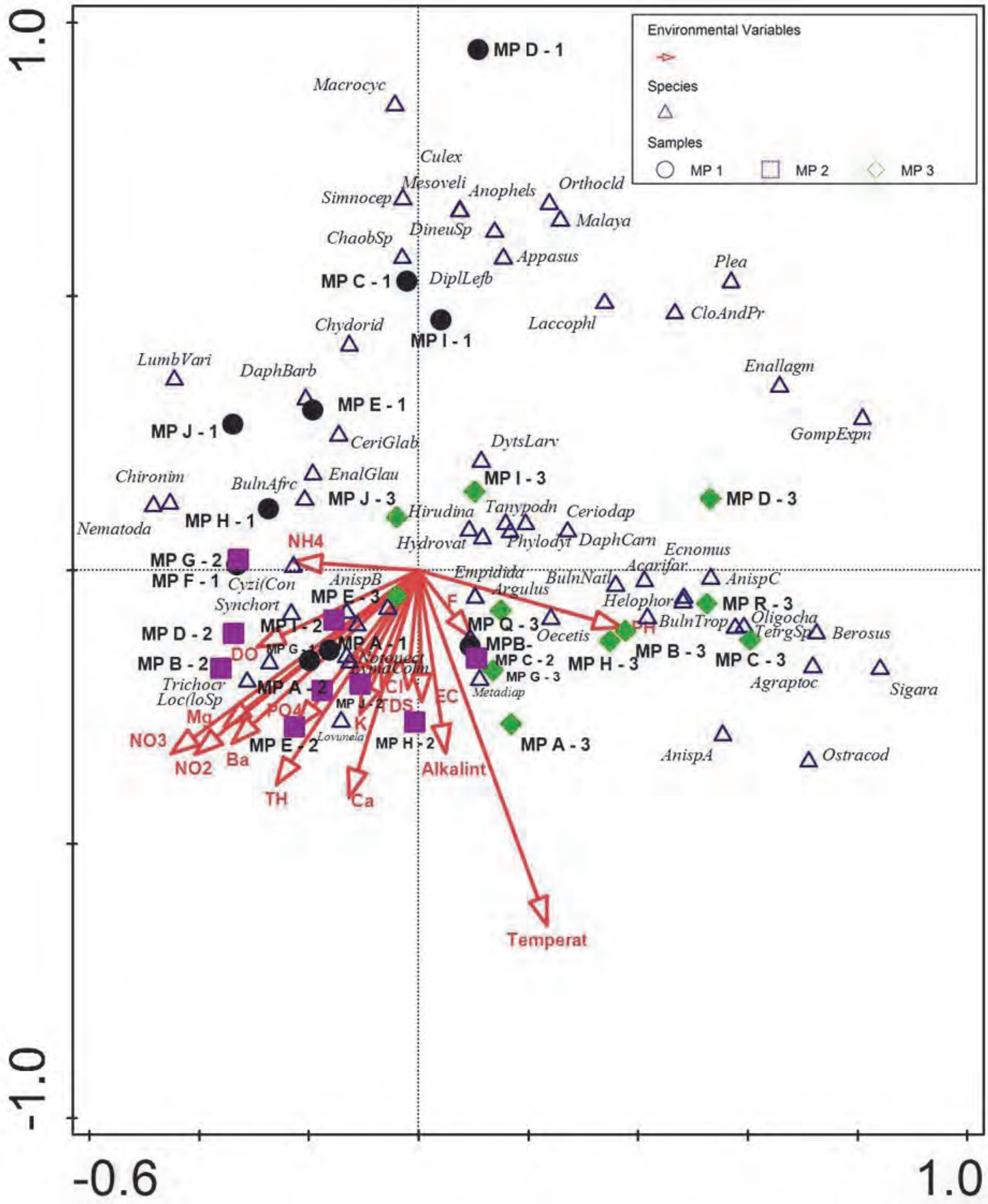


Figure 7-12: RDA tri-plot illustrating similarities between the three seasons of sampling from Mpumalanga sites. This tri-plot explains 36.43% of all variation with 23.52% explained on the first axis and 12.91% on the second axis.

7.4 Discussion

Aquatic organisms in South Africa have had to adapt to highly variable daily and seasonal fluctuations as South Africa is classified as a semi-arid country with uneven rainfall distribution that is both unpredictable and variable (DWAF, 1994; Minshall *et al.,* 1985). Many environmental factors naturally affect invertebrate distributions including geography, habitat size, depth, permanence, food availability and chemical factors (Hutchinson *et al.,* 1932; Ferreira, 2010). Change in water temperature is considered to be one of the more important driving factors for seasonal variation in aquatic invertebrate community structure and function (Eady *et al.,* 2013). Studies on freshwater and saline lakes in South Africa have however indicated that there is no distinct separation between invertebrate communities found in saline and freshwater environments. Many times highly tolerant species of freshwater taxa such as *Lovunela sp.* and *Metadiaptomus sp.* will be present in saline environments (F Seaman *et al.,* 1991; Ferreira, 2010). Pans are considered to be the least diverse wetlands and temporary bodies of water when compared to other similar sized systems across the world (Lake *et al.,* 1989; Timms and Boulton, 2001). However when taking into account their harsh physico-chemical environments, short inundation periods and small size pans are surprisingly diverse (Hutchinson *et al.,* 1932).

The different univariate statistical analyses indicate seasonal variation and this is expected as variation in number of taxa, number of aquatic invertebrates, species richness, evenness distribution and diversity is expected and a common phenomenon as both temperature and rainfall fluctuate seasonally (Water and Rivers Commission, 2001) and change in invertebrate communities because of this has been well studied in many rivers in Mpumalanga (Dallas, 2004; Ferreira, 2009). It is therefore not unexpected to have seen variation in invertebrate community structure between the three seasons during which sampling took place. In all provinces the greatest variety of taxa and number of aquatic invertebrates occurred during the colder periods specifically during the autumn survey which is to be expected as rainfall, particularly in Mpumalanga, occurs during the summer period and so by autumn the pans are at their fullest (Ferreira, 2010). As the seasons progress less rain falls from autumn to spring and so evaporation causes the pans to lose water until many of them are dry by the end of spring. The loss of water also concentrates salts, which has been known to negatively impact aquatic species diversity and biomass (Hammer, 1986) and so as water levels decrease across the seasons so does abundance and number of aquatic invertebrates present in the pans.

Spatial variation between the three provinces is also indicated by the univariate statistical analyses. Mpumalanga pans had the most taxa, number of aquatic invertebrates, species richness, evenness and diversity. This was expected as Mpumalanga is a sub-tropical region with more rainfall and less evaporation than the other provinces in this study, meaning that pans in Mpumalanga are inundated for longer periods of time and studies have shown that longer inundation not only has an additive effect on taxa and number of aquatic invertebrates (Studinski and Grubbs, 2007) but leads to colonisation by species that are not adapted to temporary conditions (Schneider and Frost, 1996).

The North West sites have the least taxa and number of aquatic invertebrates while some of the sites had a large number of aquatic invertebrates but lacked evenness indicating a dominance of certain species at these sites. The North West is an extremely dry region with very little rainfall and high evaporation and so pans contain water for much shorter periods of time than the other provinces. This prevents colonisation by a greater variety of taxa and only species that have developed mechanisms to survive in the harsh and extremely temporary conditions will be successful and thus present (Meintjes *et al.,* 1994). It is difficult to compare the Free State to the other provinces as only one site was sampled in the Free State but from the single site it can be inferred that the Free State pans may not have the abundance and diversity of Mpumalanga but also does not carry the low numbers seen in the North West. The Free State had a relatively low number of taxa and a low number of individuals and this may be due to salinity as pans with greater salinity will have more specialised invertebrate species that are tolerant to salts (Wolfram *et al.,* 1999).

Using the Bray-Curtis matrix based on 40% similarity the sites separated distinctly into two large groups representing the North West and Mpumalanga provinces and a smaller grouping representing the Free State (Figure 7.6). It is difficult to compare the Free State to the other two provinces as only one site was sampled in the province. The separation is based on dis/similarities in invertebrate community structure and it is clear that the majority of sites from each province had similar invertebrate species. This may be attributed to similar environmental factors such as climate in each of the provinces that affects the assemblages of invertebrate species. As stated before Mpumalanga is a sub-tropical region whilst the North West is more arid, therefore water composition and thus invertebrate assemblages are expected to differ between the provinces. The second Bray-Curtis matrix indicates a separation has occurred according to season in which aquatic invertebrates were sampled. A pattern does emerge as most of the sites sampled during the same season have clustered together. This may be attributed to similar seasonal variation as water temperature as well as seasonal water availability and abundance affects invertebrate community structure and function.

The invertebrate community appears to be different between seasons (first axis) as well as provinces (second axis) in the RDA tri-plot (Figure 7.10). Both Mpumalanga and the Free State have separated into autumn groups on the right and winter and summer group on the left and the North West sites have separated from the other provinces on the second axis due to water quality and invertebrate communities. The seasonal separation on the first axis may be due to water availability and abundance as the pans have more water during autumn which allows a wider diversity of aquatic invertebrates to establish whilst in the summer and winter periods pans have less water or are desiccated and so do not carry as many aquatic invertebrates. Water temperature was also a driving factor for the seasonal separation in terms of invertebrate community differences. Eady *et al.* (2013) found that total taxon richness increased from winter to autumn between streams in the Western and Eastern Cape and Dallas (2004) found that taxa richness varied spatially due to temperature differences associated with latitude and climate for example between the Western Cape which is temperate and Mpumalanga which is sub-tropical. Ferreira (2010) also found similar seasonal variations in invertebrate communities from pans in Mpumalanga. The RDA tri-plot indicates that the North West has separated from the Mpumalanga pans due to temperature, salts as well as aquatic invertebrates. The temperature difference is due to seasonal variation as the North West sites that are grouped together were mostly sampled during summer and so water temperature is much higher, particularly in warm, arid areas such as the North West. Salts have also contributed to the separation as pans in arid areas such as the North West are more ephemeral while pans in areas such as Mpumalanga are more permanent and pans that are more permanent are known to be less saline (Ferreira, 2010). Finally, the three invertebrate species that were only found in the North West also contributed to the separation as these species prefer more saline water, specifically *Artemiidae* which are commonly known as brine shrimp and prefer extreme saline conditions (Day *et al.,* 1999), and as explained before the North West sites are more saline than the Mpumalanga sites.

The Free State and North West have not separated from one another in the RDA tri-plot (Figure 7.11). This may be due to similar climate that affects the pans in similar ways as both these provinces are semi-arid regions. Separation in this tri-plot has occurred based on water composition and aquatic invertebrates on the first axis and season and water composition on the second axis. On the first axis Cl, Mg, Ca and total hardness indicate that the sites on the right of the axis may be chemically harder than sites on the left which may have influenced invertebrate assemblages as well. In the presence of chlorides, alkalinity and hardness there is a reduction in taxon richness as more sensitive species do not occur and an increase in density by the more tolerant taxa which leads to decreased invertebrate diversity (Buss *et al.,* 2002). This could be seen as on the right many of the species that were found in these sites are more tolerant and hardy species. These include *Tanypodinae* (Family Chironomidae) and Class Oligochaeta and are known to be highly tolerant species capable of living in highly polluted and degraded systems (Buss *et al.,* 2002; Gerber and Gabriel, 2002). Other tolerant species found in these sites in high abundance were *Gomphocythere expansa* and *Anisops sp.* (Day *et al.,* 2010; Gerber and Gabriel, 2002). Other important drivers included phosphates and salts. Increased phosphates may be attributed to surface runoff as it is not present at high levels in ground water (a major source of water for pans) while increased salts may indicate that these particular pans are in the process of drying up and so there is concentration of solutes in the water (Dallas and Day, 2004; Russell, 2008). Increased salinity in these pans may also be cause for the presence of Artemiidae (brine shrimp) in NW B-2 and NW E-2 and none of the other sites.

In Mpumalanga the three seasons in which sampling took place have separated from one another on the RDA tri-plot (Figure 7.12). Separation between the three seasons is due to water quality, quantity, temperature and invertebrate community assemblage. On the first axis groupings are due to differences in invertebrate communities between the sites. On the right of the axis all the autumn sites have clustered together while the summer and winter sites are separated into two groups on the left. As explained before autumn is the season in which pans are at their fullest as summer is the rainy season which starts to fill pans up and winter is the dry season. This may explain the differences seen in water quality as well as invertebrate communities as in the autumn a greater diversity of various invertebrate species were found which include species less adapted to dry conditions such as Oligochaetes. These aquatic invertebrates, although hardy and cosmopolitan, cannot tolerate dry conditions and so will only occur in pans that have been inundated for an extended period of time (Day *et al*., 1999) such as would occur during the period from summer to autumn. Water quality parameters do affect the sites here as well as on the left sites are more affected by salts and nutrients, specifically sites sampled during the summer period. This may be due to a concentration of nutrients and salts in summer when there is little water in which these constituents can dissolve whereas in the autumn there is more water and constituents are more dilute. Exhaustion of resources during the winter period may be the reason as to why fewer nutrients are present and also explain less diversity and abundance of aquatic invertebrates as species need resources to survive. On the second axis the same pattern is seen as on the first axis. Below the axis the sites contain more concentrated nutrients and salts. Temperature has a more important role on the second axis as during the summer and autumn seasons' water temperature was warmer than during the winter season. This difference in water temperature has most likely also caused the difference in invertebrate community structure between the warmer seasons (summer and autumn) and the colder season (winter).

7.5 Conclusion

After analysis of all parameters of the various sites it is clear that pans vary between both sites and seasons. This variation is caused by a number of natural factors such as climate, rainfall, temperature and inundation period. During autumn, pans are at their fullest and a greater diversity and abundance of species is found as the pans stay inundated for longer periods of time. Pans that are inundated for extended periods of time tend to have greater diversity and abundance of invertebrate communities, some of which are not necessarily permanent residents of pans or specifically adapted to living in temporary or saline environments. This is observable by the presence of Oligochaetes, which cannot tolerate drought, at several of the sites.

The objective was to determine spatial and temporal variation between sites from different provinces as well as between seasons. Results of water quality and invertebrate analysis indicate that both spatial and temporal variation is indeed present and is caused by natural variations in season and climate. Spatial variation between the three provinces was seen most prominently in the invertebrate communities and was affected by climate. The sub-tropical region of Mpumalanga had greater diversity and abundance of aquatic invertebrates when compared to the drier arid regions of the North West and Free State. Temporal or seasonal variation between sites was mostly seen in water quality parameters which also affected the community structure of invertebrate species. This was observable between seasons in Mpumalanga where the warmer seasons of summer and autumn had greater diversity and abundance of invertebrate species along than the colder season of winter with water temperature as the driving factor. The first section of the hypothesis that spatial variation exists amongst pans between different provinces is proven to be true and the second section of the hypothesis that seasonal variation is present amongst pans from each province is also proven to be true.

8 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 General Discussion

Temporary wetlands are highly variable ecosystems and undergo changes in physical and chemical characteristics on a regular basis. The invertebrate fauna that inhabit these systems are highly adapted to survive these constant changing environments. A particularly important group of fauna that inhabit these ecosystems are the Branchiopoda. The class Branchiopoda, descriptively termed phyllopods, consist of the orders Anostraca (fairy and brine shrimp), Notostraca (tadpole shrimp), Conchostraca (clam shrimp) and Cladocera (water fleas). Branchiopods and a few other zooplankton taxa make use of the escape in time survival strategy, achieved through the production of an egg bank.

Branchiopod diversity and successional patterns could be determined from small amounts of sediment. Abundances obtained here are comparable to other studies assessing the hatching success of zooplankton communities. The North West and Free State provinces in particularly had high cumulative abundances. The Mpumalanga pans had the lowest abundance of hatchlings emerging from the sediment. This was not an unusual result as pans sampled in the North West and Free State provinces were largely ephemeral in nature, while those sampled in the Mpumalanga Province were of a perennial nature. The patterns of hatching found during the study, throughout all three provinces, were similar to in situ patterns of pan succession observed in other studies. Anostracans were generally the first group of crustaceans to be identified after inundation followed by the Cladocera. The Ostracoda and Conchostraca were the last group to be identified. It is not unusual to find a single representative of Branchiopoda in a pan. The data will prove very useful in future studies and monitoring of these pans. Baseline biodiversity of pan communities can effectively be obtained through egg bank analysis. The diversity of pan communities was different between pans and between regions for the larger part of the pans studied. From this it seems that pans have their own unique communities, and each one contributes towards the regional diversity.

Recovery experiments were performed after the initial hatching experiments on the sediment exposed to AMD only. The hatching of branchiopod crustaceans was inhibited by the presence of AMD. An explanation for eggs not hatching in the presence of acid mine drainage is that AMD has a high concentration of mineral salts (consisting of toxic metals) and a low pH. The high concentration of mineral salts increases the osmotic pressure of the water. Should enough water pass through the tertiary membrane into the egg metabolic processes within the metanauplius will be activated. Should the metabolism be activated glycerol will start building up inside the egg, creating an osmotic gradient for more water to pass into the cyst. This water build up creates an osmotic pressure inside the egg which results in the bursting of the outer membrane enabling hatching.

It was also demonstrated that the recovery of these aquatic invertebrates after AMD exposure was low. When compared to the diversity of aquatic invertebrates obtained from the controls it could clearly be seen that AMD altered the community structure of the branchiopods which recovered. The diversity of individuals was much lower as a result of the AMD. This shows how poorly the community will respond to the removal of this stressor. A possible factor that could have played a role in the survival of eggs is the type of dormancy. Diapausing eggs may be more tolerant to stressors than quiescent eggs as they rely on internal conditions to hatch regardless of external conditions being favourable. The diapausing eggs of copepods have been found to be less sensitive to metal pollution due to the thick chorion membrane surrounding it. Quiescent eggs relying directly on external conditions could possibly be stimulated to hatch by favourable light and temperature conditions, but conditions such as pH and conductivity which were likely unfavourable could counteract this and inhibit hatching. In this regard diapausing eggs can lie dormant for longer without external factors interfering in the hatching process. The effect that low pH has on hatching has to do with the optimal functioning of the hatching enzyme. The enzyme is secreted by the metanauplii allowing it to break free of the inner membrane, the final membrane that has to be broken through allowing the release of the free-swimming nauplii. The low pH of the AMD may have denatured this enzyme which would prevent hatching even if the metanauplii were successful in bursting through the outer membrane. Even though recovery did take place in a few pans, the number of individuals may be too low to replace the number of eggs affected by the AMD. The buffering capacity of the egg bank will be lost, and the egg bank will eventually deplete itself in inundations to come. Species extinctions as a result are inevitable, which raises the concern that wetlands impacted to such an extent by such a stressor may be beyond rehabilitation.

Pan ecosystems are some of the most variable systems in South Africa. Their endorheic nature and the resultant variability make comparison to other wetlands and even other pans problematic. To determine the spatial and temporal variation in the physico-chemical characteristics of the water from the various pans in the Mpumalanga, North West and Free State provinces, surface water samples were collected during each of the surveys. The water analysis included nutrients, salts and metals in addition to *in* situ variables. Large variability was observed in the nutrient and salt concentrations of the selected study sites. This variability observed on a spatial scale was expected. The climate and rainfall varies between the different provinces. The pans from Mpumalanga generally have a more stable hydroperiod when compared to pans in the North West and Free State. This was evident in this study as most of the pans in Mpumalanga had water during both surveys while only on the second survey water was present in North West. No water was present in the Free State although in previous seasons water was present throughout a whole year indicating the importance of rainfall in the pan catchment for filling of the pans. The more perennial a pan is the more stable the physico chemical variables will be and vice versa for pans that are more ephemeral. The EC, for example, ranged between 0.19 Ms/cm and 9.06 Ms/cm in Mpumalanga. In comparison the EC in the Free State and North West provinces ranged from 0.81 Ms/cm to 110.56 Ms/cm.

In addition to the spatial variability, large temporal variability in water quality characteristics was also observed. The current study did initially attempt to classify the pans from Mpumalanga, Free State and North West based on their salinity and nutrient data based on the Hutchinson *et al.* (1932). However, as was seen in the statistical analysis no real classification or different groups were identified based on the water quality. The classification of pans based on their water quality variables were first attempted by Hutchinson *et al*. in 1932 on various pans in the Lake Chrissie area. Since then a few studies (Ferreira, 2010) have attempted to classify pans based on their water quality as well as their biological communities. However, no one method has really been successful. This is due to the inherent variability within these pan systems. The dataset generated in this project was used to create ranges for especially the nutrient and salinity variables for pans in the three provinces. The ranges provides a way to interpret data from impacted pans to determine if it falls within the natural or near natural ranges identified in this project.

8.2 Conclusions

It can be concluded that Branchiopoda can successfully be hatched from sediment collected *in situ* under controlled laboratory conditions. The hatching patterns are also closely related to patterns observed during in situ studies. This clearly indicates that egg bank analysis can be used as a monitoring tool and can aid in the determination of diversity when pans are desiccated. This becomes essential in any impact assessment. It is further concluded that AMD has a negative impact on the egg banks within pans and causes a loss of biodiversity. Ultimately this demonstrates how AMD will degrade these unique environments should its disposal not be properly managed and should mining activities continue to encroach upon the vicinity of these wetlands. It is critical that the integrity of these ecosystems be maintained for all that depend on them.

Due to the inherent variability within endorheic wetlands, attempts to classify these systems according to trophic state or salinities have been unsuccessful. However, water quality ranges for the nutrient and salinity have been established for pans from Mpumalanga, North West and Free State. The variability observed in water quality was also observed in the invertebrate communities that inhabit these ecosystems. There was large spatial and temporal variation in biodiversity when comparing the communities from the various sites selected in the three provinces.

8.3 Recommendations

From chapter 3 and 4 it was evident that a number of variables may have a potential impact on the hatching success of egg banks from sediment collected in situ. It is therefore recommended that further studies be completed to refine the methodology for hatching experiments as egg banks can be an important consideration in biodiversity studies and impacts assessments involving pans. It will also be important to complete future studies which compare the hatching success from egg banks collected from impacted sites.

From chapter 5 it was evident the AMD has a definite effect on the hatching success of Branchipoda and that this effect may be permanent. During the current study the effect of AMD was studied, but no dilution series was made for the AMD medium. This will allow the assessment of relevant end-points and further research is therefore recommended.

From Chapter 6 it was evident that further research is required with regards to the water quality of pans and the possible classification of pans based on physico-chemical characteristics. Studies completed on the perennial pans of Mpumalanga produced similar results. It has become evident that the variability in water quality is of such an extent that each pan could be considered unique. This has major implications for water resource management and the protection of the ecosystems.

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APPENDIX A:

Table 9-1: In situ, nutrient and salt variables for Mpumalanga pans MPpan A to MPpan G for the May 2012 survey.

Analysis	Unit	DL*	MPpanA	MPpanB	MPpanC	MPpanD	MPpanE	MPpanF	MPpanG
pH	-	-	8.99	8.99	9.07	9.26	8.93	8.62	8.55
Temperature	°C	-	19.2	19.9	18.2	10.3	12.5	15.5	16
EC	Ms	-	4.55	4.29	3.77	3.8	2.49	1.641	2.1
TDS	ppm	-	3550	3140	2500	2610	1650	1092	1400
Oxygen saturation	%	-	95.4	89.9	110.1	59.6	86.8	89.7	89.9
Oxygen content	mg/l	-	8.9	8.47	10.2	6.43	7.88	8.99	8.86
Alkalinity	(mg/l)	0.1	1813.0	524.0	571.0	429.0	361.0	316.0	347.0
Ammonia	(mg/l)	0.01	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TH (CaCO3)	(mg/l)	0.1	170.0	123.0	63.0	64.0	59.0	88.0	98.0
Fluoride	(mg/l)	0.037	2.0	0.7	0.7	0.8	0.8	0.7	0.5
Chloride	(mg/l)	0.031	968.0	906.0	778.0	790.0	376.0	258.6	367.0
Nitrite	(mg/l)	0.07	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070
Nitrate	(mg/l)	0.07	4.1	<0.070	<0.070	<0.070	0.3	0.1	0.5
Phosphate	(mg/l)	0.065	10.1	0.1	4.4	<0.065	4.5	2.2	1.1
Sulphate	(mg/l)	0.053	4.4	36.5	189.0	105.6	189.3	96.5	75.5
Ca	(mg/l)	0.009	47.1	27.0	15.9	14.0	11.0	9.0	18.3
K	(mg/l)	0.007	134.2	84.5	27.3	36.3	39.0	16.0	33.8
Mg	(mg/l)	0.001	12.8	13.7	5.7	7.1	9.7	7.6	12.8
Na	(mg/l)	0.009	803.7	668.0	655.9	619.4	448.3	235.5	487.6

Table 9-2: Metal concentrations in water for Mpumalanga pans MPpanA to MPpanG for the May 2012 survey.

Analysis	Unit	DL*	MPpanA	MPpanB	MPpanC	MPpanD	MPpanE	MPpanF	MPpanG
Ag	(mg/l)	0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
As	(mg/l)	0.005	4.1	2.2	1.0	1.3	5.8	3.4	3.3
Al	(mg/l)	0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
B	(mg/l)	0.004	0.4	0.2	0.1	<0.004	0.1	0.1	0.1
Ba	(mg/l)	0.004	0.4	0.3	0.1	0.1	0.2	0.1	0.2
Cd	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Co	(mg/l)	0.009	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Cr	(mg/l)	0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Cu	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Fe	(mg/l)	0.004	4.6	2.8	1.2	0.7	4.6	4.0	3.2
Mn	(mg/l)	0.01	0.0	<0.01	<0.01	<0.01	0.1	0.0	0.0
Mo	(mg/l)	0.009	<0.009	<0.009	<0.009	<0.009	<0.009	<0.009	<0.009
Ni	(mg/l)	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Pb	(mg/l)	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010

Se	(mg/l)	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Si	(mg/l)	0.002	4.1	2.7	3.1	1.1	2.8	1.8	2.0
Sr	(mg/l)	0.004	0.4	0.4	0.1	0.2	0.1	0.1	0.2
Ti	(mg/l)	0.007	0.1	0.0	0.0	0.0	0.1	0.0	0.0
U	(mg/l)	0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
V	(mg/l)	0.003	0.1	0.0	0.0	<0.003	0.0	0.0	0.0
Zn	(mg/l)	0.01	0.0	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010

Table 9-3: In situ, nutrient and salt variables for Mpumalanga pans MPpanH to MPpanO during the May 2012 survey.

Analysis	Unit	DL	MPpanH	MPpanI	MPpanJ	MPpanK	MPpanL	MPpanM	MPpanO
pH	-	-	8.82	8.92	8.62	7.58	8.61	7.11	8.36
Temperature	°C	-	15.3	14.6	10	12.6	12.5	13.5	10
EC	Ms	-	2.7	2.15	0.1704	1845	4.57	0.81	0.1704
TDS	ppm	-	1900	1530	122.4	1500	3160	553	102.4
Oxygen saturation	%	-	85.6	94.6	73.9	55.7	92.4	49.9	81.3
Oxygen content	mg/l	-	8.48	1.52	8.41	5.88	9.82	4.8	8.73
Alkalinity	(mg/l)	0.1	415.0	273.0	45.0	236.0	918.0	151.0	18.0
Ammonia	(mg/l)	0.01	0.1	0.0	0.1	0.0	0.0	0.0	0.8
TH (CaCO3)	(mg/l)	0.1	138.0	55.0	33.0	139.0	90.0	49.0	23.0
Fluoride	(mg/l)	0.037	0.4	0.7	0.1	0.4	0.7	0.4	0.2
Chloride	(mg/l)	0.031	551.0	416.0	15.3	504.0	999.0	167.4	12.4
Nitrite	(mg/l)	0.07	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070
Nitrate	(mg/l)	0.07	0.2	0.3	0.4	0.2	0.1	<0.070	0.3
Phosphate	(mg/l)	0.065	2.9	3.6	<0.065	<0.065	5.3	<0.065	<0.065
Sulphate	(mg/l)	0.053	99.0	139.0	19.7	1.6	302.9	14.3	28.6
Ca	(mg/l)	0.009	27.0	12.9	6.6	19.4	19.3	8.7	4.2
K	(mg/l)	0.007	69.0	24.5	3.9	11.8	42.9	15.4	2.2
Mg	(mg/l)	0.001	17.2	5.6	4.1	22.5	10.2	6.7	3.1
Na	(mg/l)	0.009	738.6	478.6	9.3	278.6	775.9	98.9	7.2

Table 9-4: Metal concentrations in water for Mpumalanga pans MPpanH to MPpanO during the May 2012 survey.

Analysis	Unit	DL	MPpanH	MPpanI	MPpanJ	MPpanK	MPpanL	MPpanM	MPpanO
Ag	(mg/l)	0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
As	(mg/l)	0.005	1.5	4.8	0.1	0.0	4.7	0.0	0.2
Al	(mg/l)	0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
B	(mg/l)	0.004	0.1	0.0	<0.004	<0.004	0.1	<0.004	<0.004
Ba	(mg/l)	0.004	0.3	0.3	0.1	0.3	0.2	0.3	0.1
Cd	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Co	(mg/l)	0.009	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Cr	(mg/l)	0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Cu	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005

Fe	(mg/l)	0.004	2.0	4.8	0.4	3.7	6.8	2.1	0.7
Mn	(mg/l)	0.01	<0.01	0.1	<0.01	0.5	0.1	0.1	<0.01
Mo	(mg/l)	0.009	<0.009	<0.009	<0.009	<0.009	<0.009	<0.009	<0.009
Ni	(mg/l)	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Pb	(mg/l)	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Se	(mg/l)	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Si	(mg/l)	0.002	2.0	2.1	0.1	0.4	3.6	0.5	0.1
Sr	(mg/l)	0.004	0.3	0.2	0.1	0.3	0.2	0.1	0.0
Ti	(mg/l)	0.007	0.0	0.0	<0.007	<0.007	0.0	<0.007	<0.007
U	(mg/l)	0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
V	(mg/l)	0.003	<0.003	0.0	<0.003	<0.003	0.0	<0.003	<0.003
Zn	(mg/l)	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010

Table 9-5: In situ, nutrient and salt variables for the North West pans that contained water during the May 2012 survey.

Analysis	Unit	DL	NWpanE	NWpanI
pH	-	-	9.79	9.2
Temperature	°C	-	22.3	15
EC	Ms	-	11.88	1.318
TDS	ppm	-	8530	903
Oxygen saturation	%	-	222	96.6
Oxygen content	mg/l	-	21.57	9.59
Alkalinity	(mg/l)	0.1	511.0	358.0
Ammonia	(mg/l)	0.01	0.0	0.2
TH (CaCO3)	(mg/l)	0.1	247.0	190.0
Fluoride	(mg/l)	0.037	0.5	0.4
Chloride	(mg/l)	0.031	1012.6	176.9
Nitrite	(mg/l)	0.07	<0.070	<0.070
Nitrate	(mg/l)	0.07	23.0	0.8
Phosphate	(mg/l)	0.065	<0.065	<0.065
Sulphate	(mg/l)	0.053	368.1	20.0
Ca	(mg/l)	0.009	11.4	14.9
K	(mg/l)	0.007	121.9	30.3
Mg	(mg/l)	0.001	53.4	37.2
Na	(mg/l)	0.009	1859.5	121.2

Table 9-6: Metal concentrations for the North West pans that contained water during the May 2012 survey.

Analysis	Unit	DL	NWpanE	NWpanI
Ag	(mg/l)	0.004	<0.004	<0.004
As	(mg/l)	0.005	0.1	0.2
Al	(mg/l)	0.003	<0.003	<0.003

B	(mg/l)	0.004	1.7	0.1
Ba	(mg/l)	0.004	0.1	0.0
Cd	(mg/l)	0.005	<0.005	<0.005
Co	(mg/l)	0.009	<0.005	<0.005
Cr	(mg/l)	0.006	<0.006	<0.006
Cu	(mg/l)	0.005	<0.005	<0.005
Fe	(mg/l)	0.004	0.1	0.1
Mn	(mg/l)	0.01	<0.01	<0.01
Mo	(mg/l)	0.009	0.0	<0.009
Ni	(mg/l)	0.01	<0.010	<0.010
Pb	(mg/l)	0.01	<0.010	<0.010
Se	(mg/l)	0.01	<0.010	<0.010
Si	(mg/l)	0.002	0.2	0.3
Sr	(mg/l)	0.004	0.8	0.1
Ti	(mg/l)	0.007	<0.007	0.0
U	(mg/l)	0.004	<0.004	<0.004
V	(mg/l)	0.003	0.0	0.0
Zn	(mg/l)	0.01	<0.010	<0.010

Table 9-7: In situ variables for the Free State pans for the December/Janurary 2012/2013 survey.

	Unit	FSpanK	FSpanL	FSpanM	FSpanN	FSpanO
EC	ms/cm	108.4	6.54	37.3	9.04	53.8
TDS	ppt	54.6	4.45	18.6	6.44	27.1
Temperature	°C	25.7	29.5	34.2	32.5	38.5
pH	-	7.93	8.93	9.57	8.92	8.84
Oxygen content	mg/l	5.91	8.08	6.28	7.38	5.01
Oxygen saturation	%	75.3	105.5	89.3	104.1	74
Secchi readings	cm	3-3	3-3	1-1	28-23	3-3
Chlorophyll a	µg/l	0.02	ND	24.75	0.35	ND

Table 9-8: In situ variables for Mpumalanga pans for the December/Janurary 2012/2013 survey.

	Unit	MPpanA	MPpanB	MPpanC	MPpanD	MPpanE	MPpanG	MPpanH	MPPanI	MPPanJ	MPPanK	MPPanO
EC	ms/cm	OL	9.06	5.06	6.02	2.8	2.3	3.52	2.96	0.249	2.88	0.212
TDS	g/l	OL	4.42	3.78	3.1	1.9	1.12	2.47	2.04	0.1068	2	0.135
Temperature	°C	24	23	28.2	22.3	24.5	21.8	24.5	26.6	20.6	25.7	21.9
pH	-	9.39	9.38	9.2	8.96	8.98	8.92	9.07	9.31	9.52	7.94	9.9
Oxygen content	mg/l	5.87	7.44	7.98	7.66	7.8	6.19	6.59	6.69	9.01	6.33	9.51
Oxygen saturation	%	0.689	0.888	1.05	0.895	0.91	0.698	0.844	0.796	0.989	0.824	1.131
Chlorophyll a	µg/l	8.9	3.62	0.49	1.63	0.02	3.26	0.2	0.17	0.67	0.15	36.55

Table 9-9: In situ variables for North West pans for the December/Janurary 2012/2013 survey.

	Unit	NWpanA	NWpanB	NWpanE	NWpanF	NWpanG	NWpanH	NWpanI	NWpanJ
EC	ms/cm	1.69	33	5.21	4.56	3.58	151.6	1.54	0.824
TDS	g/l	1.201	23	3.64	3	2.66	76.9	1.09	0.555
Temperature	°C	33.2	32.8	23.3	31.9	36.6	32.4	28.8	31.8
pH	-	10.08	9.32	9.57	8.95	9.17	9.2	9.14	9.11
Oxygen content	mg/l	7.93	5.9	6.59	6.92	8.04	5.2	7.25	6.35
Oxygen saturation	%	97.3	88	77.3	95.4	140.2	65.4	90.5	88.9
Secchi readings	cm	3-3	8-7	5-3	14-11	2-2	4-4	44-36	5-3
Chlorophyll a	µg/l	0.83	0.96	0.22	0.1	2.01	13.98	0.08	0.1

Table 9-10: Ions and nutrient data from the December 2012 survey of the water quality of the selected Mpumalanga pans.

Analysis	**Unit**	**DL**	**MPA**	**MPB**	**MPC**	**MPD**	**MPE**	**MPG**	**MPH**	**MPI**	**MPO**	**MPK**	**MPJ**
Alkalinity	(mg/l)	0.1	2200	1100	1000	800	480	440	600	420	48	368	64
Ammonia	(mg/l)	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	44.4	<0.01	<0.01	4.74	<0.01	<0.01
Total Hardness	(mg/l)	0.1	155	238	99	123	51	94	152	47	39	183	62

Fluoride	(mg/l)	0.037	0.35	0.8	1.28	1.13	1.32	0.65	0.76	1.04	0.2	0.54	0.32
Chloride	(mg/l)	0.031	7470	1940	1261	1369	418	388	678	525	17.28	701	21.98
Nitrite	(mg/l)	0.07	2.59	5.67	4.84	5.8	2.91	2.67	2.89	2.95	<0.070	0.23	0.14
Nitrate	(mg/l)	0.07	0.4	2.66	3	4.5	2.77	2.27	1.88	2.43	0.15	1.85	0.11
Phosphate	(mg/l)	0.065	17.1	12.3	7.79	1.43	5.11	1.68	4.33	4.92	<0.065	<0.065	<0.065
Sulphate	(mg/l)	0.053	4241	667	325	203.4	235	85.4	105.6	174	31.19	1.03	24.6
Ca	(mg/l)	0.009	38.125	58.571	27.255	27.231	12.893	20.027	32.636	14.613	7.843	24.631	12.163
K	(mg/l)	0.007	843.451	168.175	27.94	77.213	43.272	37.865	101.88	29.028	5.022	17.754	7.107
Mg	(mg/l)	0.001	14.488	22.384	7.377	13.293	4.47	10.625	17.224	2.674	4.767	29.72	7.581
Na	(mg/l)	0.009	7784.835	1665.845	1293.255	1258.065	711.547	607.731	761.732	687.247	11.582	641.936	18.005

Table 9-11: Metal concentrations in water samples from the Mpumalanga pans for the December 2012 survey.

Analysis	Unit	Detection	MPA	MPB	MPC	MPD	MPE	MPG	MPH	MPI	MPO	MPK	MPJ
Ag	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Al	(mg/l)	0.003	0.04	0.021	0.039	0.006	0.01	<0.003	0.019	0.018	0.047	0.032	0.006
As	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
B	(mg/l)	0.004	0.789	0.284	0.159	0.069	0.128	0.092	0.123	0.082	0.031	0.015	0.026
Ba	(mg/l)	0.004	0.617	0.411	0.101	0.322	0.152	0.208	0.277	0.166	0.219	0.536	0.261
Cd	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Co	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cr	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Cu	(mg/l)	0.005	0.06	0.021	0.015	<0.005	0.011	<0.005	<0.005	0.014	<0.005	<0.005	<0.005
Fe	(mg/l)	0.004	0.03	0.009	0.047	0.016	0.024	0.007	0.008	0.012	0.981	2.133	0.03
Mn	(mg/l)	0.001	0.027	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.251	<0.001	<0.001
Mo	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Ni	(mg/l)	0.001	0.038	0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pb	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Se	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Si	(mg/l)	0.002	0.419	0.512	0.734	0.437	0.334	0.287	0.356	0.208	0.027	0.113	0.002
Sr	(mg/l)	0.004	1.132	0.859	0.254	0.352	0.124	0.234	0.32	0.164	0.068	0.396	0.112
Ti	(mg/l)	0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
U	(mg/l)	0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
V	(mg/l)	0.003	0.067	0.042	<0.003	<0.003	0.015	<0.003	<0.003	0.031	<0.003	<0.003	<0.003
Zn	(mg/l)	0.001	0.333	0.032	0.054	0.12	0.059	0.043	0.042	0.036	0.013	0.154	0.161

Table 9-12: Ions and nutrient data from the January 2013 survey of the water quality of the selected North West pans.

Analysis	Unit	Detection	NWpanG	NWpanH	NWpanE	NWpanI	NWpanF	NWpanJ	NWpanA	NWpanB
Alkalinity	(mg/l)	0.1	1120	6400	376	488	132	500	680	596
Ammonia	(mg/l)	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Total Hardness	(mg/l)	0.1	59	45	18	373	174	85	20	32
Fluoride	(mg/l)	0.037	1.19	<0.037	<0.037	0.68	0.45	0.5	1.24	1.31
Chloride	(mg/l)	0.031	903	92800	1528	244.5	1209	113.2	216.5	666
Nitrite	(mg/l)	0.07	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070
Nitrate	(mg/l)	0.07	1.86	<0.070	<0.070	<0.070	<0.070	0.31	0.45	<0.070
Phosphate	(mg/l)	0.065	4.61	63.2	6.25	<0.065	<0.065	0.92	9.49	0.13
Sulphate	(mg/l)	0.053	85.84	22620	51.38	19.07	182.9	115.4	51	724
Ca	(mg/l)	0.009	16.103	1.712	6.611	22.923	24.415	22.281	7.011	9.876
K	(mg/l)	0.007	117.202	7508.61	250.505	71.155	100.919	26.032	7.22	1160.91
Mg	(mg/l)	0.001	4.489	9.961	0.355	77.109	27.631	7.007	0.589	1.825
Na	(mg/l)	0.009	869.447	187631.8	1623.927	241.622	1154.897	143.805	664.42	8838.987

Table 9-13: Metal concentrations in water samples from the North West pans for the January 2013 survey.

Analysis	Unit	Detection	NWpanG	NWpanH	NWpanE	NWpanI	NWpanF	NWpanJ	NWpanA	NWpanB
Ag	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Al	(mg/l)	0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
As	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
B	(mg/l)	0.004	3.129	11.843	0.798	0.297	1.474	0.759	1.18	5.662
Ba	(mg/l)	0.004	0.343	0.154	0.39	0.22	0.259	0.184	0.089	0.304
Cd	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Co	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cr	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Cu	(mg/l)	0.005	0.041	<0.005	0.028	0.027	0.017	0.012	0.03	0.017
Fe	(mg/l)	0.004	0.028	0.494	0.041	0.027	0.029	0.03	0.077	0.028
Mn	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Mo	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Ni	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pb	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Se	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Si	(mg/l)	0.002	0.53	0.111	1.055	0.745	0.279	1	1.8	0.651
Sr	(mg/l)	0.004	0.227	0.348	0.055	0.217	0.791	0.3	0.024	0.88
Ti	(mg/l)	0.007	<0.007	0.019	0.019	<0.001	<0.007	<0.007	0.025	<0.007
U	(mg/l)	0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
V	(mg/l)	0.003	0.219	<0.003	0.158	<0.003	<0.003	0.097	0.457	0.45
Zn	(mg/l)	0.001	0.071	0.082	0.014	0.058	0.083	0.028	0.01	0.005

Table 9-14: Ions and nutrient data from the January 2013 survey of the water quality of the selected Free State pans.

Analysis	Unit	Detection	FSpanM	FSpanN	FSpanO	FSpanL	FSpanK
Alkalinity	(mg/l)	0.1	1200	300	36	52	392
Ammonia	(mg/l)	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Total Hardness (CaCO3	(mg/l)	0.1	392	1310	9430	2650	20000
Fluoride	(mg/l)	0.037	<0.037	<0.037	<0.037	0.46	<0.037
Chloride	(mg/l)	0.031	11350	2793	19300	1141	53700
Nitrite	(mg/l)	0.07	<0.070	<0.070	<0.070	<0.070	<0.070
Nitrate	(mg/l)	0.07	<0.070	<0.070	<0.070	<0.070	<0.070
Phosphate	(mg/l)	0.065	<0.065	<0.065	<0.065	<0.065	<0.065
Sulphate	(mg/l)	0.053	1181	307.1	3996	1887	11260
Ca	(mg/l)	0.009	46.862	9.374	2410.06	950.236	1192.07
K	(mg/l)	0.007	152.227	106.973	215.007	82.553	1944.58
Mg	(mg/l)	0.001	67.069	313.693	831.196	66.053	4161.21
Na	(mg/l)	0.009	7579.307	1433.987	11574.77	949.726	62550.67

Table 9-15: Metal concentrations in water samples from the Free State pans for the January 2013 survey.

Analysis	Unit	Detection	FSpanM	FSpanN	FSpanO	FSpanL	FspanK
Ag	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Al	(mg/l)	0.003	<0.003	<0.003	<0.003	<0.003	<0.003
As	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005
B	(mg/l)	0.004	4.7	0.374	2.828	1.518	1.708
Ba	(mg/l)	0.004	0.62	0.701	0.316	0.119	0.247
Cd	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Co	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cr	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Cu	(mg/l)	0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Fe	(mg/l)	0.004	0.053	0.041	0.038	0.027	0.035
Mn	(mg/l)	0.001	0.079	0.479	<0.001	<0.001	0.796
Mo	(mg/l)	0.001	<0.001	<0.001	0.099	<0.001	<0.001
Ni	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pb	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Se	(mg/l)	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Si	(mg/l)	0.002	0.219	1.255	0.162	0.657	0.543
Sr	(mg/l)	0.004	3.339	5.952	47.238	8.274	65.186
Ti	(mg/l)	0.007	<0.007	<0.007	0.032	0.011	0.019
U	(mg/l)	0.004	<0.004	<0.004	<0.004	<0.004	<0.004
V	(mg/l)	0.003	0.023	<0.003	<0.003	<0.003	<0.003
Zn	(mg/l)	0.001	0.022	0.019	0.101	0.011	0.169

Table 9-16: In situ variables for Mpumalanga pans for the March/April 2013 survey.

		MPA	MPB	MPC	MPD	MPE	MPG	MPH	MPI	MPJ	MPK	MPL	MPM	MPO	MPP	MPQ	MPR
Oxygen saturation	%	58.6	79	88.8	68	68.7	76.1	83.5	53.3	74.1	33	67.6	39	89.4	56.6	74	75.1
Oxygen content	mg/l	4.68	5.85	6.97	5.65	6.48	7.79	8	5.13	6.96	3.32	6.63	4.26	8.73	4.8	7.13	7.23
TDS	g/l	13.6	5.63	4.87	5.01	9.02	1.7	2.74	2.3	0.235	1.73	5.96	0.443	0.132	0.285	1.84	1.62
pH	-	9.1	9.15	9.14	9.45		9.04	9.24	9.03	8.99	7.89	9.15	8.77	8.19	8.43	9.04	8.99
EC	ms	19.6	8.06	7.03	7.24	3.3	2.56	3.8	3.36	0.329	2.51	8.65	0.622	0.192	0.45	2.63	2.42
Temperature	°C	27.6	26.1	27.5	24.1	22.6	16.6	17.4	17.4	16.5	16.1	16.8	10.7	17.2	26.6	17.7	16.7

Table 9-17: In situ variables for the North West and Free State pans for the March/April 2013 survey.

		FSpanM	FSpanN	NWpanA	NWpanH	NWpanI
Oxygen saturation	%	68	142	80	49.6	70.7
Oxygen content	mg/l	6.03	11.45	7.87	3.97	6.5
TDS	g/l	4.59	11.79	0.00418	27.3	1.136
pH	-	9.29	9.1	10.35	9.67	9.08
EC	ms	6.68	16.81	0.607	54.7	1.73
Temperature	°C	21.3	24.5	16.7	25.5	18.6

Table 9-18: Chemical water quality from the inundated pans in the North West and Free State provinces from the March/April 2013 survey.

	Unit	FSM3	FSN3	NWA3	NWI3	NWK3
Alkalinity	mg/l	242.64	517.15	188.52	462.16	1681.14
Ammonium	mg/l	<0.01	<0.01	<0.01	<0.01	<0.01
Total Hardness	mg/l	392	1310	9430	283	18
Fluoride	mg/l	0.2	0.7	0.22	0.53	0.13
Chloride	mg/l	1716	3150	123.7	231.8	2408
Nitrite	mg/l	<0.070	<0.07	<0.07	<0.070	<0.070
Nitrate	mg/l	0.16	15.63	0.68	1.06	0.9
Phosphate	mg/l	10.01	<0.065	1.46	<0.065	<0.065
Sulphate	mg/l	488	325.6	30.08	18.69	4109
Ag	mg/l	<0.001	0.132	<0.001	<0.001	0.376
Al	mg/l	0.921	<0.003	1.108	0.073	0.118
As	mg/l	0.115	0.12	<0.001	<0.001	0.028
B	mg/l	0.464	0.07	0.049	0.052	1.596
Ba	mg/l	0.031	0.729	0.102	0.066	0.031
Ca	mg/l	5.745	131.433	2.825	15.991	2.739
Cd	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001
Co	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001
Cr	mg/l	<0.005	0.022	<0.005	<0.005	<0.005
Cu	mg/l	0.02	0.038	0.012	0.01	0.025
Fe	mg/l	0.636	0.057	0.94	0.065	0.052
K	mg/l	25.772	122.128	21.484	21.948	1 106.70
Mg	mg/l	6.643	294.149	1.118	59.205	2.633
Mn	mg/l	0.018	0.168	0.033	<0.001	<0.001
Mo	mg/l	<0.001	<0.002	<0.003	<0.001	<0.001
Na	mg/l	1007.174	1766.464	99.946	155.201	15 707.23
Ni	mg/l	0.031	0.042	<0.001	0.023	0.026
Pb	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001
Se	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001
Si	mg/l	0.062	<0.002	0.161	0.023	<0.001
Sr	mg/l	0.195	5.525	0.012	0.146	0.127
Ti	mg/l	<0.007	<0.007	<0.007	<0.007	<0.007
U	mg/l	<0.004	<0.004	<0.004	<0.004	<0.004
V	mg/l	0.089	0.04	0.051	0.026	0.133
Zn	mg/l	<0.001	<0.002	<0.003	<0.001	<0.001

Table 9-19: Chemical water quality from the inundated pans in the Mpumalanga Province from the March/April 2013 survey.

	Unit	**MPA3**	**MPB3**	**MPC3**	**MPD3**	**MPE3**	**MPG3**	**MPH3**	**MPI3**
Alkalinity	mg/l	1047	726.26	858.48	808.64	473.44	382.63	628.88	396.87
Ammonium	mg/l	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Total Hardness	mg/l	103	111	58	59	37	96	113	38
Fluoride	mg/l	0.11	0.95	0.87	0.5	1	0.61	0.66	0.85
Chloride	mg/l	2012	963	851	1126	352	363	612	427
Nitrite	mg/l	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070
Nitrate	mg/l	0.36	<0.070	<0.070	<0.070	1.89	<0.070	<0.070	0.17
Phosphate	mg/l	6	5.19	3.78	0.62	3.58	<0.065	<0.065	3.6
Sulphate	mg/l	713	266	175.8	142	15.59	73.5	73.3	110.1
Ag	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Al	mg/l	<0.003	0.126	0.186	<0.003	0.354	0.509	0.087	1.224
As	mg/l	0.074	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
B	mg/l	0.128	0.064	0.032	<0.004	0.033	0.023	0.035	0.005
Ba	mg/l	0.309	0.187	0.058	0.225	0.079	0.198	0.195	0.139
Ca	mg/l	29.763	28.531	17.09	15.886	8.985	20.829	26.123	11.304
Cd	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Co	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cr	mg/l	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.01
Cu	mg/l	0.019	<0.005	0.021	<0.005	0.013	<0.005	<0.005	<0.005
Fe	mg/l	0.006	0.069	0.092	<0.004	0.288	0.224	0.037	0.801
K	mg/l	233.515	113.621	28.819	65.292	25.689	27.783	94.504	26.203
Mg	mg/l	7.087	9.578	3.642	4.701	3.488	10.667	11.667	2.22
Mn	mg/l	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	<0.001	0.018
Mo	mg/l	<0.0011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Na	mg/l	502.11	810.521	787.792	883.777	484.986	421.59	518.539	434.98
Ni	mg/l	0.025	<0.001	0.019	0.021	0.02	<0.001	0.023	<0.001
Pb	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Se	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Si	mg/l	0.123	0.127	0.182	0.126	0.156	0.164	0.149	0.128
Sr	mg/l	0.508	0.378	0.158	0.214	0.101	0.24	0.254	0.13
Ti	mg/l	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	0.011
U	mg/l	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
V	mg/l	0.059	0.03	0.026	<0.003	0.048	0.024	0.021	0.045
Zn	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 9-20: Chemical water quality from the inundated pans in the Mpumalanga Province from the March/April 2013 survey (Continued from Table 9.17).

	Unit	MPJ3	MPK3	MPL3	MPM3	MPO3	MPP3	MPQ3	MPR3
Alkalinity	mg/l	79.09	259.94	669.96	83.36	45.46	45.02	422.61	520.54
Ammonium	mg/l	1.27	<0.01	<0.01	<0.01	1.38	<0.01	<0.01	<0.01
Total Hardness	mg/l	81	107	63	37	55	37	44	72
Fluoride	mg/l	0.33	0.34	0.85	0.31	0.14	0.42	0.94	1.49
Chloride	mg/l	27.96	422	855	90.9	22.35	45.4	280	289.8
Nitrite	mg/l	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070	<0.070
Nitrate	mg/l	0.22	0.15	<0.070	0.1	<0.070	8.46	0.32	0.12
Phosphate	mg/l	<0.065	<0.065	<0.065	<0.065	<0.065	<0.065	2.08	1.23
Sulphate	mg/l	24.52	1.22	216.4	4.04	31.85	44	8.94	66.1
Ag	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Al	mg/l	0.123	<0.003	0.39	<0.003	<0.003	0.621	0.252	0.435
As	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
B	mg/l	<0.004	<0.004	0.023	<0.004	0.007	0.011	0.008	0.003
Ba	mg/l	0.191	0.294	0.105	0.266	0.289	0.254	0.108	0.055
Ca	mg/l	15.703	14.907	19.216	6.622	11.047	7.279	12.373	16.621
Cd	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Co	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cr	mg/l	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Cu	mg/l	<0.005	<0.005	<0.005	<0.005	0.01	<0.005	<0.005	<0.005
Fe	mg/l	0.611	0.616	0.225	0.817	1.925	0.394	0.192	0.298
K	mg/l	11.99	13.655	25.243	11.538	6.993	20.581	23.948	5.869
Mg	mg/l	10.148	17.095	3.552	5.013	6.753	4.559	3.277	7.444
Mn	mg/l	0.155	0.075	<0.001	<0.001	0.259	<0.001	<0.001	<0.001
Mo	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Na	mg/l	20.287	259.529	616.005	60.096	13.809	47.458	266.681	174.662
Ni	mg/l	0.022	<0.001	0.018	<0.001	<0.001	0.019	<0.001	<0.001
Pb	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Se	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Si	mg/l	<0.002	0.028	0.165	<0.002	0.04	0.208	0.174	0.039
Sr	mg/l	0.158	0.232	0.237	0.083	0.101	0.08	0.115	0.152
Ti	mg/l	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007	<0.007
U	mg/l	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004	<0.004
V	mg/l	0.024	<0.003	0.015	<0.003	<0.003	0.019	0.043	0.046
Zn	mg/l	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

APPENDIX B:

Table 9-21: Site photographs of all the pans from Mpumalanga, North West and Free State during the March/April 2013 field surveys. (FS = Free State; NW = North West; MP = Mpumalanga).

FS pan A	FS pan B	FS pan C
		
FS pan D	FS pan E	FS pan F
		

FS pan G	FS pan H	FS pan I
		
FS pan J	FS pan K	FS pan L
		

FS pan M	FS pan N	FS pan O
		
NW pan A	NW pan B	NW pan C
		

NW pan D	NW pan E	NW pan F
		
NW pan G	NW pan H	NW pan I
		

NW pan J	NW pan K	MP pan A
		
MP pan B	MP pan C	MP pan D
		

MP pan E	MP pan G	MP pan H
		
MP pan I	MP pan J	MP pan K
		

MP pan M	MP pan O	MP pan P
		
MP pan Q