Department of Environmental and Aquatic Sciences

Nitella congesta - a charophyte as a tool for the rehabilitation of sand mine-void wetlands at Capel, Western Australia

Isaac Kwamina Eshun Annan

This thesis is presented for the Degree of
Doctor of Philosophy
of
Curtin University of Technology

November 2008

DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: ……..

Date: …….

DEDICATION

This work is dedicated to Mrs. Sarah Dosoo (nee Annan) whose selfless sacrifice twenty five (25) years ago paved way for me to come this far in my academic pursuit.

Sarah, may the Good Lord richly bless you for your selflessness.

ACKNOWLEDGEMENTS

To God Be The Glory; Great Things He Has Done! I thank God for His abundant grace that saw me through this work successfully.

I wish to thank my supervisor, Asso. Prof. Jacob John for introducing me to this field of study with his directions, constructive criticisms and valuable feedback. I also thank my associate supervisor, Dr. Lynne Robert Jones for his valuable contributions and guidance in this work.

I also wish to thank the Administrative staff of the Department of Environmental and Aquatic Sciences, Prof. Jonathan Majer, Head of Department, Mrs. Enid Holt and Glenice Carmody for the administrative assistance. I extend my gratitude to the chairperson of my thesis committee, Dr. Beng Tan for his concern and support. My sincere appreciation goes to Mr. Charles Lacoste and the Laboratory Technicians, Mr. William Parkinson and Ms. Lydia Kupsky for their assistance in my field and laboratory works. My special thanks go to Mr. Peter Mioduszewski who wholeheartedly accompanied and assisted me in all my field works. Peter, I am very grateful. My gratitude also goes to Ann Bentley of Illuka Resources, Capel for her assistance and cooperation during my filed trips. Many thanks to Dr. Adriana García who assisted with the identification of the alga in this project.

I also express my appreciation to Mr. & Mrs. Max Morgan who gave me and my family a home where a greater part of this work was completed. Thanks to my lovely friends, Dr. Benjamin Abgenyegah, Nino & Linda, John & Monica, Kow & Irene, Jane Namono, Mrs. Baaba Sackey-Clarke, Comfort Afoani, Mr. & Mrs. Bendov Ansah-Otu and Mr. Robert Lugushie whose inspiration and support urged me on in difficult times.

A special word of thanks go to my parents, siblings and in-laws for their prayer support, encouragement and the confidence reposed in me. I also wish to thank Mr. and Mrs. Ato Essuman for their guidance and support that

has brought me this far. Ato and Sally, I am very very grateful to you. Thanks to Mr. Kow Otoo for his support and assistance towards my studies.

Finally, I wish to thank my lovely wife, Mary for her support in diverse ways and patience during difficult times, in most instances bearing a greater part of my stress. I specially thank my precious lovely kids, Daryl and Danyl for their understanding and patience when Daddy has to leave them some times to concentrate on this work.

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ABSTRACT

This research is the outcome of investigations of the ability of *Nitella congesta*, a charophyte, to hyperaccumulate metal contaminants, as well as contribute to the sustainable development of a chain of lakes derived from the sand mine voids at Capel 250km south of Perth, Western Australia. Studies were conducted to ascertain the taxonomy of *Nitella congesta* as well as its life cycle pattern in relation to the hydrological regime of the lakes of the wetlands. It was observed that a decrease in the availability of water in the lakes particularly on the onset of summer, initiated the production of fruiting bodies whiles prolonged availability of water ensured a prolonged vegetative growth.

Aquatic plants play an important role in the structuring of freshwater communities. Freshwater macrophytes such as *Nitella congesta* have been reported to serve as food source as well as provide refuge and shelter for macroinvertebrates. Thus the presence of freshwater macrophytes in one way or the other has a direct impact on the species abundance and diversity of macroinvertebrates that use them as their habitat. A study of the impact of *Nitella congesta* as a suitable macrophyte on diversity and abundance of macroinvertebrates showed a positive outcome. It was observed that species richness and diversity were high in *Nitella congesta* dominated sites of the lakes. Experimental outcome showed that *Nitella congesta* is a hyperaccumulator of metals. Both the mucilage and the thallus displayed concentrations of few metals.

The disappearance of submerged macrophytes such as charophytes in shallow lakes is a major problem caused by eutrophication. There has been an approved proposal to discharge treated waste water with a phosphorus concentration of about 4,000µg/L into the lakes of the Capel Wetlands Centre. This necessitated a study of the impact of eutrophication on the establishment of *Nitella congesta* as a functional macrophyte for the enhancement of the ecological structure of the wetlands.

Results showed that though eutrophication will initially increase the primary productivity of the lakes of the wetlands, the eventual consequence will be the loss of *Nitella congesta* as a functional macrophyte in the wetlands.

At the last stage of the study, a consistent decrease in pH readings of the lakes and lack of successful germination of *Nitella congesta* as a result of prolonged drought and exposure of the lake sediment was observed. This necessitated a thorough study of the impact of climate change on the establishment of *Nitella congesta* in the wetlands. In conclusion, it was observed that *Nitella congesta* could serve as a suitable tool for the rehabilitation of the wetlands.

1 GENERAL INTRODUCTION

1.0 Scope of research

There has been a growing interest in the use of plants as agents for the remediation of contaminated soil and water by means of a process called phytoremediation. Decades of research has led to the discovery of metal accumulating plants that have the ability not only to tolerate but accumulate unusually high concentrations of metal ions in their tissues. Many terrestrial plants as well as some aquatic plants are known to hyperaccumulate heavy metal ions. Charophytes, a group of green algae, have been known to play a significant role in the enhancement of water quality of aquatic systems by taking up heavy metals. They have been reported to improve water quality and enhance macroinvertebrate species diversity and abundance thereby attracting water birds. This thesis is the outcome of the investigations on the ability of *Nitella congesta*, a charophyte, to contribute towards the development of a sustainable wetlands system derived from sand mining and its ability to hyperaccumulate metal contaminants.

Charophytes have been found to dominate a number of artificial lakes at Capel. The ecological significance of charophytes to the development of these artificial wetlands has been studied. High concentration of heavy metal ions, high electrical conductivity and slow rate of development were of great concern in the rehabilitation of these sand mine voids. The sand mineral mining activities resulted in the deposition of associated heavy metals (Ca, Mg, Mn, Fe, Zn and Al) into the lake sediment (Claridge 1978). This study looked at the ability of *N. congesta* to hyperacummulate these metals with special emphasis on zinc.

Submerged macrophytes have major effects on the productivity and biogeo-chemical cycles in fresh water as they occupy key interfaces in stream and lake ecosystems. Most macrophytes are rooted, constituting a living link between sediments and the overlying water (Carpenter and Lodge 1986). Some of the roles played by the macrophytes are biotic interactions, which involve their use as habitat or food by other aquatic organisms. Therefore, any changes in the macrophyte component would have detrimental impacts on freshwater ecosystems.

The establishment of self-sustaining wetlands requires the presence of a diverse range of aquatic invertebrates to contribute to the successive ecological link of the food chain by providing a protein-based food source. Therefore, it is expected that an increase in macrophyte populations would enhance the development of macroinvertebrate communities. In this project, a study of the epifauna, of charophytes (*N. congesta*) was conducted, determining the contribution by the charophyte to the enhancement of species abundance and diversity of the macroinvertebrates.

One of the serious problems caused by eutrophication of shallow waters is the loss of submerged macrophytes. The loss of macrophytes results in the loss of faunal habitats with the subsequent loss of species diversity, abundance and biomass, thus negatively affecting food-webs (Korner 2002). Charophytes, like other submerged macrophytes have been found to decline during eutrophication (Blindow 1992a; Scheffer 1998; van den Berg *et al.* 1998) and can thus be used as indicators of eutrophication. Since charophytes are the major submerged macrophytes of the Capel Wetlands, their decline by eutrophication would negatively affect the sustainability of the wetlands. Therefore, in the light of an imminent plan to discharge waste water from the local Water Corporation processing plant into the lakes, a study of the effect of eutrophication on *N. congesta* was conducted.

Close to the termination of the study, the lakes experienced acidification due to prolonged drying up of the lakes and exposure of sulphidic sediment. Finally the impact of low pH on the charophytes was also investigated.

1.1 Introduction

Major mining activities to a large extent have affected most of the ecosystems of the earth in various ways. The direct impacts of mining activities on land are mostly severe, resulting in biodiversity loss due to removal of natural soils, plants and animals. Among ecosystems which have been severely affected by surface mining activities are wetlands.

Wetlands occupy about 6% of the world's land surface and vary according to their origin, geographical location, water regime, chemistry and dominant plants (Gosselink 1990). Wetlands are known for their ability to filter nutrients and other contaminants from water. These functions to a greater extent, have led to the widespread use of wetlands for wastewater treatment (Kadlec and Knight 1995). Wetlands also provide important habitat for many species of wildlife: fish, waterfowl, shorebirds and other organisms. Wetlands are also used for the removal of toxic residues. Toxic residues such as heavy metals, pesticides and herbicides can be removed from water by ion exchange and absorption in the organic and clay sediments (Kadlec and Knight 1995). However, wetland destruction has plagued many countries for decades (Williams 1990). It is therefore important not only to rehabilitate these important ecosystems, but also protect them from further destruction.

The increase of industralisation, modern agricultural practices and population growth has lead to the degradation of wetlands and in some cases, complete disappearance (Findlay and Houlahan 1997). Wetlands have only been recognised recently as areas of ecological complexity and conservation importance worldwide. In spite of the fact that some cultures have existed and made sustainable use of wetlands for many years, the modern history of wetlands in many parts of the world is of their destruction and degradation (Mitsch and Gosselink 2000). Wetlands provide critical habitats for many rare and endangered species, therefore their loss and degradation is likely to cause a significant reduction in local biodiversity.

There has been a considerable decline of wetlands in Western Australia since the European settlement in early Nineteenth Century. There are over 9,600 wetlands covering more than 25% of the Swan Coastal Plain in Western Australia (Balla 1994). The Swan Coastal Plain is the geographic feature lying directly west of the Darling Scarp. It contains the Swan River which flows west into the Indian Ocean. Estimates however, show that more than two thirds of the wetlands of the Swan Coastal Plain have been destroyed or degraded by various forms of land use (Brooks 1992). Additionally, a number of mine voids are being created by surface mining in

Western Australia. Mine void-related impacts have been of long-term concern. A final mine void is defined as that remaining when an opencut mine has ceased operations and is not planned to be mined or used as access for underground mining in the near future (Mallet and Mark 1995).

Opencut mining is predominant throughout Western Australia with subsequent mine voids extending below the water table. There are currently about 1800 existing mine voids and more than 150 mines that are operating below the water table in Western Australia (Johnson and Wright 2003). At the end of the removal of water to facilitate mining, there is a recovery of the water level and the mine voids are filled with underground water thus becoming a "window" to the water table. Mine voids vary in size ranging from borrow pits (about 100m in diameter) to very huge pits. The wetlands formed from the mine-voids are also referred to as mine pit lakes. Many pit lakes have the potential to be made point sources of hypersaline water (especially in Western Australia) thus impacting on the surrounding groundwater resources. Low annual rainfall coupled with high evaporation with the resultant rainfall deficit in most parts of Australia, subsequently contribute to the development of hypersaline water bodies (Hall 1998). Additionally, there are factors which may also lead to the generation of acidic conditions in the pit lakes.

Western Australia is a leading producer of titaniferous minerals such as ilmenite, rutile, zircon and monazite in the world. Since 1956, sand mining has been practiced in Capel, 200km south of Perth (33^{o}S, 116^{o}E) in Western Australia resulting in mine voids after the extraction of the minerals. These voids, intercepting the water table were converted into artificial wetlands with permanent water most of the year from 1975 to 1979. The most common problems associated with mine void lakes are salinisation and acidification which in turn have severe adverse effects on local and regional groundwater resources and generally natural environment on the whole. However the extent of the impact ranges from very minimal to considerably significant (Commander *et al.* 1994).

The extent of impact on the surrounding groundwater environment is significantly dependent on the hydrogeology of the area thus determining whether the mine void will act as either a groundwater sink or groundwater - through flow cell. In the groundwater sink regime, the rate of groundwater inflow into the void is exceeded by the rate of evaporation, a typical occurrence in most Western Australian hard-rock mines. In the groundwater - through flow type, the reverse situation occurs whereby the rate of groundwater inflow exceeds the rate of evaporation resulting in saline plumes moving out of the void and affecting other groundwater resources (Commander *et al.* 1994).

In Australia, the focus of mine void issues predominantly has been on the coal and sand mining industries. The lack of empirical hydrochemical and post-mine closure data makes it very difficult to assess the impact of salinity in Western Australia (Hall 1998). Mine voids that form “groundwater sinks” eventually become more saline. A long-term concern is the down-gradient movement of saline plumes from “throughflow” mine voids, which may eventually extend widely impacting on other groundwater resources (Commander *et al.* 1994).

As a major issue elsewhere in Australia, the generation of acidic waters is associated with the coal mining industry in the higher rainfall southwest region and a handful of metalliferous mines. Most abandoned mine voids in the Collie Basin, Western Australia’s premier coal mining area have highly acidic water. Opencut strip-mining method with its waste rock and spoil deposited in voids has become an issue of concern. This is because the void is immediately adjacent to an active strip thereby creating a direct contact with water resulting in the leaching of solutes (Johnson and Wright 2003). On the other hand, the potential for acid-water generation in most metalliferous mines is comparably minimal, with little sulphide-rich ore mined in Western Australia. Generally, the high acidity of acid mine drainage and the high amounts of dissolved heavy metals make acid mine drainage highly toxic to most organisms (Pentreath 1994).

In spite of the problems associated with mine void lakes, they have considerable benefits which are often untapped in pursuance of lease viability and profitability. Some of these benefits such as recreational have been well established in some arid mining areas taking into consideration the quality of the pit water (Pentreath 1994).

1.2 Creation of artificial wetlands

Artificial wetlands are being created worldwide for their habitat value, often to compensate for the loss of wetlands elsewhere (Mitsch 1998). The science of wetland restoration and creation has taken a slow pace in the area of research. The construction of wetlands requires specific goals (Hammer 1992) which help define monitoring elements and establish standards for determining and following the progress of the wetland (Zentner 2001). The creation of wetlands for wildlife is a greater challenge than for the purpose of preservation or restoration (Hammer 1992).

In the creation of artificial wetlands, where possible, larger systems should be created as they have greater potential for supporting and sustaining high biodiversity (Hammer 1992; Zedler 1997). However, in most cases, land and financial constraints determine the size of a constructed wetland (Kent 2001). The created wetland develops best if it is located in close proximity to other established wetlands, thus encouraging wildlife establishment (Hammer 1992); animals are more likely to colonise habitats in close proximity (Kent 2001). More importantly there should be fewer barriers to water flow and movement of fauna. The long-term success of wetland creation or restoration in any form depends largely on restoring, establishing and managing the appropriate hydrology because it controls the nutrient cycling and availability. Wetland hydrology influences abiotic factors such as water availability, nutrient availability and other related conditions such as water depth and water chemistry (Hammer 1992).

Another important component that needs consideration when creating artificial wetlands is the soil. Wetland soils provide support for wetland plants.

They serve as medium for many chemical transformations, the principal reservoir for minerals and nutrients needed by the plants as well as many other substances (Hammer 1992). The composition of wetland soil in addition to other environmental conditions can affect the binding capacity of the soil to certain nutrients and thus determine their availability within the water body (Chambers and McComb 1996).

1.3 Wetland rehabilitation

Wetland rehabilitation can restore most wetlands though some of the damages are irreversible; it can be achieved despite the difficulties posed by irreversible abiotic and/or biotic conditions. Any rehabilitation attempt in this case should begin at the landscape scales (Williams 1990). Wetland rehabilitation can be hampered by soil conditions as soil conducts groundwater, transforms nutrients, improve water quality, support rooting and mycorrhizae, symbiotic bacteria and other soil macrofauna. Therefore if the soil texture, nutrient status, microbiota or seed bank are varied, then the related functions will be subsequently affected. The simple act of draining the soil in some coastal wetland restoration triggers chemical reactions that aerate the soil and change conditions from reducing to oxidizing with resultant detrimental conditions to microorganisms, plants and soil fauna species (Zedler and Kercher 1995). Most wetlands with extremely rich species diversity and composition are not able to recover their full potential during restoration or rehabilitation (Zedler 2000). In the case where animal populations have been depleted, habitat reconstruction and reintroduction can be considered. On the whole, the landscape should be equipped in order to attract various animal species (Zedler 2000).

1.4 Background Information and Literature Survey

1.4.1 The use of plants as hyperaccumulators

Plants may contain elements (micro- and macronutrients), ranging from trace amounts to large quantities, depending on the importance of such elements to their growth. Depending on the concentration of elements found in plant

tissue, it could be defined as “normal” or “abnormal”. Plants that contain unusually high concentrations of particular elements are termed hyperaccumulators and this phenomenon has attracted much interest from researchers.

1.4.2 Phytoremediation

Over the past century, anthropogenic activities (e.g. agricultural and industrial) have contributed to the contamination of soil and water through aqueous discharges. For example, in the United States alone, there are over 20,000 sites contaminated with heavy metals that would need remediation (Ensley 2000). Physical, chemical and biological processes have been applied extensively to remediate contaminated soil and water. Common among such methods are soil washing, excavation and reburial of metal-contaminated soils, and the pump-and-treat systems applicable for water (i.e. *ex situ* techniques) (Glass 1999).

The discovery of plants with metal hyperaccumulating properties suggested the feasibility of the use of such plants for the cleanup of heavy metal contaminated soil and water. The use of these plants for remediation purposes was termed phytoremediation. Phytoremediation is a new technology that uses specially selected metal-accumulating plants to remediate soil and water, contaminated with heavy metals. It offers an attractive and economical alternative to conventional methods such as currently practised soil removal and burial methods (Blaylock 2000). Phytoremediation employs *in-situ* remediation of metals involving the use of plants (Cunningham *et al.* 1995).

Phytoremediation has been defined differently by different authors amongst as shown below;

- the use of green plants to remove pollutants from the environment or to render them harmless (Cunningham and Berti 1993; Raskin *et al.* 1994).

- the use of plants to remove, destroy or sequester hazardous contaminants from soil, water and air (Gardea-Torresdey 2003; Prasad 2003).

All plants take up metals to varying degrees from the substrates in which they are rooted (Baker *et al.* 2000). However, some take up more metals than others. Thus in selecting plants for successful implementation of phytoremediation, those with the ability to take up significant quantity of metal ions from the soil and effectively and efficiently decrease the soil metal concentration should be considered.

A significant quantity of the metal should be removed from the soil through plant uptake with a residual reduction of the soil metal concentration to culminate in a successful implementation of phytoremediation. However, the first critical factor for an effective and efficient phytoremediation is the availability of the metal in soil (Blaylock 2000). Thus soils with metal contaminants that are not available for uptake by plants will adversely affect the success of phytoremediation.

1.4.2.1 Advantages and disadvantages of phytoremediation

The most widely acclaimed advantage of phytoremediation is its cost; it is believed to have far lower capital and operational cost compared with conventional methods of remediation (Glass 2000). Phytoremediation is relatively inexpensive because it takes place *in situ* and is also solar-driven or solar-powered (Salt *et al.* 1995; Salt *et al.* 1998). Phytoremediation as a remediation process eliminates the creation of sludge and plant biomass is generally easier to handle and dispose of than sludge.

Phytoremediation can also be applied concurrently with other treatments, for example, as a polishing step to remove relatively low metal concentrations remaining in treated waste streams (Glass 2000). However, as a remediation technique, it is limited by the time involved for the remediation process. More time may be required for phytoremediation of a site than conventional technologies such as excavation and landfill or incineration. For example, the

complete removal of metal ions from soil, even with hyperaccumulating plants, could take 15-20 years depending on the concentration (Glick 2003). This timeframe is considered to be too slow for practical application. However, the economic and environmental advantages far outweigh the time taken for remediation. On the other hand, phytoremediation can take place more gradually, on a human scale and can welcome public involvement.

Moreover, like all biological methods, the use of plants for metal uptake may not allow 100% removal or reduction of contaminants. This is because reaction rates decrease with time. Moreover, depending on the concentration, toxicity of contaminants may adversely affect the plants and hence their metal uptake ability. However, in order to meet expected efficiency output, some mechanisms such as the use of chelators to enhance the solubility of metals in the soil (Salt *et al.* 1995; Huang *et al.* 1997) and the application of classical genetics or advanced molecular biology tools to improve plant varieties (Cunningham and Ow 1996; Raskin 1996), have been employed.

1.4.3 Hyperaccumulation; its history and definition

The use of plants for remediation of the environment is historically dated from the 16th Century. This was when Andrea Caesalpino, a Florentine botanist, described *Alyssum bertolonii* (Brassicaceae) as a hyperaccumulator of minerals from Tuscany, Italy. In the year 1948, another discovery was made by a Florentine couple, C. Minguzzi and O. Vergnano. They described the unusual accumulation of nickel ions by *Alyssum bertolonii* (Brassicaceae) from the Impruetal region near Florence (Brooks 1998). They found up to 0.79% (7900 μg g^{-1}) nickel ions in dried leaves of plants growing in soils containing only 0.42% of this element whiles on an ash-weight basis the leaves contained 9.21% nickel. In 1972 and 1973, Severe and Brooks, and Coles reported the unusual accumulation of nickel by the West Australian species *Hybanthus floribundus* (Violaceae). This plant was found to have contained 1.38% nickel in soils containing only 0.07-0.10% of the element (Brooks 1998).

The term “hyperaccumulator” was first coined by Brooks *et al.* (1977) to describe plants with Ni concentrations higher than 1000 µg g^{-1} dry weight (0.1%). This value was not chosen arbitrarily as Ni as a micronutrient for plants is mostly found in the vegetative organs of most plants in the range of 1-10 µg g^{-1} dry weight (Assunção *et al.* 2003). Critical toxicity levels in crop species are in the range of 10-50 µg g^{-1} (Marschner 2002). A preliminary survey showed that a handful of plants contain nickel in the range of 300-1000 µg g^{-1} dry weight (Brooks *et al.* 1977). This depicted a clear distinction between hyperaccumulators and non-hyperaccumulators with the 1000 µg g^{-1} dry weight threshold as a criterion for the definition of Ni-hyperaccumulation (Assunção *et al.* 2003).

It is worth noting that the threshold that defines hyperaccumulation depends on the element involved. Hyperaccumulation thresholds for the best studied metals are 10000 µg g^{-1} dry weight for Mn and Zn; 1000 µg g^{-1} dry weight for Ni, Cu and Se; and 100 µg g^{-1} dry weight for Cd, Cr, Pb and Co (Reeves and Baker 2000). In many plants, the concentration of zinc is between 30-100 µg g^{-1} dry weight. Concentrations above 300µg g^{-1} are considered toxic (Marschner 1995). For an element such as cadmium, a foliar concentration above 100 µg g^{-1} dry weight (0.01%) is considered exceptional and is therefore used as a threshold value for Cd hyperaccumulation (Baker *et al.* 2000). The identified metal hyperaccumulators account for less than 0.2% of all angiosperms with majority being Ni hyperaccumulators with at least 317 species identified (Baker *et al.* 2000).

Hyperaccumulator plants are considered endemic to metalliferous substrates and are also characterized by their tolerance and sequestration of exceptional quantities of metals like Zn, Ni and Cd in their shoots at concentrations that would be toxic to “normal” plants (Baker and Whiting 2002).

1.4.3.1 Functions of metal hyperaccumulation

An intriguing question regarding hyperaccumulation is *"why some plants do or can accumulate elements to concentrations that are toxic to most other organisms"?* (Pollard 2000). Various adaptive explanations have been given to answer this question. There have been various hypotheses to explain the functions of hyperaccumulation (Boyd 2004). However the most tested to any extent is the hypothesis of "elemental defense" by Boyd (1998) that hyperaccumulated metals defend the plants against pathogens and herbivores. Apparently, an initial review by Boyd and Martens (1992) suggested four benefits of metal hyperaccumulation to plants which has attracted considerable experimental findings in the last decade to support or dispute the hypothesis.

The first hypothesis termed the tolerance/disposal hypothesis is of the view that the mechanism or process of hyperaccumulation by plants is to allow them to sequester metals in their tissues (i.e. tolerance), coupled in some instances with the elimination of such metals by shedding off those tissues (i.e. disposal) (Boyd 2004). However, genetic analysis has disproved the interrelationship between Zn hyperaccumulation and Zn tolerance, thus arguing against the tolerance aspect of the hypothesis in discussion (Macnair *et al.* 1999). Moreover, there has not been enough evidence to emphatically support the disposal aspect of the said hypothesis (Boyd 1998).

The second hypothesis, the interference hypothesis suggests that perennial hyperaccumulator plants use the mechanism of hyperaccumulation to enrich the surface of the soil on which they grow with high metal concentration and by this, prevent the growth and establishment of less tolerance plant species. This hypothesis was re-named "elemental allelopathy hypothesis" by Boyd and Martens (1998). However, though Boyd and Jaffre (2002) have reported the high metal concentration on the surface soil under high Ni accumulator plant species such as *Sebertia acuminata* (Sapotaceae), the hypothesis under discussion still remains untested or unproven.

The third hypothesis is the drought resistance hypothesis, according to which the mechanism of high metal accumulation offers the hyperaccumulator plants resistance to drought (Boyd 2004). However, there has been insufficient evidence to support this hypothesis and coupled with that, elaborate experiments performed by Whiting *et al.* (2003) have suggested no evidence that plant species that hyperaccumulate high concentrations of Ni and Zn such as *Alyssum murale* (Brassicaceae) and *Thalspi caerulescens* (Brassicaceae), are drought resistant.

The fourth hypothesis, termed 'the defence hypothesis' suggests that plants are protected from some herbivores and pathogens by the high concentration of metals they hyperaccumulate. Among the above mentioned hypotheses, the most tested with much research and continuing exploration is this hypothesis of "elemental defence" postulated by Boyd (1998).

1.4.3.2 The hypothesis of "Elemental Defence"

Plants have developed a variety of defence mechanisms to overcome some of the peculiar constraints resulting from a stationary or static way of life. Among such mechanisms is hyperaccumulation of metals. Most plant chemical defences such as alkaloids, glucosinolates terpenes are produced from the chemical products of photosynthesis - photosynthates (Boyd 2004). Hyperaccumulated metals on the other hand, represent a set of defences coined as "elemental defences" (Martens and Boyd 1994). The characteristic of the elemental defence is such that the toxic principle is rather an element or suite of elements taken up by the plants from the soil as compared to that obtained as a photosynthate. More so, their elemental nature makes them highly biochemically nondegradable by counter-defence chemicals secreted by herbivores (Martens and Boyd 1994). Elaborate experimental investigations performed to support this hypothesis have been mostly focused on the hyperaccumulation of nickel, and to some minor extent zinc and copper (Boyd 1998).

Information regarding the possible defence function(s) of metals in hyperaccumulator plants was initially provided by Ernst (1987). He had observed European populations of *Silene vulgaris* (Caryophyllaceae) which can accumulate high amounts of copper (≥ 1400 μg g^{-1} dry weight in its leaf tissues). He observed populations of the above named species growing on copper-rich or chalk grassland soils in which the seed capsule of the chalk grassland population were destroyed by moth larvae. On the other hand, this did not occur in the population grown on high copper soils, though the concentration of copper in the capsules and seeds were similar. The caterpillars died upon transferring them to plants grown on metalliferous soils (Ernst 1987). The explanation given for these observations was that the caterpillars may have been killed after they had fed on the leaves of plants which followed their consumption of the available seed capsules. Presumably, it was the highly copper concentrated leaves that killed the caterpillars.

Boyd and Martens (1994) and Martens and Boyd (1994) pioneered the great effort to test the elemental defence hypothesis. They used the nickel hyperaccumulators, *Streptanthus polygaloides* (Brassicaceae) and *Thlaspi montanum var. montanum* (Brassicaceae) employing larvae of the cabbage white butterfly, *Pieris rapae* L. as insect herbivores. In a “force-feeding” or an “induced-feeding” experiment, the insect herbivores were fed with leaf material harvested from plants grown on soils with either high or low Ni concentrations and those with none serving as a control. Eventually, the responses of insects showed acute toxicity in both cases of high and low Ni concentrations. They also observed that insect herbivores fed on leaves with high nickel concentration showed decreased growth; leading to the conclusion that the insect herbivores were killed by the high-nickel leaves as a defence mechanism.

Nickel, however was not the only element or metal found to be present in the plant tissues from analytical results. Therefore it was uncertain to attribute the lethal effect to the presence of nickel ions. To ascertain this, Boyd and Martens (1994) and Martens and Boyd (1994) resorted to the use of artificial

insect diet amended with nickel for the *Pieris rapae* L. In this experiment, they observed diminished survival of the larvae when the artificial diet nickel concentrations were above 1000µg g^{-1}. From this observation, they concluded that the high nickel concentration was lethal enough to have caused the decrease in survival of the insect herbivore. A defence is most effective if it deters attack rather than acting on an attacking organism after damage has occurred (Boyd 1998). This was evident in an experiment by Pollard and Baker (1997) and Jhee *et al.* (1999), where caterpillars (*Pieris brassicae and Pieris napi oleracea*) were found to have fed on low-Zn leaf (25-90% of total leaf area eaten) leaving the high-Zn leaf untouched; with no visible damage even after examination under microscope. In this case, the high concentration of zinc served as a defence preventing feeding by caterpillars.

All the afore-mentioned experiments were laboratory based. To supplement these experiments with field investigations, Martens and Boyd (2002) reported the first and most current experiment to examine the defensive effectiveness of Ni hyperaccumulation under field conditions as well as the types of herbivores involved. The experiment was conducted on a site naturally inhabited by the Ni hyperaccumulator, *Strepthanthus polygaloides* (Brassicaceae). Expectedly, initial findings were that, low-Ni plants were destroyed by insects on a high scale compared to the high-Ni plants. However, there was no observable distinction in terms of level of destruction between low-Ni plants and high-Ni plants (Boyd 1998).

Boyd (1998) has argued that the outcome of this experiment depicted a potential problem of field-based tests of the defence hypothesis with two explanations. The first being that the metals in plants do not have a defensive function and secondly, the experiment was not conducted at the right ecological scale in order to detect a genuinely-extant defensive function. He therefore suggested that defences may be important only against specific organisms depending on site and time.

1.4.3.3 Elemental Defence - Storage Location

Another question regarding the hypothesis of elemental defence is *"where exactly is the metal located in the hyperaccumulator plants?*". The Western Australian nickel hyperaccumulator *Hybanthus floribunduns* (Violaceae) was shown to have had high levels of nickel in the epidermal cells of its leaves (Farago and Cole 1988) cited in Boyd (1998). Recent studies of other hyperaccumulator species have shown highest concentration of metals in outer layers of plants leaves and roots (Boyd 1998). For example, most zinc in the zinc hyperaccumulator *Thlaspi caerulescens* (Brassicaceae) was found to be located in the epidermal and subepidermal layers of the leaves and roots (Vazquez *et al.* 1994) cited in Boyd (1998). In the nickel hyperaccumulator, *Berkheya zeyheri* (Asteraceae), the highest concentration of nickel was found to be in the epidermis and adjacent parenchyma tissues. The concentration of metals in the peripheral tissues in plants is consistent with a defensive function since the outer tissues are the first to be attacked by herbivores or pathogens (Boyd 1998).

However, the question that comes to mind is, "*What defends the plants against herbivores and pathogens – presence of metals or the concentration of metals?"*. Experiments have shown that what may be minimal metal concentrations in one case can be the highest concentration that may cause acute toxicity in another (Boyd 1998). Boyd and Martens (1994) and Martens and Boyd (1994) reported that the mortality of *Pieris rapae* increased when the nickel concentration was above 1000 μg g^{-1} nickel. In another study, nickel had no detrimental effect on pea aphids *Acyrthosiphon pisum* until the concentration reached 2500μg g^{-1} at which there was evidence of increased mortality (Boyd and Martens 1999).

1.4.4 Hyperaccumulation by aquatic and wetland plants

Aquatic plants just like the terrestrial plants can be employed for phytoremediation of metal contaminated waters. They are known to accumulate and bioaccumulate heavy metals (Prasad *et al.* 2001) and play an important role in oxygen production, nutrient cycling, water quality,

sediment stabilization and provide habitat and shelter for various aquatic organisms as well as aquatic wildlife (Mohan and Hosetti 1998). Aquatic plants are represented by a variety of species of algae and macrophytes which are found in various habitats (Mohan and Hosetti 1999). Two natural divisions of plants are involved in aquatic phytoremediation; the purely aquatic plants such as the floating water hyacinth (*Eichhornia crassipe*) and the terrestrial plants with the submersion of the rhizosphere in which removal of metal pollutants is by rhizofiltration (Brooks and Robinson 1998).

Freshwater vascular plants (FVPs) and macroscopic algae are collectively known as macrophytes (Brooks and Robinson 1998). Differences in trace-element accumulation between aquatic and terrestrial plants species has been reported by Outridge and Noller (1991). In metal accumulation by terrestrial plants, solubilisation of the metal in the rhizosphere is necessary to allow root uptake of the metal. In the aquatic system, the metalloid is already present in a bioavailable form which can be either adsorbed or absorbed by the leaves. The ability of macrophytes to concentrate elements or metals from the aquatic environment was reviewed by Hutchinson (1975). He reported that levels of potentially toxic elements such as cadmium, lead and mercury were higher compared to that in the surrounding aqueous medium.

The basis of terrestrial phytoremediation is the accumulation of metals by plants. There are more terrestrial plants than aquatic plants and aquatic plants suitable for metal accumulation are not always available. However, in spite of these drawbacks, the use of 26 genera of aquatic plants in phytoremediation has been documented (Guntenspergen *et al.* 1989). Examples of these are Water hyacinth (*Eichhornia crassipe*) (Outridge and Noller 1991), Duckweed (*Lemna trisulca* L.) (Wahaab *et al.* 1995; Bonomo *et al.* 1997; Zayed *et al.* 1998; Prasad *et al.* 2001), *Typha* and *Phragmites* (Mungur *et al.* 1997; Ait Ali *et al.* 2004), Umbrella plant (*Cyperus alternifolius* L.), Water zinnia (*Wedelia trilobata* Hitchc.), Smartweed (*Polygonium hydropiperoides* Michx.), Water lettuce (*Pistia straiotes* L.), Mare's tail (*Hippuris vulgaris* L.), Stripped brush (*Baumia rubiginosa*), Monkey flower (*Mimulus guttatus* Fisch.) (Qian *et al.* 1999), Parrot's feather (*Myriophyllum*

sp.) (Qian *et al.* 1999; Kamal *et al.* 2004; Skinner *et al.* 2007), Creeping rose (*Ludwigina palustris*), Water mint (*Mentha aquatic*) (Kamal *et al.* 2004; Skinner *et al.* 2007), Water fern (*Azolla caroliniana*) (Skinner *et al.* 2007).

1.4.5 Hyperaccumulation by algae: Phycoremediation

Most of the literature dealing with metal accumulation by algae are concentrated on seaweeds. For example, it has been reported that the brown seaweeds *Sargassum* (gulf weed), *Egregia* (feather boa kelp), *Macrocystis* (giant kelp) and *Nereocystis* (bull kelp) accumulate arsenic to the highest level (Dunn 1998). Nonetheless, a freshwater filamentous alga (*Mougeotia sp*.), has been reported to be a metal hyperaccumulator (John 2003). Laboratory culture and field investigations showed that this green alga was able to sequester iron and aluminium (John 2000).

The term phycoremediation was introduced by John (2000) referring to the remediation by algae. He defined phycoremediation as "the remediation of aquatic environments by the use of algae as tools, resulting in the enhancement of water quality, including reduction of heavy metals, leading to the sustainable development of aquatic systems." Phycoremediation has also been defined to include the process of nutrient removal by Olguín (2003). Dresback *et al.* (2001) also defined phycoremediation as the use of algae to remove pollutants from the environment or to render them harmless. In a study using two green algae, they concluded that these algae have the capability to degrade Trichloroethylene (TCE).

1.4.6 Hyperaccumulation by charophytes

Another group of algae found to accumulate metals are the charophytes.

Hyperaccumulation of metals by charophytes was initially reported by Hutchinson (1975). They were found to contain more manganese than any other plant material. Outridge and Noller (1991) found that the level of manganese within the mucilage and plant tissue of *Nitella hyalina* was very high.

Nitella congesta (R. Br.) A. Br. a charophyte (initially reported as *Nitella hyalina*) was discovered by John (2003) to have the ability to hyperaccumulate metals such as P, N, Ca, Mg, Mn, Fe, Cu and Zn from lake water and sediment at the RGC Wetlands. This was after several years of observation and laboratory findings (John 2004, pers. com). *N. congesta* is a rare species, endemic to Australia and Tasmania. It is one of the many charophyte species that produces mucilage covering the vegetative growing tip of the alga in the form of a sheath (Wood and Imahori 1965). The mucilage is suspected to be made up of a mucopolysaccharide probably containing alkaline phosphatase (John and Gayton 1994). It was shown that in the lakes where *N. congesta* was found to be growing, the plant tissue contained much higher amount of metals than the surrounding water (John and Gayton 1994). Another study showed high concentration of metals (Al, Fe, Ca and Mn) in the plant tissue and mucilage of *N. congesta*, with calcium concentration being the highest (Burkett 1998). This supports the assertion that charophytes may require calcium for the maintenance of cell wall integrity, by increasing its rigidity and minimising its susceptibility to damage at higher calcium concentrations (Starling *et al.* 1974).

Despite all these findings about the accumulation abilities of *Nitella congesta*, there has been very little effort to use them for the remediation and rehabilitation of degraded wetlands. Although charophytes are widespread, little is known about their ability to improve water quality and rehabilitation. Much study have been conducted on the establishment of *Nitella congesta* as a suitable submerged macrophyte in the lakes of Capel most of which concentrated on its colonization of lakes. However, not much knowledge exists on its use as a tool for phycoremediation which according to John (2000) leads to the sustainable development of aquatic systems. Also in all attempts to rehabilitate the sand mine wetlands, no consideration has been made to employ a biological component of the lakes with the ability to effectively and efficiently develop the various components of the aquatic systems concurrently. It is expected that the use of *Nitella congesta* as a tool for phycoremediation will result in a cost-effective, efficient and effective method for the rehabilitation of aquatic ecosystems.

1.5 Introductory overview of charophytes

Charophytes are a group of green algae with apical growth and differentiation of the body into nodes and internodes. Charophytes are very complex in their vegetative and reproductive morphology, with chlorophyll a and b, apical growth and an oogamous life cycle (García 2001; García 2002). They are macroscopic, multicellular green algae found in permanent and temporary waters (Casanova and Brock 1996). They are common submerged algae in freshwaters (with only a few species occurring in brackish water (Lee 1989) where they grow in shallow, quiet or gently flowing waters (Wood and Imahori 1965; Bold and Wynne 1978). Many of them are significantly calcified, with concentration of meadows at the bottom of lakes historically resulting in the formation of marl ($CaCO_3$ and $MgCO_3$) and hence their common name, Stoneworts (Lee 1989). They form a conspicuous part of the submerged aquatic vegetation in many lakes, ponds and reservoirs. Their propagation is by both vegetative means (bulbils, vegetative fragments) and oospores (de Winton *et al.* 2004). Charophytes have gained recognition for ecological value (Coops 2002) though their ecological significance has been overlooked for many years until recently, and their role in the conservation and restoration of water bodies is being recognized only recently (van den Berg *et al.* 1998).

Charophytes occur on all continents except Antartica. There are approximately 440 taxa existing world-wide with 62% being endemic. Proportions of taxa are, *Nitella* 216; *Chara* 194; *Tolypella* 15; *Lamprothamnium* 8; *Lychnothamnus* 4 and *Nitellopsis* 3. Australia has a total of 62 taxa, with *Nitella* 35; *Chara* 24; *Lamprothamnium* 1; *Lychnothamnus* 1 and *Tolypella* 1 (Khan and Sarma 1984; García and Chivas 2006). The primary centre of origin for almost all six genera of charophyte was considered as the Indian sub-continent. The gradual divergence of geographical separation of populations may have resulted in speciation (Khan and Sarma 1984).

1.6 Geographical distribution of Australian charophytes

Of the charophyte species in Australia, 26% have a wider distribution outside Australia and the Western Pacific, 12% are found in the Western Pacific region (Australia, New Zealand, Pacific Islands and South East Asia) whiles 62% are endemic to Australia (García 2001; García 2002; García and Chivas 2006). Major characteristics of the Australian charophyte flora are high proportion of (1) dioecious taxa (≈ 50% of all species present in Australia), (2) endemic taxa (≈ 60%) and (3) non-gyrogonites producing taxa (≈ 80% of the species present in Australia) (García 2001; García 2002; García and Chivas 2006).

1.7 Aims and objectives of research

The principal aim of this research is to investigate the ability of the charophyte to enhance the rehabilitation of sand mine-wetlands at Capel, Western Australia into productive wetlands. The specific objectives are:

- to study the life cycle pattern of *N. congesta*, in relation to the hydrological regime of the Capel Wetlands, as a suitable macrophyte for rehabilitation;
- to investigate the role of *N. congesta* in the ecological rehabilitation of sand mine wetlands at Capel, by focusing on the limnology, macroinvertebrates and diatom communities associated with the charophyte;
- to investigate the ability of *N. congesta* to (hyper)accumulate heavy metals;
- to investigate the impact of eutrophication on the establishment of *N. congesta* in the lakes at the Capel Wetlands Centre.
- to investigate the impact of acidification on *N. congesta*.

1.8 Structure of thesis

This thesis is comprised of nine chapters which have been structured in the following order;

- The first chapter details the scope of the thesis, an introduction and background information and literature survey and objectives of the project.
- Chapter two gives a description of the location of the research, its historical background, climate, hydrology and biological resources.
- Chapter three deals with the identification of the charophyte of interest, its morphology, taxonomic features, growth and life-cycle pattern in relation to the hydrological regime of the lakes at Capel.
- Chapter four deals with the ability of *N. congesta* to hyperaccumulate heavy metals from the sediment and water of the lakes.
- Chapter five examines the macroinvertebrate diversity and abundance associated with *N. congesta* in the wetlands.
- Chapter six investigates the impact of eutrophication on the establishment of *N. congesta* meadows.
- Chapter seven was devoted for the acidification of the lakes observed at the end of the study after prolonged drought. This chapter looked at the possible causes of the lake acidification and its impact on the establishment of *N. congesta*.
- Chapter eight deals with diatoms as indicators for hyperaccumulation of metals and acidification of the lakes at Capel Wetlands Centre.
- The final chapter is comprised of a general discussion, conclusion and recommendations.

Summary

The scope of the study, introduction, background information and literature survey, the objectives of the study and structure of the thesis were discussed in this chapter. The following chapter discusses the site of the research, its geographical location, historical background and climate.

2 THE STUDY SITES AT CAPEL WETLAND CENTRE

2.0 Introduction

The town of Capel (33°S, 115°E) is midway between the regional centre, Bunbury and Busselton, 200km south of Perth (32°S, 115°E), Western Australia.

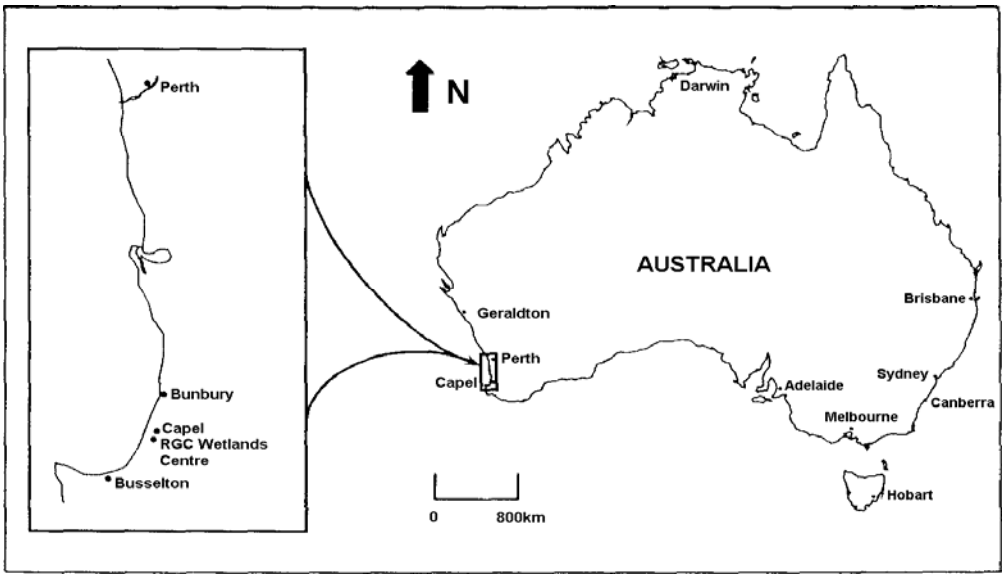

Figure 2.1 Map of Australia showing site.

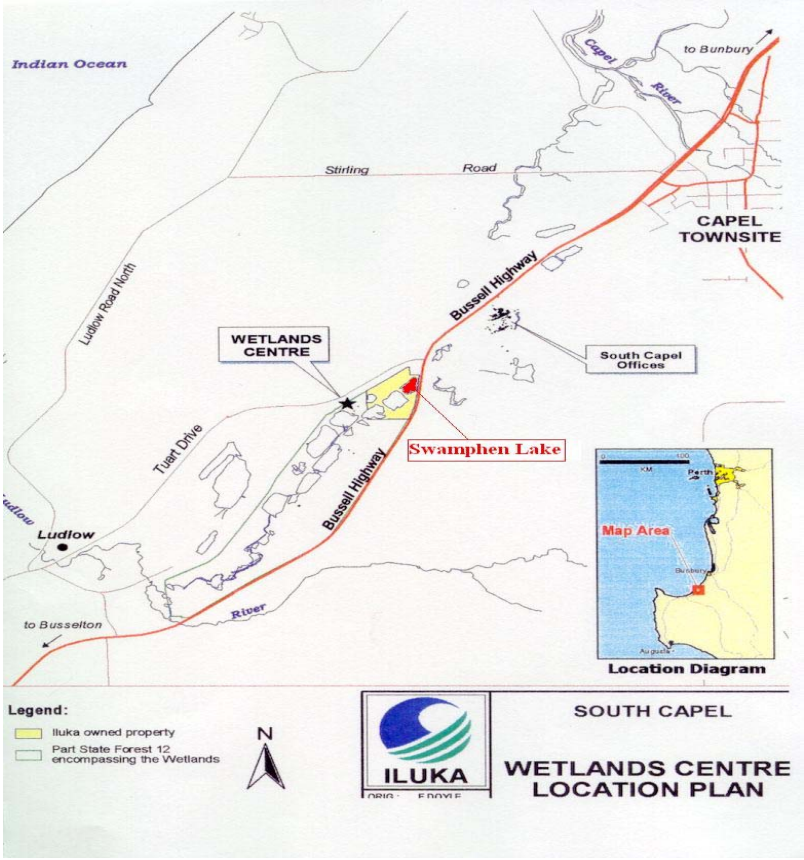

Figure 2.2 Location of Capel Wetlands Centre.

2.1 Sandmining at Capel and the creation of artificial lakes

Since 1956, sand deposits, mainly titaniferous minerals, ilmenite and rutile have been mined in Capel (Brooks 1992; Qui *et al.* 2002). The mining was of very high grade but there was significantly no backfilling. The mining operations were extensive with the removal of large quantities of sand from particular areas that were rich in ore bodies. The mining pits created after the extraction of mineral sands intercepted the water table with the resultant formation of mine voids pits. These pits were later converted into artificial wetlands between 1975 and 1979.

2.2 The Capel Wetland Centre

The Capel Wetlands Centre (CWC) was originally known as RGC (Renison Goldfields Consolidated) Wetlands. The Capel Wetlands Centre was established in 1985, replacing a pine plantation and pasture, for water birds conservation site (Davies 2002). The area has been subjected to a number of

disturbances including clearing, agriculture and mining. The mining company RGC later merged with Iluka Resouces in 1998. The Capel Wetlands Centre consists of a chain of 15 artificial water bodies, making up a total area of 52 hectares (Fig. 2.3). The source of water was retention ponds used during mineral sand mining operations for mineral processing at one end, with an outlet discharge point at the other end (Davies 2002). The surrounding areas of these mine pits had been cleared resulting in banks too steep for possible re-vegetation. Rehabilitation was not a high priority with the initial plan to keep the surrounding land for pasture while planting trees around the wetland (Davies 2002).

After consultation with the various stakeholders the following objectives for creating the wetland centre were set out by the management committee;

- To develop a self-sustaining wetland ecosystem for the conservation of water birds in mine-void lakes at Capel.
- To facilitate research into wetland ecosystems including their development and management.
- To develop the potential and facilities for public education and recreation at the Capel Wetlands.
- To develop and demonstrate rehabilitation technology for wetlands created by human activities.

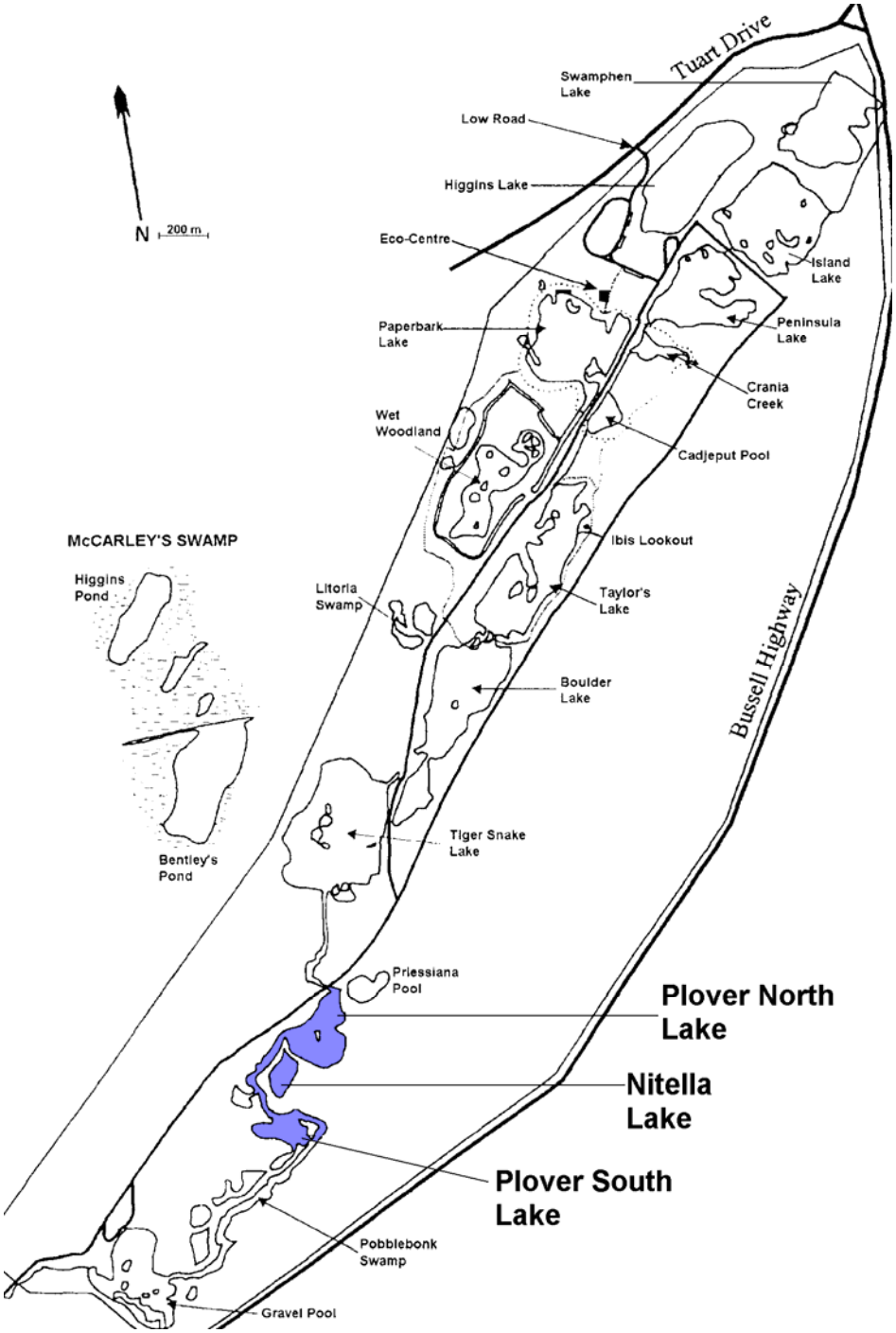

Figure 2.3 Chain of lakes that make up the Capel Wetlands Centre.
The lakes coloured blue are the three main lakes selected for the project.
Northern lake groupings = Swamphen, Island, Peninsula, Cadjeput lakes
Middle lake groupings = Paperbark, Taylor's, Boulder and Tiger Snake lakes
Southern lake groupings = Nitella, Plover North, Plover South lakes and Gravel Pool.

2.2.1 Climate, groundwater and lake levels

The average annual rainfall for the Capel region is 730.3 mm and the climate is Mediterranean, which is characterised by dry summers and wet winters. (Bureau of Meteorology, 2008).

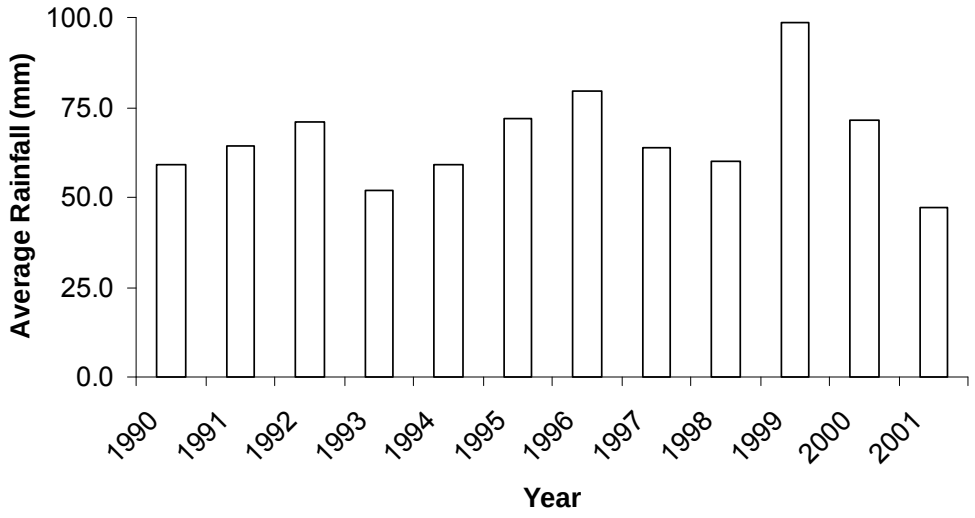

Figure 2.4 Annual average rainfall for Capel Wetland Centre from 1990 to 2001.

Surface-water bodies are in one way or the other connected to groundwater in most types of landscapes. Thus surface-water bodies serve as an integral part of groundwater flow systems. In nearly all landscapes, ranging from small streams, lakes, and wetlands, groundwater interacts with surface water in headwater areas. As a general assumption, topographically high areas are groundwater recharge areas whiles topographically low areas are groundwater discharge areas (Winter 1999).

Hydrologic processes associated with the surface water bodies themselves, such as seasonally high surface-water levels and evaporation and transpiration of groundwater from around the perimeter of surface water bodies, are a major cause of the complex and seasonally dynamic groundwater flow fields associated with surface water (Winter 1999). The movement of surface water and groundwater is controlled mainly by topography and the geologic framework of an area.

The source of water to and losses of water from the earth's surface are controlled by climate. Thus it is important to understand the effect of interaction between topography and climate on the groundwater flow system of an area (Winter 1999). Consulting hydrologists (Nield and Townley 1987) showed that the groundwater movement at the Capel Wetlands Centre is from east to west and leans north to south towards the Ludlow River (Fig. 2.2). They also suggested that the groundwater table level on which the lakes of the wetland depend would fall with the eventual stoppage of processing water into the lakes with a resultant shift from permanent lakes to seasonal lakes. Lake water levels measured from 1991 to 1998 showed that there was a remarkable fluctuation by Swamphen Lake as a result of isolation from the input of processing water (Qui *et al.* 2002). Island lake on the other hand showed a constant level until after August 2005 where there was a fluctuation similar to that of Swamphen Lake. Local groundwater hydrology was monitored by a series of piezometers located within the Capel Wetlands Centre (Staaden 2002); though depths were not known from literature. Piezometers are instruments that allow the measurement of hydraulic head at a certain depth of an aquifer (Freeze and Cherry 1979). They are pipes with slotted screens at one end, which are then inserted into the ground to the depth at which the hydraulic head of the water table is to be measured (Sanders 1998).

In 1994, piezometers were used to measure the cross-flow transverse around the middle, the northern as well as the eastern ends of the chain of lakes and bore data was then recorded in meters to Australian Height Datum (AHD) (Staaden 2002). The Australia Height Datum is a geodetic datum for altitude measurement in Australia. The depths of the lakes range from 1 m to more than 6 m and the water level depicts the seasonal fluctuation of the ground water table (Brooks 1992).

2.2.2 Wetland dimensions

Based on the height fluctuations and location within the Capel Wetlands Centre, the 15 lakes were arranged into three main groups to determine the

water balance of the wetland (Staaden 2002). The lakes were earlier divided into three groups based on correlation of their lake levels and sensitivity to receiving the plant discharge (Qui *et al.* 2002). Island, Peninsula and Cadjeput lakes were more sensitive to the discharge with their lake levels influenced by the timing and point of discharge. Paperbark, Taylor, Boulder, Tiger Snake, Plover North, Plover South and Gravel Pool (Fig. 2.3) lakes maintained stable and similar water levels irrespective of the point of plant discharge. Swamphen, Litoria and Wet Woodland lakes were however isolated from the discharge with their lake level fluctuations occurring seasonally and on a larger scale than the other groups (Qui *et al.* 2002). The groupings had the northern group comprising of Swamphen, Island, Peninsula and Cadjeput lakes as a result of their different lake heights (ADH) and fluctuations compared to the second and third groups (Staaden 2002). The second group comprised of Paperbark, Taylor's, Boulder and Tiger Snake lakes apparently located in the middle of the Capel Wetlands Centre (Fig. 2.3). The third group comprised of Nitella Lake, Plover North, Plover South and Gravel Pool. In the study, the surface areas and volume of the lake-groups were calculated by summing the surface areas or the volumes of the individual lakes in each group. The dimensions of each lake-group are displayed in Table 2.1 below.

Table 2.1 The dimensions of lake groups in the Capel Wetland Centre.

Physical parameters	**Lake Groupings**		
	Northern	**Middle**	**Southern**
Mean surface area ($\times 10^3$) (m^2)	68.2	190.2	26.4
Mean radius (m)	147.2	246.0	91.7
Mean diameter (m)	294.4	492.1	183.4
Perimeter (m)	924.9	1545.9	576.2
Mean volume ($\times 10^3$) (m^3)	77.2	334.3	15.4

Adopted from Staaden (2002)

Table 2.2 Physical parameters of some lakes at the Capel Wetland Centre studied in June/July 1999.

	LAKES									
PARAMETERS	**Swamphen**	**Island**	**Peninsula**	**Paperbark**	**Cadjeput**	**Taylor's**	**Boulder**	**Tiger Snake**	**Plover North**	**Gravel Pool**
Maximum water level (AHD)	15.20	15.40	11.80	10.50	11.20	10.30	10.40	10.40	10.30	10.20
Plan area @ max. Water Level ($\times 10^3$) (m^2)	22.8	41.8	25.1	38.1	5.3	52.0	52.1	59.7	12.6	19.5
Volume of water @ max. Water Level ($\times 10^3$) (m^3)	25.5	59.3	27.8	68.4	5.0	108.0	152.6	119.3	11.4	19.5
Maximum depth @ max. Water Level (m)	2.10	3.00	2.30	3.10	3.10	3.50	4.50	3.90	1.50	1.70
Average depth @ max. Water Level (m)	1.10	1.40	1.10	1.80	0.90	2.10	2.90	2.00	0.90	1.00
Minimum water level (AHD)	13.40	13.80	11.30	9.30	10.50	9.20	9.20	8.80	9.40	9.20
Plan area @ min. Water Level ($\times 10^3$) (m^2)	2.0	14.2	21.2	27.5	3.6	43.2	46.8	40.2	6.6	12.7
Volume of water @ min. Water Level ($\times 10^3$) (m^3)	0.3	11.3	16.2	25.9	1.7	54.4	92.2	37.1	1.8	3.1
Maximum depth @ min. Water Level (m)	0.30	1.40	1.80	1.90	2.40	2.40	3.30	2.30	0.60	0.70
Average depth @ min. Water Level (m)	0.15	0.80	0.80	0.90	0.50	1.30	2.00	0.90	0.30	0.20
Volume of Slime ($\times 10^3$) (m^3)	64.8	102.4	53.5	30.0	1.6	55.7	28.1	56.7	11.2	2.2
Maximum thickness of slime (m)	6.00	6.20	4.20	4.40	1.10	2.50	1.60	3.10	1.70	0.50
Average thickness of slime (m)	2.80	2.40	2.10	0.80	0.30	1.10	0.50	0.90	0.90	0.10

Adopted from (Surveys 1999)

2.3 Baseline studies at the Capel Wetland Centre

In order to provide a basis for planning future development of the wetlands, baseline studies were undertaken in 1987 to establish base line status of the wetlands. This was done by using nearby wetlands as control sites (Brooks 1992). It was established from hydrological studies that the water levels of the lakes were artificially being maintained by the input of wastewater from the mine processing. This was an indication that the water levels were not in line with the seasonal fluctuations of the ground water table thus negatively affecting the development of fringing vegetation and invertebrates that depended on the lakes (Brooks 1992; Qui *et al.* 2002). Results of analysis showed that the lakes had low pH (3-4), very high ammonium levels (22,300-82,700 µg L^{-1}), low phosphorus levels (4-20 µg L^{-1} total P) and high concentrations of iron, manganese and sulphates (Brooks 1992; Doyle

2000). It was found that phosphorous from the water body was being removed by iron and magnesium compounds within the sediment thus accounting for the low phosphorous concentrations in the lakes. Coupled with these chemical factors, steep slope, hard substrate and poor water quality all contributed to the low productivity of the lakes. Therefore the development of emergent and submerged vegetation as well as invertebrates, were inhibited by the above stated factors. Also the lakes lacked oxygen and temperature stratification and showed limited diurnal changes (Brooks 1992).

Studies of water bird was conducted from November 1984 to November 1986 (Doyle and Davies 1998; Doyle 2000). A total of 24 bird species were recorded to be using the wetlands during this time frame with the natural wetlands attracting more species. The study indicated that the main problem was the lack of habitat supplying food and shelter. Apparently, the lakes were used by the birds as a refuge in dry summer with some birds breeding on the lake system though with virtually little success (Brooks 1992). The studies also showed lack of sufficient aquatic invertebrates thus contributing to the poor breeding success of birds since the aquatic invertebrates could serve as a protein source for the birds. Overall, the lakes did show a high species richness, however, with low abundance as compared to the natural wetland (Brooks 1992).

Another result from the study showed the algal populations of the lakes depicting the poor water quality, with diatoms dominating with a well-established benthic diatom community of low diversity (Brooks 1992; John 1993b). The species of diatom recorded were indicators of low pH with little biological symptoms of eutrophication. Out of the 67 algal taxa recorded, 55 were diatoms, whereas a nearby natural wetland displayed a maximum species richness of 62 taxa. These results confirmed that the lake system was in a relatively underdeveloped state (John 1988; Brooks 1992).

2.4 Development of specific habitat at the Capel Wetland Centre

Results from the baseline studies supported the need for the lakes to be developed into a wetland ecosystem and to achieve that there was the need for improved productivity at all levels of food chain (Brooks 1992). Initial priority was given to water and sediment chemistry, re-shaping of shorelines, the establishment of vegetation as well as the development of specific habitat needs of fauna, including invertebrates, birds and other vertebrates (Brooks 1992; Doyle 2000). To be able to address the above issues, a plan was adopted not only to see to habitat development but also to attend to the need for public facilities such as visitor's car park, picnic areas, walking trails and bird hides. This called for an extensive site development which was carried out in 1987 thereafter a continuous development has been regular and gradual over the years. Public facilities were provided as the wetland's productivity developed making the wetlands more aesthetically attractive (Brooks 1992). Each species of water bird has specific habitat requirement for breeding, feeding and loafing. Therefore, in planning the habitat development of Capel Wetlands Centre, all these were taken into consideration. As a result, various habitat types such as trees, reeds; food sources such as macrophytes, invertebrates, frogs, reptiles and loafing areas, shallow and deep were all considered during the habitat development (Brooks 1992).

2.5 Water quality at the Capel Wetland Centre

It was found that poor water quality of lakes at the Capel Wetlands Centre was a contributing factor limiting their productivity. This was associated with the quality of effluent water that flowed into the former mine pits from the mineral processing plant. In order to address the water quality problem, there was an improvement of the mineral processing plant between 1987 and 1989 with a resultant improvement of water quality of the lakes (John 1993a; Doyle 2000). Nitrogen and phosphorus levels also required substantial improvement as a result of release of ammonium in effluent streams (Brooks 1992). The low pH of some of the lakes, for example the pH of 2.4 for Island lake in 1987 was due to the lake serving as the entry point for the acidic

mineral sands processing water (Brooks 1992). By raising the pH and decreasing the concentrations of soluble ions such as Fe^{2+}, Mn^{2+} and SO_4^{-2}, there was an increased species richness of algae thus highlighting the need for biomonitoring the development of the Capel Wetlands Centre (John 1993b).

Table 2.3 Average water chemistry for Mineral Sands Mining Lakes from 1987 to 1992.

Parameter	Year					
	1987	**1988**	**1989**	**1990**	**1991**	**1992**
Mean pH	3.64	6.82	7.53	7.43	7.50	7.60
Fe (mg/L)	1.40	0.40	0.30	0.05	0.24	-
Mn (mg/L)	22.00	9.40	0.30	0.10	0.21	-
Ca (mg/L)	>350	250	250	<350	<350	350.00
SO_4^{-2} (mg/L)	1003	866	1360	856	929	-
PO_4^{-3} (mg/L)	0.001	0.001	<0.015	<0.015	0.015	-
NO_3^{-1} (mg/L)	0.01	0.10	<0.05	<0.05	0.50	-
SiO_2 (mg/L)	1.50	0.50	0.50	0.50	0.50	-
$NH4^+$ (mg/L)	>50	<50	<50	<50	<50	-
Cl^- (mg/L)	>500	<500	<500	<500	250.00	-
Conductivity (mS/cm)	>5000	4000	<4000	<4000	<4000	3499

Adopted from John (1993b) and Doyle and Davies (1998)

2.6 Selection of lakes for the study

Among the chain of 15 lakes, Nitella Lake (33^o 36.10S, 115^o 30.23E), Plover North Lake (33^o 36.09S, 115^o 30.33E) and Plover South Lake (33^o 30.17S, 115^o 30.25E) were selected due to the fact that *Nitella congesta* was found to be well established in these lakes for the past few years (John 2004, pers. com). Plover North and Plover South lakes are interconnected by a drainage channel whiles Nitella Lakes stood alone (Fig. 2.3). There was the inclusion of some other lakes such as Taylor's lake, Gravel Pool, Tiger Snake lake, Penninsula lake, Island lake, Swamphen lake, Boulder lake and Higgins lake in the study as a basis for comparison with the selected study lakes.
Photographs of some of the lakes are shown in figures 2.5 to 2.10.

Figure 2.5 Plover south lake in winter 2006.

Figure 2.6 Nitella lake in winter 2007.

Figure 2.7 Nitella lake in summer 2007.

Figure 2.8 Plover North lake in winter 2006.

Figure 2.9 Island Lake in summer 2007.

Figure 2.10 Pobble Bonk Lake in summer 2007.

Table 2.4 Activities performed in the lakes.

LAKE	ACTIVITIES
Nitella Lake	- Growth rate and life cycle studies of *N. congesta* conducted in 2004. - Sediment and water collected for the laboratory culture of *N. congesta* in 2004 and 2005. - *N. congesta* sampled for hyperaccumulation of metals in 2004. - Macroinvertebrates sampled in 2004 and 2005. - Diatom assemblages sampled in *N. congesta* mucilage in 2004 and standing water in 2004 - 2007.
Plover South	- Growth rate and life cycle studies of *N. congesta* conducted in 2004. - Sediment and water collected for the laboratory culture of *N. congesta* in 2004 and 2005. - *N. congesta* sampled for hyperaccumulation of metals in 2004. - Diatom assemblages sampled in *N. congesta* mucilage in 2004 and standing water in 2004 - 2007.
Plover North	- Growth rate and life cycle studies of *N. congesta* conducted in 2004. - Sediment and water collected for the laboratory culture of *N. congesta* in 2004 - 2007 - *N. congesta* sampled for hyperaccumulation of metals in 2004. - Diatom assemblages sampled in *N. congesta* mucilage in 2004 and standing water in 2004 -2007.
Higgins	- Macroinvertebrates sampled 2004 and 2005. - Diatom assemblages sampled in standing water in 2004 - 2006.
Island, Taylor's, Cadjeput, Penninsula, Boulder's, Tiger Snake, Gravel Pool, Swamphen, Paperbark	- Diatom assemblages sampled in standing water in 2004 - 2007.

Summary

The Capel Wetlands Centre was established in 1985 for the rehabilitation of large pits left after sand mining to attract water birds. In this chapter, the historical development of the site, its climate, hydrology, biological resources and rehabilitation techniques employed for the successful rehabilitation programme has been discussed. The lakes sampled for various research activities have been described. The next chapter deals with the identification, morphology, growth and life cycle studies of *Nitella congesta,* the dominant macrophyte in the lakes.

3 GROWTH, MORPHOLOGY AND LIFE CYCLE STUDIES OF *Nitella congesta*

3.0 Introduction

The growth and reproduction of charophytes is affected by temperature, light availability and water level. Both water regime and the life-cycle of individual species influence the allocation of resources to vegetative growth and sexual reproduction (Casanova 1994). Increase in temperature between spring and summer results in the diversion of plant resources to enhance sexual reproduction instead of vegetative growth (Casanova 1994).

Fluctuations in water level affect charophyte growth; a rise in water level results in an increase in vegetative growth. On the other hand, after an appropriate level of vegetative growth, a decrease in water level stimulates sexual reproduction (Casanova 1994). Charophytes exhibit apical growth and basal decay in water bodies deeper than six meters, thereby ensuring resources to be concentrated in the actively growing region (Andrews *et al.* 1984a).

Charophytes can be classified as annuals or perennials depending on the life-cycle pattern (Casanova and Brock 1999). In the case of annuals, resources are used for early sexual reproduction with subsequent prolific production of spores instead of vegetative growth and shoot proliferation to ensure species succession (Casanova and Brock 1999). Perennial species on the other hand, tend to allocate more resources to vegetative growth. In this case they proliferate by vegetative means and, produce bulbils (vegetative reproductive organs) in summer and autumn; shoots re-establish from bulbils in spring and after floods and sexual reproduction is stimulated by decreasing water depth (Casanova 1994).

3.1 Morphology of charophytes

The morphological features of charophytes used here were adopted from Sharma (1986) and Bryant and Stewart (2002) unless otherwise stated. The entire thallus is differentiated into rhizoids and the main axis with branchlets. The rhizoids are whitish, thread-like, uniseriate (arranged in one row or series), multicellular and branched. They function to anchor the alga in the

sediment. The main axis is erect, long, branched and differentiated into nodes and internodes. There are four types of appendages of which some or all develop from the nodes of the main axis and branchlets. These appendages are branchlets, bracteoles, bract-cells and stipulodes (Fig. 3.1).

- **Branchlets**

Branchlets are the whorled laterals of limited growth. They have nodes and internodes and in many species of *Chara*, *Lamprothamnium*, *Lychnothamnus* and *Nitellopsis* they are undivided. Branching in *Nitella* is by forking and is sympodial but monopodial in *Tolypella*.

- **Bract-cells**

The bract-cells vary in length and if on the inner side of the branchlets (i.e. the side facing towards the main branch), they are adaxial; if they are on the outer side they are abaxial. When they are well developed, the bract-cells can be mistaken for small side branches.

- **Bracteoles**

Bracteoles are elongated, unicellular processes that are found one on either side of the oogonium in *Chara*, *Lamprothamnium* and *Lychnothamnus*. They resemble the bract-cells but may be more slender and slightly longer. In dioecious species of *Chara* there is another small unicellular process called bractlets that lie immediately beneath the oogonium (taking the place of the absent antheridium).

- **Stipulodes**

Stipulodes are unicellular outgrowths (of varying length and size in the different species) produced from the outermost cells of the branch nodes and lie immediately beneath the branchlet whorls in *Chara*, *Lamprothamnium* and *Lychnothamnus*. Depending on the number and arrangement of the stipulodes if present, there are various conditions recognised. If one stipulode is present, the condition is termed as unistipulate. The presence of two stipulodes is termed as bistipulate. The stipulodes either form a single ring (haplostephanous) or a double ring (diplostephanous). When elongated and

in double ring they may be more or less adpressed and point upwards and downwards from the nodes. The number of stipulodes in each ring is equal to the number of primary cortical rows below and to the number of branchlets above the axial node.

In most species of *Chara* and *Lychnothamnus* the axis (and branchlets in *Chara*) are overlaid by cells adpressed to the internodal cell to produce a cortex, which appears as lines along the axis. The structure of the cortex is diagnostic in determining the species of *Chara*.

- **Rays**

The branchlets of *Nitella* are divided one or more times. The lowest segment (the internode nearest the main axis) is termed the primary ray and subsequent segments are known in turn as secondary, tertiary etc. The end segment (ultimate ray) is known as the dactyl and these may be single-celled or made up of two or more cells. All the rays in *Nitella*, apart from the dactyl are single-celled. The branchlets of *Tolypella* may be divided or, if simple, made up of several segments but lack dactyls.

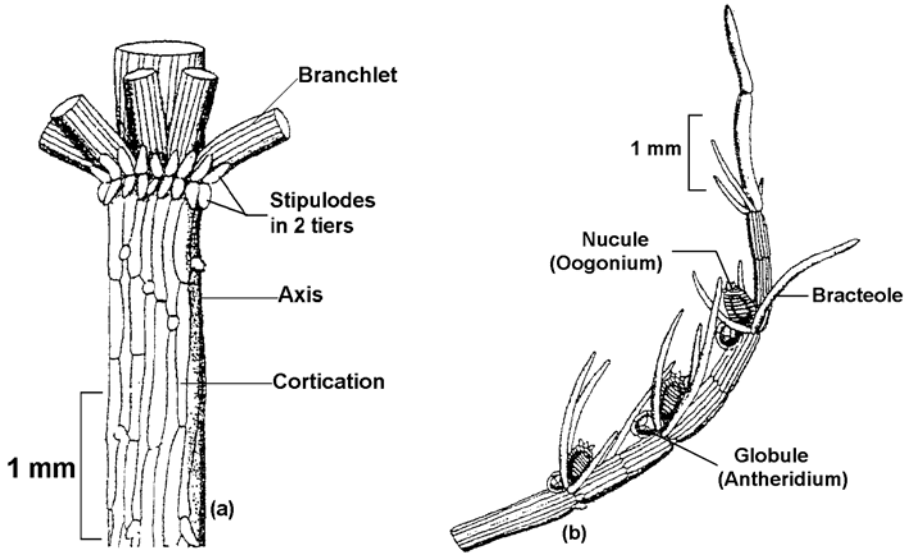

Figure 3.1 General morphology of charophytes showing characteristics of *Chara* sp.
(a) Part of the axis showing axial node with 2-corticated cortex and stipulodes
(b) Branchlet with conjoined gametangia at nodes (lateral).
Adopted from Wood and Imahori (1965).

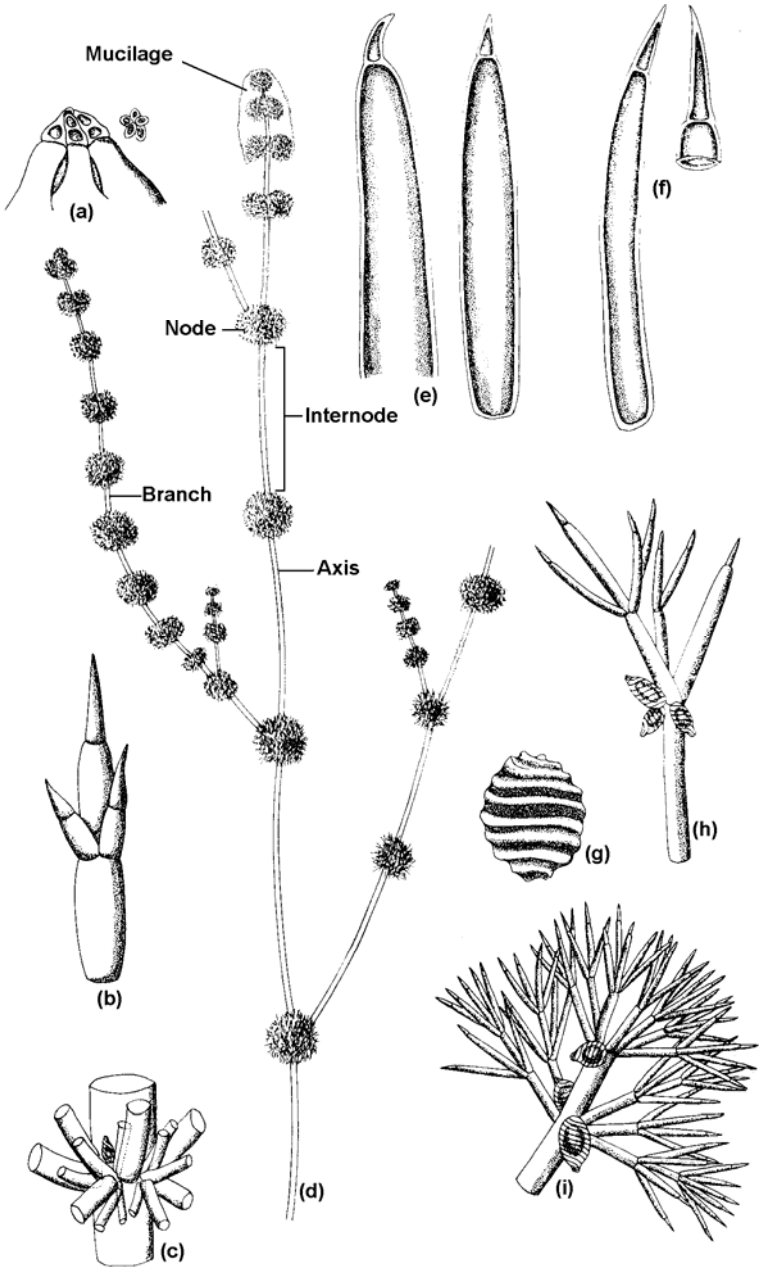

Figure 3.2 General morphology of charophytes showing characteristics of *N. congesta*. (Adopted from Wood and Imahori 1965)

(a) Coronula side and top; (b) Small sterile branchlet (from a fertile whorl)

(c) Axial node with numerous small branchlets arising between large ones and an oogonium replacing small branchlet.

(d) Habit; (e) and (f) Dactyls and end cells

(g) Oospore; (h) Small fertile branch

(i) Branchlet with oogonia and percurrent central ray.

3.2 Taxonomy of charophytes

The taxonomy of charophytes is based on various criteria including morphological features, the ultrastructure of the oospore wall and chromosomes numbers (John and Moore 1987; Casanova 1997; Sakayama *et al.* 2002; Sakayama *et al.* 2004; Sakayama *et al.* 2005). The oospore of charophytes is protected by a sheath of five spirally disposed cells known as the tube cells and when fertilised, there is the formation of a resistant, multi-layered wall. Ridges, which ascend spirally from right to left are formed from the lower lateral walls of these spiral cells and later develop into a prominent flange or wing-like protrusion. The area of the wall lying between each spirally disposed ridge is called the fossa. The fossa is often ornamented and this is a species-specific feature. Fully mature oospores enhance adequate investigation of their ornamentation, offering reliable information than non-mature ones since the latter either lack, or have weakly developed ornamentation (John and Moore 1987).

The number and height of ridges, shape, length and width of oospore, presence of a flange, colour, width of fossa and oospore ultrastructure are some of the characteristics of the oospore used for taxonomic identification. Membrane ultrastructure is described as smooth, granulate, verrucate, reticulate, finely or minutely granulate or minutely reticulate (Haas 1994). Using three species of *Chara*, the taxonomic value of the different characteristics of oospores variation within species and populations was investigated by Casanova (1997). It was observed that within species and populations, characteristics of the oospores such as length, width and number of striae or flanges varied considerably, whereas the ultrastructure of the oospore wall showed consistency in each species.

3.3 The ecology of charophytes

Charophytes are often pioneer colonisers of newly created or inundated wetlands (Casanova and Brock 1996) forming a conspicuous part of the submerged aquatic vegetation. Their principal ecological role is always near the base of the trophic pyramid serving as primary producers (Mann *et al.*

1999). They are common submerged macrophytes in freshwaters (with only a few species occurring in brackish water (Lee 1989)) and have gained recognition for their ecological value (Coops 2002). Charophytes are known to give way to angiosperms and other macrophytes with increasing eutrophication (Blindow 1992a; van den Berg *et al.* 1998) finally disappearing from the water bodies with extreme turbidity (van den Berg *et al.* 1998; Kufel and Kufel 2002). The range of aquatic environments occupied by charophytes, coupled with their response to lake restoration, make them relevant in the management of wetlands (Coops 2002). The growth and mortality of charophytes may be influenced by factors such as temperature, water depth and transparency, pH, flow and turbulence, the presence of herbivores, shading by other macrophytes and nutrient concentrations in the water and sediment (Meiers *et al.* 1999; Casanova and Brock 1999). Some charophytes are able to tolerate low nutrient levels, high salinities and high metal and mineral ion concentrations; characteristics of many natural and artificial wetlands as a result of poor waste water management and agricultural practices.

3.3.1 Ecological factors that affect distribution of charophytes

Charophyte distribution is affected by different ecological factors some of which have been discussed below;

- ***pH***

Some charophytes species are commonly found in alkaline and hard waters that have high calcium bicarbonate content. The amount of free carbon dioxide in waters with high alkalinity is usually minimal and charophytes found to grow in such conditions have a high photosynthetic rate (Crawford 1977). In such waters, there is precipitation of calcium carbonate ($CaCO_3$) from the internodal cells and is deposited on the charophytes by encrustation. In the process, the effluxion of hydroxyl ions (OH^-) from the internodal cells raises the CO_3^{-2} concentration with a resultant supersaturation and precipitation of $CaCO_3$ (Prescott 1968; Lee 1989; Blindow 1992b). For example, *Chara tomentosa* was found to have $CaCO_3$

concentrations as high as 53% in a calcium rich lake (Blindow 1992b). Such high level of bicarbonate alkalinity and hardness of water can be minimised by corresponding high charophyte productivity. In some instances, it may be possible to remove the calcium carbonate rich deposits and use for commercial purposes such as lime fertiliser and cement manufacturing (Prescott 1968; Crawford 1977).

- ***Salinity***

Charophyte distribution is also influenced by the salinity of water bodies. Some charophyte species are tolerant to a wide range of salinity; such species are known as euryhaline species. An example of such species is *Chara vulgaris* and *Lamprothamnium papulosum* with the latter surviving salinity twice that of seawater. There are other species of charophytes which are completely intolerant of high salinity and are therefore restricted to freshwater bodies. This type of charophytes are said to be obligate freshwater species (Winter *et al.* 1987). The cells of charophytes have large vacuoles with variable ionic composition; the predomin'.;jnv hbjjny ijoj nbant cations composition being Na^{+}, K^{+}, Ca^{+2} and Mg^{+2} while the anion is Cl^{-} (Winter *et al.* 1987; Davenport *et al.* 1996). The salt tolerant species are able to alter the ionic concentration of the vacuolar sap by the accumulation of sucrose. This offers them the ability to control their internal osmotic pressure. Thus euryhaline charophytes are able to regulate their turgor pressure so as to keep the external osmotic pressure constant, when under salinity stress. The ability to control internal osmotic pressure by altering the ionic concentration is a unique physiological adaptation of charophytes which other aquatic macrophytes lack (Winter *et al.* 1987).

- ***Nutrient and Turbidity***

One of the most important factors limiting Characean growth is the amount of nutrients in the water body. Charophytes usually thrive in waters with comparatively low phosphorous concentrations; an exception being *Chara coralina* which can thrive in nutrient rich waters (Wells *et al.* 1998). Studies have shown that charophytes are amongst the first submerged macrophytes that disappear during eutrophication and extreme turbidity (Blindow 1992a;

van den Berg *et al.* 1998; Kufel and Kufel 2002). Established populations of charophytes however, may be able to stabilise moderately eutrophic conditions by rapidly taking up and accumulating phosphorus (Kufel and Ozimek 1994).

- ***Water depth and transparency***

The maximum depth which charophytes spread in wetlands depends on factors such as water temperature, topography which causes shading of the water column, the gradient of the bottom and grazing and trampling pressure from herbivores and other animals. Of all these, the most important factor determining the depth ranges of charophytes is the under water light conditions (Vant *et al.* 1986). There is a wide range of differences in the depth ranges of Characean species. A specie of charophytes, *Nitella opaca* Bruzelius was reported to have thrived at depth up to 40 meters while some species of *Chara* may grow to a depth of up to 30 meters in European and Japanese lakes (Stross 1979).

An increase in depth has a corresponding decrease in light irradiance resulting in the decrease in rates of photosynthesis and respiration (van den Berg *et al.* 1998; Schwarz *et al.* 1999). The ability of charophytes to tolerate lower light levels is dependent on the accumulation of starch which is the carbohydrate food reserve of Characeans. The accumulation of starch takes place before the onset of unfavourable or adverse conditions (Schwarz *et al.* 1999). A decline in the starch reserve to minimum concentrations triggers a decay of the basal cells and the algae get detached from the substrate (Schwarz *et al.* 1999). The growth of charophytes is light inhibited (Crawford 1979). A rapid increase in growth due to favourable light conditions is an important adaptation of perennial charophytes. *Chara* species were observed to be short and bushy in morphology under high light intensity. The algae became pale with long internodes as the water become turbid and decreased light penetration (Crawford 1979). The clarity of the water column also influences the maximum depth of charophytes penetration (Stross *et al.* 1995). Nutrient enrichment of the water can contribute to phytoplankton and periphyton bloom which will eventually reduce water clarity. Reduction in

water clarity can also occur as a result of the influx of suspended sediments by seasonal flooding (Schwarz and Hawes 1997; van den Berg *et al.* 1998). A study by Schwarz and Hawes (1997) showed that when the light irradiance falls below limiting levels, the rate of decline of a Characeae population could be attributed to factors such as the previous light history, the amount of stored photosynthate to serve as an adaptive mechanism to a more shaded environment and the ability to reduce respiration rates.

- ***Mineral and mineral ions***

Depending on the season, sediment type and other factors such as wind and wave action, the concentration of ions in water can undergo fluctuations. The level of ions in water can possibly be increased as a result of flooding of dried sediment at the onset of winter with subsequent rainfalls. The release of ions into water can also occur under strong winds and wave action. In this case the sediment in the shallow region of water bodies is stirred up causing the release of sediment stored ions. In wetlands that have high productivity and subsequently high organic matter, anoxic conditions may develop as a result of excessive microbial action and oxygen depletion. This results in the precipitation of metal ion complexes from the sediment (Ward *et al.* 1997). Charophytes have the ability to survive in waters with fluctuating ionic concentrations. This is due to the fact that their thallus is able to accumulate concentrations of certain ions that would be toxic to most green plants (Ward *et al.* 1997). The growth of most aquatic plants may be retarded by excessive levels of ammonium, sulphate, calcium, iron and manganese present in the waters they inhabit. *Nitella congesta* (mistakenly reported as *Nitella hyaline* by John and Gayton 1994, Ward *et al.* 1997) have established in the Capel Wetland Centre in Western Australia, have a mucilage envelope of mucopolysaccharides secreted around its growing axis meristems (John and Gayton 1994). They speculated that possible presence of alkaline phosphatase in the mucilage might be responsible for the conversion of organic P, HCO_3 and Fe^{+3} into inorganic P, CO_2 and Fe^{+2} respectively for easy absorption. The mucilage sheath covering *Nitella congesta* has an additional advantage of minimising the loss of these converted nutrients into

the surrounding water column, serving as an adaptive mechanism to ensure adequate nutrient availability to the alga.

3.4 *Nitella congesta* as the dominant macrophyte in the lakes at Capel

Nitella congesta, a charophyte, was found to have colonised a number of the lakes at the Capel Wetlands Centre. Its presence was found to have significantly contributed to the development of the lakes at Capel Wetlands Centre as a suitable macrophyte by enhancing the water quality of the lakes. Initially studies at Capel Wetlands Centre by John 2000 had identified this *Nitella* sp. as the dominant charophyte thriving in the selected lakes though two *Chara* species were also identified. Samples of charophytes collected from the field showed consistent resemblance with the characteristics of *Nitella hyalina*. Most morphological features were similar. However, a closer look at the reproductive stages revealed slight differences which prompted a more careful study in order to ascertain the identity of the specie. This was done by studying the morphological features in comparison with specimen described in the literature.

The objectives of this part of the project were;

- To investigate the morphological features of *Nitella* species and identify it at the species level.
- To study the viability and germination of its oospores under laboratory conditions.
- To study the growth of *Nitella* species in the field and the laboratory in relation to environmental factors.
- To study the life-cycle pattern of the *Nitella* in relation to the hydrological regime of the lakes of Capel Wetlands Centre as a suitable rehabilitation tool.

3.5 Materials and methods

The following methods were used in the identification of *Nitella congesta* and its life cycle: Observation of morphological features, identification of the oospores, spore bank quantification in the selected lake sediments,

germination, growth studies in the laboratory and the field, and life cycle studies in the laboratory and in the field.

3.5.1 Morphology and taxonomy

Samples of the cultured charophytes were placed in Petri dishes and washed to remove as much debris as possible and observed under a Leica Stereomicroscope. The shoots were bisected and various morphological features observed. Measurements were taken for comparison with information on other species in the literature. Where possible, photographs were taken.

3.5.2 Identification of oospore(s) of *N. congesta* by Scanning Electron Microscopy (SEM).

To identify the oospores, they were removed with a Pasteur pipette under a stereomicroscope and placed in small vials as described in section 3.5.3. Oospores were cleaned for scanning electron microscope observation using the water bath and acetic acid method (John and Moore 1987).

Oospores were then placed on glass slides using a Pasteur pipette; the glass slides were heated on a hot plate at low heat, until all the water was evaporated. Clean oospores were mounted on aluminium stubs with a double-sided transparent adhesive tape, sputter-coated with gold and viewed with a Phillip's XL 30 scanning electron microscope using secondary and back scattered electron detectors. Accelerating voltage = 15kV. Oospore characteristics are outlined using descriptions given by Haas (1994).

3.5.3 Quantification of oospore bank

Charophytes produce thick walled oospores which are drought resistant and can remain in the sediment for many years (Takatori and Imahori 1971). Some species of charophytes respond to changing environmental conditions by varying morphological and reproductive responses (Casanova 1994).

The oospore bank quantification was done to estimate the number of oospores in the lake sediments. The aim of this part of the study was to investigate and record the oospore bank of *Nitella congesta* present in Nitella Lake, Plover North Lake and Plover South Lake at the Capel Wetlands Centre. Oospores of the *Nitella* were counted from sediment collected in April 2004 (autumn), September 2004 (spring), February 2005 (summer), February 2007 (summer) and September 2007 (spring) and calculations were made for the number of oospores per gram of dry sediment collected as in Burkett (1998).

To measure the dry weight of wet sediment from each lake, an initial amount of about 50g of sediment was collected using a shovel into plastic containers, covered and labelled and taken to the greenhouse of the Department of Environmental and Aquatic Sciences, Curtin University. Approximately 10g portions of wet sediment from each lake was placed in paper bags, labeled, weighed on a Sartorius (top loading) analytical balance and oven-dried at 60^{o}C for 72 hours. The bags were then removed, allowed to cool to room temperature and weighed. This was done till weight was fairly constant. The dry weight of the sediment was calculated for each bag; a mean dry weight was calculated for each lake sediment sample. Four replicates of such were taken for each lake at each time.

To separate the oospores from the sediment in the laboratory, each sample was washed through an Endecotts 710 µm brass sieve, a 500 µm stainless steel sieve and a 300 µm bronze sieve. For each sample, the sediment remaining in the sieve was poured into a large petri dish and examined under a Leica stereomicroscope and the oospores counted. Small amounts of sediment were removed from the large petri dish and transferred to a smaller petri dish (5 cm in diameter and 1 cm in depth). The petri dish had a dot marked in the centre and a line marked on the perimeter. The entire petri dish was viewed under a stereomicroscope by starting at the perimeter mark and moving the dish in a clockwise direction until the perimeter mark was reached again. Enough water was added to ensure easy swirling. The petri dish was then swirled several times to separate the oospores from the

heavier sediment particles and also increase oospore visibility. Oospores were counted between the central dot and the perimeter mark for the entire Petri dish. This process was repeated until the entire sample had been examined. The total oospore count per dry weight of each sample collected from the lakes at each time was calculated as follows;

1. For each sample, Number of oospores per unit weight = number of oospores counted / dry weight.
2. For each sediment, Mean number of oospores per unit weight = total number of oospores / total dry weight of sediment.

The mean number of oospores per unit weight of dry sediment and the standard deviation was calculated for each of the three lakes in autumn and spring in 2004, summer in 2005, summer in 2006 and spring in 2007.

3.5.4 Germination trials of *N. congesta* oospores in aquarium tanks

Samples of 100g of sediment from Nitella, Plover North and Plover South Lakes each was placed in 500 ml rectangular plastic containers and dried at room temperature for 8 weeks (56 days). Drying promotes the breakage of dormancy and enhances germination of some species from unfavourable habitats (Brock 1991; Casanova and Brock 1996; Casanova and Brock 2000). Prior to the beginning of the experiment, the dried sediment was mixed with deionised water and small amounts of the sediment were removed from the buckets into small plastic cups (7 cm diameter, 5 cm depth) to an approximate depth of 1 cm; 15 of such cups were filled with sediments from each lake. Each set of 15 cups was placed in a rectangular aquarium (30 L glass tanks) and flooded with deionised water to a depth of about 8 cm to just cover the cups maintaining the water level for the duration of the experiment. Three replicate cups were removed from each tank randomly at intervals of 2 weeks, starting from the fourth week after the set up and examined under the stereomicroscope. The germinated sporelings were removed and counted. The remaining sediment in each cup was then washed through sieves as in section 3.6.1 and the viable oospores removed

and counted. The viable oospores were those that were undamaged and turgid. Such oospores exude white starch grains when squeezed with forceps (Casanova 1993).

The oospore germination response was calculated as follows as

$$G_p = (F_{pt}/T_s) \times 100$$

Where G_p is the final germination percentage

F_{pt} is the number of oospores germinated at the termination of trial.

T_s is the total number of germinated and apparently viable oospores

$$G_r = \sum((n_1 \times t_1) + (n_2 \times t_2) + \ldots(n_x \times t_x)) / S_g$$

Where G_r is the germination rate

n_1 is the number of germinants at the first day of germination

t_1 is the number of days from start to first germination.

S_g is the total number of oospores germinated

$$G_m = G_p / D_f$$

Where G_m is the mean daily germination

D_f is the number of days to the final germination percentage.

The formulae were adopted from Barrett and Fox (1983).

3.5.5 Growth of *N. congesta* in the laboratory

Sediment samples (5 kg each) containing oospores of charophytes and water was collected from Nitella Lake, Plover North and Plover South Lakes (at an approximate depth of 30 cm and a distance of 1 m from the western banks). These were transferred into aquarium tanks in the greenhouse at the Department of Environmental and Aquatic Sciences, Curtin University of Technology. The aquaria were made of 30 litres glass tanks (Fig. 3.2). The sediment was placed in the aquaria and 20 L of lake water was added to each. The aquaria were aerated with aquarium pumps and airstones and were left in natural daylength conditions from 30th April – 30th November 2004 (244 days).

Figure 3.3 Aquarium tank with *N. congesta* growing.

The germination and growth of sporelings was monitored. Measurement of height, number of nodes and branches were recorded for 20 different individuals (selected at random and tagged by loosely but carefully tied red coloured thread around the thallus) from each tank. This was done from the onset of germination of the sporelings. Height was measured from the upper surface of sediment where charophyte rhizoids are buried to the top most tip of the apical meristem. The number of nodes and branches were counted along the axis of tagged individuals. One-way ANOVA was used to compare means of the three different groups. Statistical package SPSS Grad Pack 15.0 was used for the analysis. The growth rates G_r (mm day^{-1}) were calculated from the shoot length data using the formula;

$$G_r = \frac{H_t - H_0}{t} \qquad (3.1)$$

where H_0 is the length of the shoot at time zero

H_t is the length of the same shoot after a period of t days.

3.5.6 Growth of *N. congesta* in the field

Charophyte samples were harvested monthly from May – November 2004 from Nitella Lake, Plover North and Plover South Lakes at sites with sufficient healthy appearing biomass into plastic bags with some of the lake water in order to maintain cellular integrity. They were transferred to the greenhouse

at the Department of Environmental and Aquatic Sciences, Curtin University and spread out in plastic trays. Measurement of plant height, number of nodes and number of branches were recorded for 25 separate individuals. *N. congesta* shoots with upper or lower part broken off were not measured in order to ensure uniformity of results. Height was measured from tip of apical meristem to the point of attachment of rhizoids. Nodes and branches were also counted along the axes. Data collected were used to calculate mean growth rates for *N. congesta* in the field using formula 3.1. One-way ANOVA was used to compare means of the three different groups. Statistical package SPSS Grad Pack 15.0 was used for the analysis.

Two sites were selected from Plover South Lake (shallow, 33^{o} 36.19" S, 115^{o} 30.11" E and deep, 33^{o} 36.17" S, 115^{o} 30.25" E) and the mean height of *Nitella congesta* shoot were measured against the water depth monthly from June to November. Data obtained were analysed by regression analysis to determine the relationship between water depth and total length of *N. congesta* shoots. Statistical package SPSS Grad Pack 15.0 was used for the analysis.

3.5.7 Life cycle studies of *N. congesta* in the laboratory

The life cycle of the *Nitella congesta* cultured in the laboratory were observed on a daily basis. Timing of production of fruiting bodies (antheridia and oogonia) and the duration for maturity were recorded. Fruiting bodies were collected at random, measurements taken and features viewed under a Leica stereomicroscope. Where possible, photographs of the growth stages were taken. Male and female shoots were counted at random to determine the sex ratio.

3.5.8 Life cycle studies of *N. congesta* in the field

The life cycles of the *Nitella congesta* cultured in the field were observed fortnightly. Timing of production of fruiting bodies (antheridia and oogonia) and the duration of their maturity were recorded. Fruiting bodies were

collected at random, measurements taken and features viewed under a Leica stereomicroscope. Where possible, photographs of the stages were taken. Male and female shoots were counted at random to determine the sex ratio.

3.6 Results

3.6.1 Description of *N. congesta* morphology

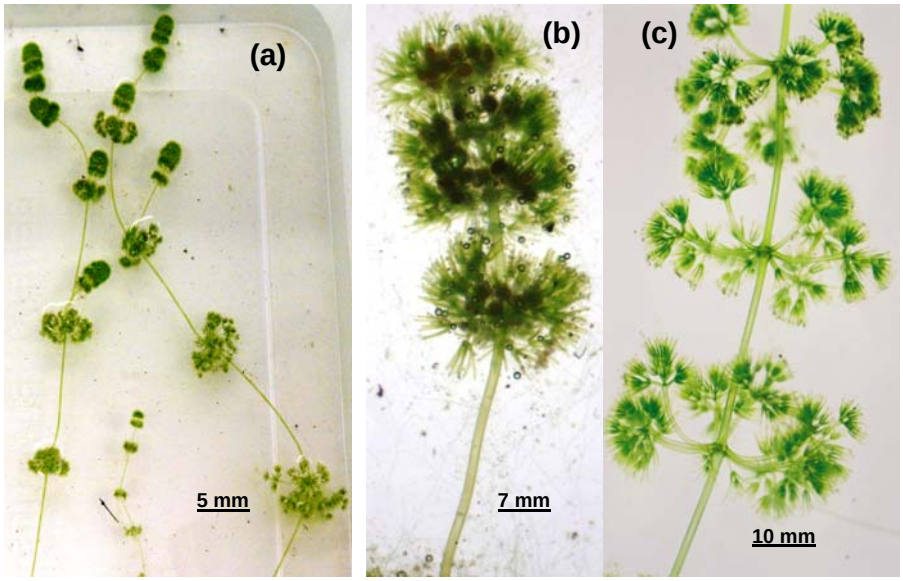

Figure 3.4 General morphology of *N. congesta*.

(a) Habit showing whorls of branchlets and internodes; towards the apex the internodes are very closely arranged.
(b) Habit showing whorls of fertile branchlets (with oogonia).
(c) Habit showing whorls of older branchlets spread out as mucilage is shed off.

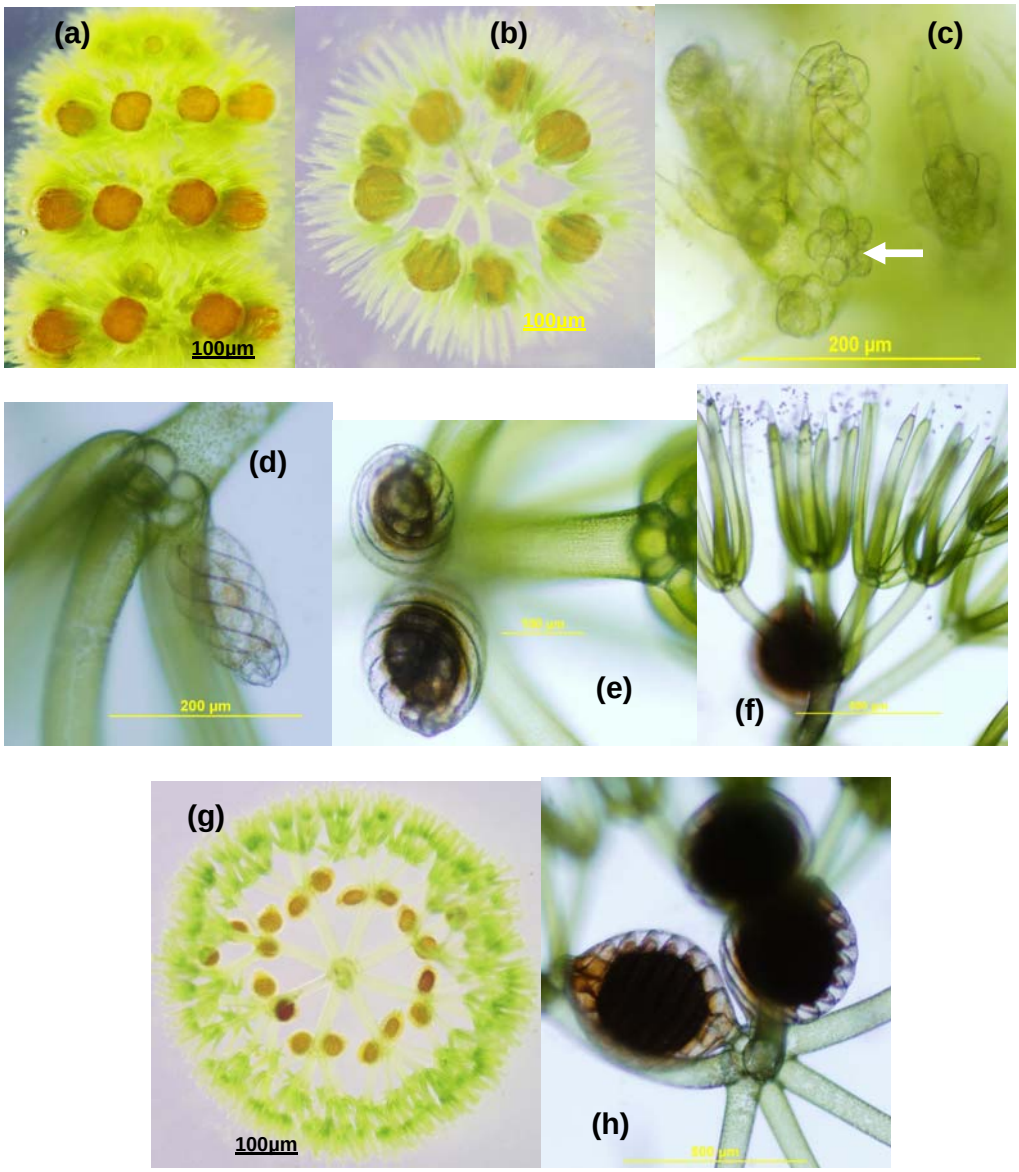

Figure 3.5 Reproductive bodies of *N. congesta*.

(a) and (b) antheridia (globules) – male sex bodies.

(c) Developing oogonia (nucules) showing 5 tube cells. Top view of the coronula cells (arrowed).

(d) Developing oogonium

(e) Fertilized oogonia at primary branchlet.

(f) Developing oospores at node and terminal branchlets.

(g) Developing oogonia at nodes forming a whorl.

(h) Mature oospores at a node replacing primary branchlets.

Description of *N. congesta* from Capel Wetlands

Description of *N. congesta* is after Wood and Imahori (1965), García (1999) and adapted from Blindow (2003) and Koistenen (2003) for *Nitella hyalina*.

***Nitella congesta* (R. Brown) A. Brauni**

The alga is dioecious, can grow up to 33 cm high, dark green with slender axes, 0.7-1.0 mm in diameter. There are 15-20 branchlets (15 seen in Fig. 3.7b) at each node in 3 rows which are long (0.3-0.7 cm) and heteroclemous. Apical part of shoot is covered with mucilage making it compact. The internode lengths vary according to water depth; shorter at the apical meristems and increases downwards along the axis. The branchlets furcate into secondary branchlets (length 0.1-0.23 cm which are 5-6 in a whorl (reduced when replaced by an oogonium), one being percurrent. The secondary branchlet furcates into 5-6 tertiary branchlets after which there were dactyls. Dactyls are 2-celled; end cell transparent in the population studied. They are elongated, 100-200 µm long (including acuminate end cell). Both the internode and whorls of branchlets are ensheathed by mucilage. As mucilage is shed off in the lower portion of shoot, branchlets spread out. Whorls of shorter branchlets (identified as accessory branchlets in literature (Wood and Imahori 1965; van Raam 1995; García 1999) are found at some nodes. They are irregularly arranged and can be up to 10-20 in number. Gametangia are born on separate individuals. Oogonia are 1-3 at a node, 650-750 µm long, 400-500 µm wide and with 8-9 convolutions. Antheridia are solitary, octoscutate in arrangement and terminal on male individual (Fig. 3.5a,b), 375-450 µm in diameter. Oospores are light brown or dark brown (when fully mature), 415-500 µm long, 300-400 µm wide; striae of 9 broad, prominent ridges with a spongious ornamentation.

Accessory branchlets

In the population studied, the accessory branchlets could not be observed as a consistent feature at all nodes. This made identification of species difficult. However, a careful observation showed that the accessory branchlets develop later at some nodes away from the top of the thallus. The first three whorls of branchlets (numbered 1-3 in Fig. 3.6) from the tip of the apical

meristems showed no presence of accessory branchlets. However, the following node (Fig. 3.7a,b) shows the development of accessory branchlets. Also some of the older nodes of the thallus were found to lack any accessory branchlets (Fig. 3.8b). The only parts of the thallus with accessory branchlets were the few nodes in-between the apical meristems and the older portion of the thallus. Thus the accessory branchlets may not be a common feature at all the nodes of *N. congesta*. However, the accessory branchlets if present can bare sex bodies.

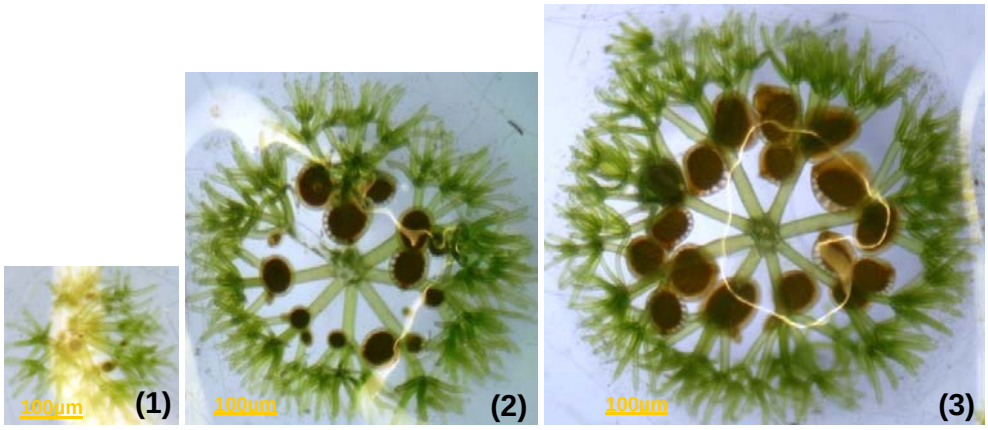

Figure 3.6 Whorls of branchlets showing the absence of accessory branchlets. **(1)** Terminal most whorl of branchlets **(2)** Whorls of branchlets at second node **(3)** Whorls of branchlets further below; third node.

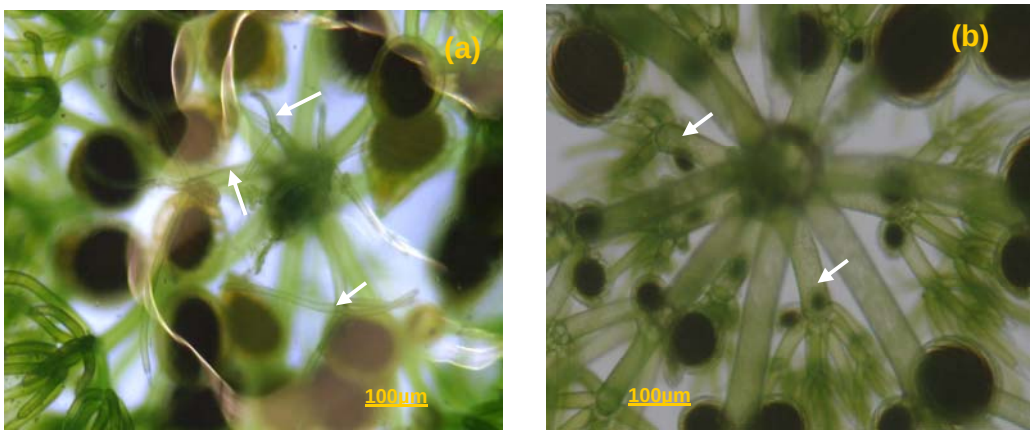

Figure 3.7 (a) Whorls of primary branchlets with developing accessory branchlets (3 arrowed).

(b) Whorls of primary branchlets and accessory branchlets (2 arrowed) at fourth node from the top on thallus.

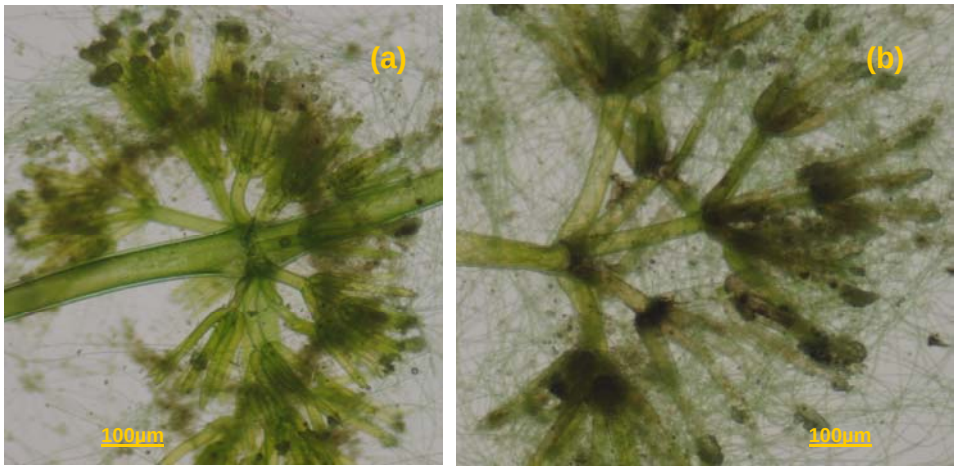

Figure 3.8 (a) Whorl of primary branchlets from the mid-portion of thallus with accessory branchlets.

(b) Whorl of primary branchlets at lower part of thallus without accessory branchlets.

Figure 3.9 Whorl of fertile branchlets with oogonia; stained with Toluidine Blue to show mucilage covering whorl.

3.6.1.1 Taxonomy of *N. congesta*

The taxonomy of *N. congesta* was deduced following García (1999), García (2001) and García (2002).

Thallus morphology: Key at the Tribe Level

Tribe Nitelleae

Coronula of 10 cells in two tiers; branchlets divided in rays of 2nd to 5th order or monopodial with 1-2 nodes bearing 1-4 pluricellular rays; in some cases with accessory branchlets; without unicellular processes; oospores/gyrogonites with 1 or 2-3 impressions of the sister cells of the oospore (García 2001; García 2002).

Thallus morphology: Key for the genera in Tribe Nitelleae

Genus *Nitella*

Branchlets generally furcate, rarely with a percurrent central axis; lateral rays generally verticillate; antheridia terminal at the end of branchlet rays, rarely at base of whorl; oogonia/oospores laterally compressed; oospores with 2-3 impressions at the base, corresponding with the 2-3 sister cells of the oosphere; not calcified (García 2001; García 2002).

Key to charophyte specie identified (*Nitella congesta*)

- Branchlets divided into segments - 3(4) rays; 3rd or 4th segments are dactyls.
- Heteroclemous - branchlet 7-8 in a whorl; 15-20 accessory branchlets found at older nodes (not consistent).
- Dioecious (García 1999; García 2001; García 2002)
- Oospores 415-500 µm long, 300-400 µm wide, antheridia 375-450 µm in diameter.

3.6.2 Identification of oospore of *N. congesta* by SEM

Oospore of *Nitella congesta* was identified using the following parameters and descriptions. Results were compared with literature according to Wood and Imahori (1965), García (1999), García (2001) and García (2002).

Length: 415 - 500 µm; width 300 - 400 µm; Length to width ratio ≈ 1.0 – 1.6

Shape: Apical view – flattened (Figure 3.11), Lateral view – compressed (Figure 3.10); ridges are prominent, 7-9 in number (Figure 3.10); colour is light brown but turns dark brown when mature; membrane ornamentation is mostly spongious, with anastomosed imperfect walls. (Figure 3.13).

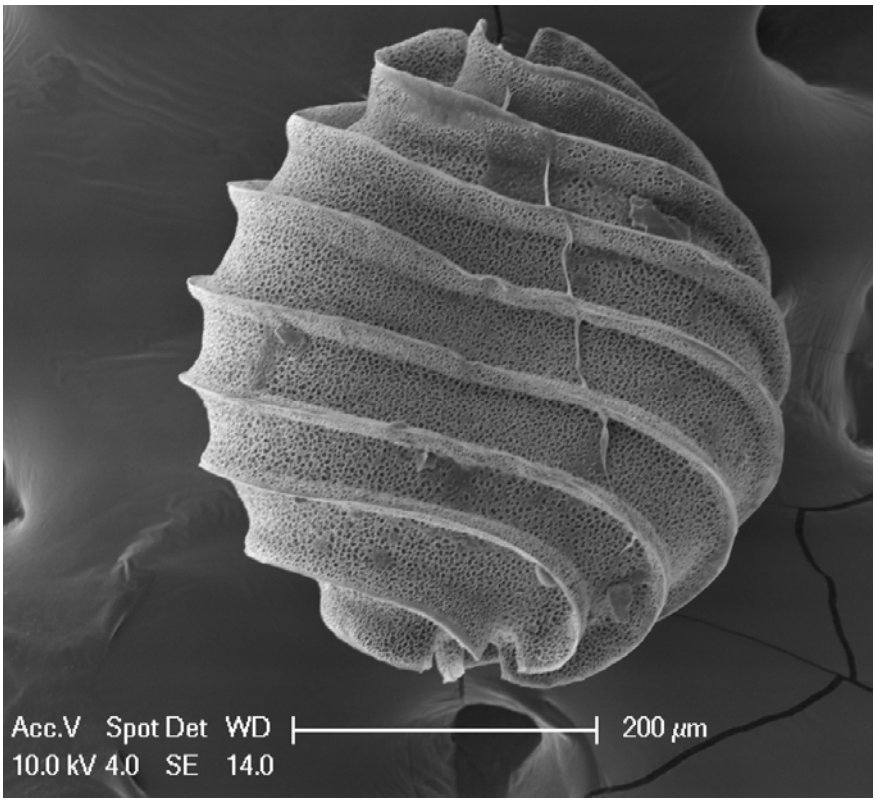

Figure 3.10 Lateral view of oospore of *N. congesta* showing ridges or flanges with fossa.

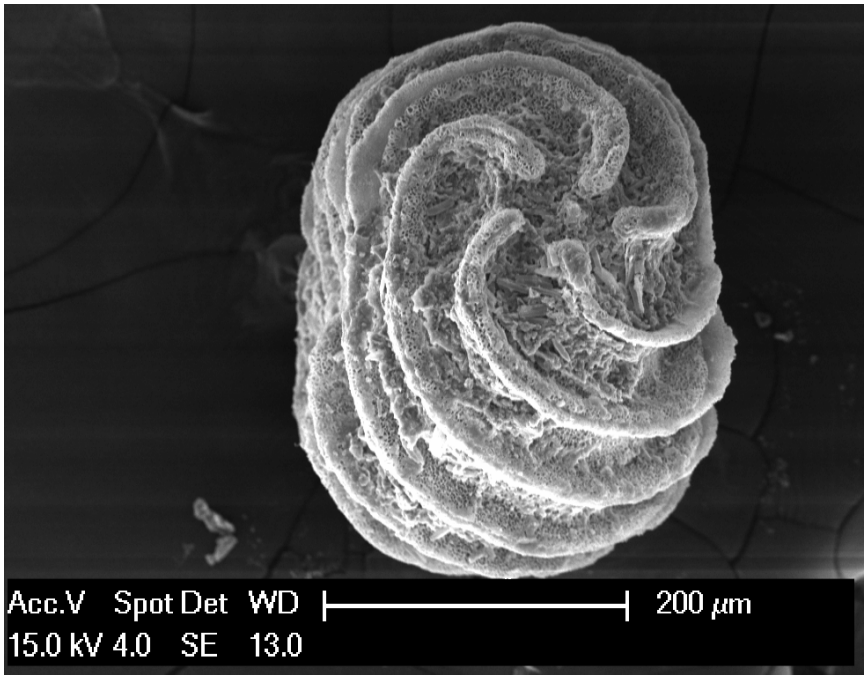

Figure 3.11 Apical view of oospore of *N. congesta*.

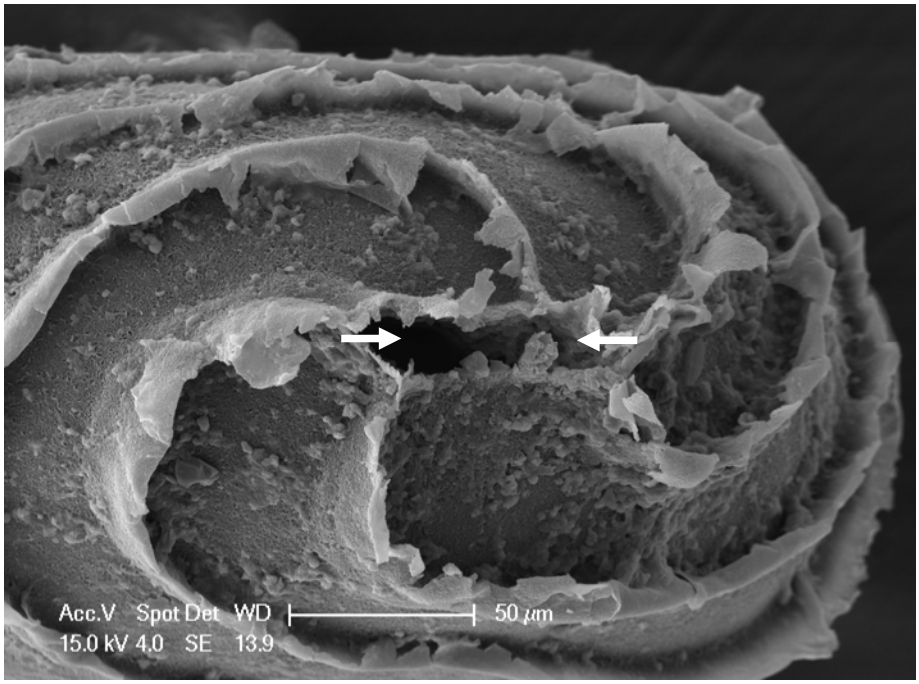

Figure 3.12 Basal view of oospore of *N. congesta* showing double basal plugs (arrowed). Left arrow showing 1st impression, right arrow showing 2nd impression.

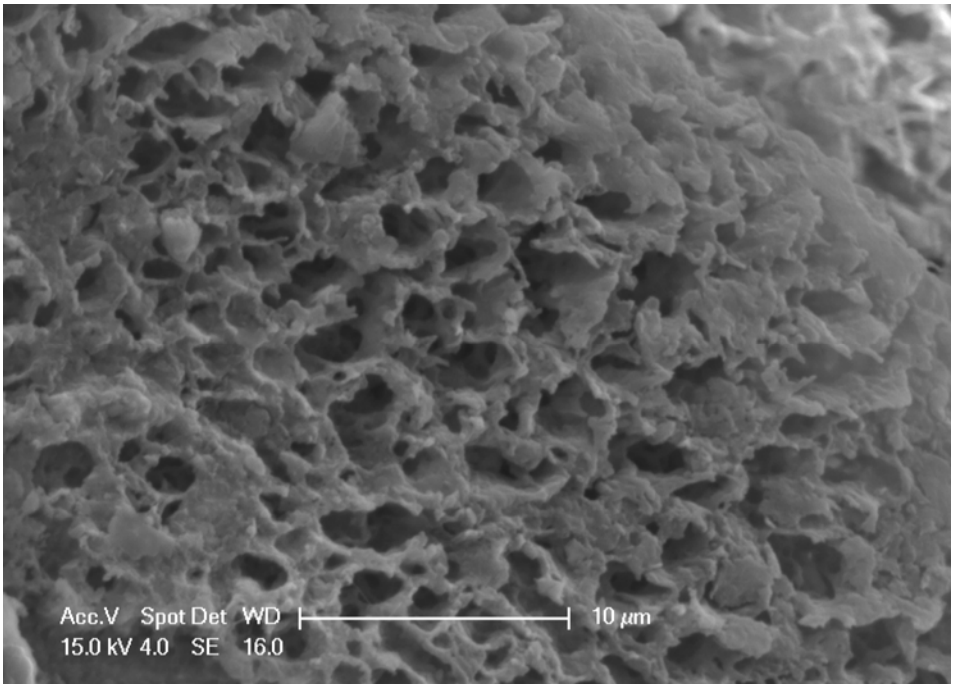

Figure 3.13 Oospore wall of *N. congesta* showing spongious ornamentation.

3.6.3 Quantification of oospore bank

Table 3.1 Mean number of oospores counted per gram of dried sediment samples from the western banks of three lakes.

Season		Nitella Lake	Plover North Lake	Plover South Lake
		Mean±sd	Mean±sd	Mean±sd
Apr-04	Autumn	0.39±0.23	0.55±0.12	0.37±0.10
Sep-04	Spring	0.51±0.12	0.48±0.20	0.40±0.11
Feb-05	Summer	2.00±0.28	1.73±0.55	1.57±0.40
Feb-07	Summer	2.07±0.28	1.72±0.36	1.64±0.44
Sep-07	Spring	1.60±0.45	1.46±0.44	1.21±0.30

The oospore bank results obtained show that the lakes maintained an oospore bank potential in the sediment irrespective of the water level though oospore count for winter was not done. The number of oospores contained in the sediment however varied in relation to the water regime as well as the life cycle stage of *Nitella congesta*. Nitella lake showed the highest oospore density (mean oospore per gram of sediment) in all spring and summer. In autumn, Plover North lake showed the highest oospore density.

3.6.4 Germination trials of *N. congesta* oospores in aquarium

Table 3.2 *N. congesta* oospore viability and germination inundated to a depth of ≈ 8cm.

	Plover North Lake		Plover South Lake		Nitella Lake	
Mean oospore bank (g^{-1} dry sediment)	1.73		1.57		2.00	
Date	**V**	**G**	**V**	**G**	**V**	**G**
01-04-05	6	0	8	0	6	0
15-04-05	21	4	14	2	6	0
29-04-05	17	6	11	4	13	3
13-05-05	18	5	8	5	16	5
27-05-05	15	8	13	10	25	4
Sum	77	23	54	21	66	12
Mean	15.40	4.60	10.80	4.20	13.20	2.40
Sd	±5.68	±2.97	±2.77	±3.77	±7.92	±2.30
Germination % (Gp)		23.00		28.00		13.64
Mean daily germination (Gm)		0.27		0.33		0.16
Germination rate (Gr)		12.60		14.00		14.67

V = No. of viable oospores, **G** = No. of germinated oospores, Total number of days = 84

Nitella lake showed the highest germination rate with the highest oospore density, however the lowest germination percentage. Eighty five percent oospores were viable but germination was low. Plover South showed highest germination percentage with the lowest oospore density.

3.6.5 Growth measurement of *N. congesta* in the laboratory

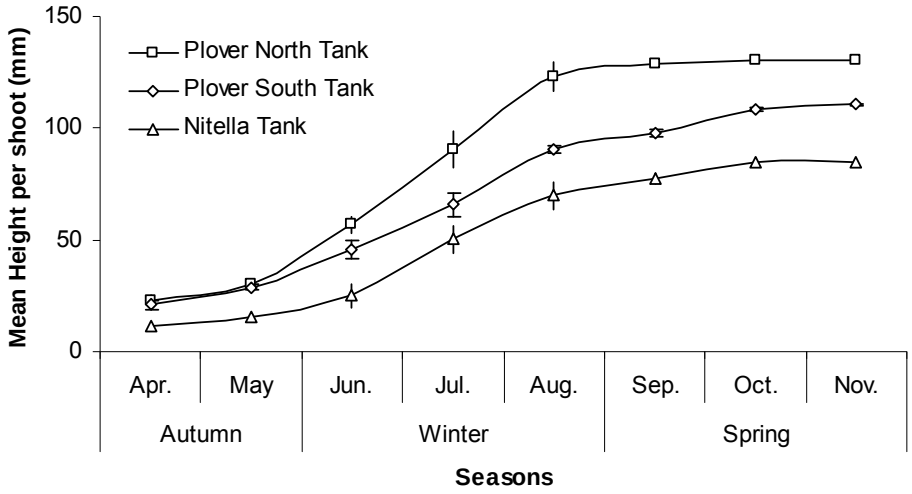

Figure 3.14 Mean height of shoots of *N. congesta* cultured in the laboratory in 2004.

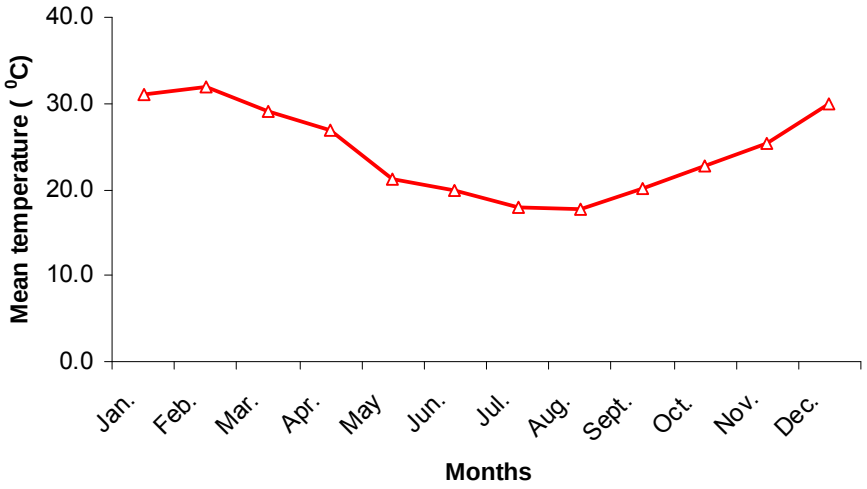

Figure 3.15 Mean temperature (^{0}C) in laboratory in 2004.

High growth rate in winter (Fig. 3.14) corresponds with lowest temperature (Fig. 3.15). As temperature increases in spring, vegetative growth rate decreases initiating the production of reproductive bodies (antheridia and oogonia).

Table 3.3 Laboratory growth rate (mm day^{-1}) of *N. congesta* in aquaria in 2004, n = 20.

	Plover South Tank	Plover North Tank	Nitella Tank
Autumn	0.47±0.30	0.50±0.37	0.26±0.16
Winter	0.67±0.12	1.01±0.11	0.59±0.26
Spring	0.22±0.14	0.07±0.10	0.43±0.34

Growth rate was highest in winter in all tanks with the highest being in the Plove North Tank. *N. congesta* meadows in Plover North Tank showed the lowest growth rate in spring; production of fruiting bodies was first observed in this tank.

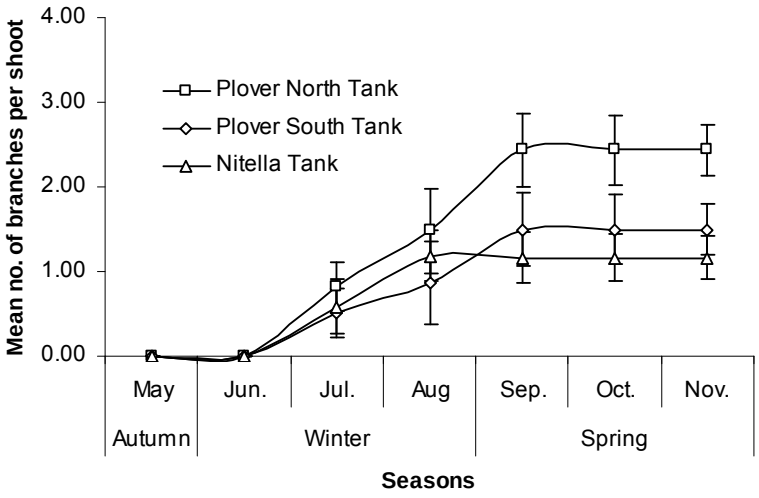

Figure 3.16 Mean number of branches per shoot of *N. congesta* cultured in the laboratory in 2004.

"Branches" refer to new shoots from a whorl of branchlets, with nodes and internodes. *N. congesta* meadows in the Plover North Tank had the highest number of branches per shoot.

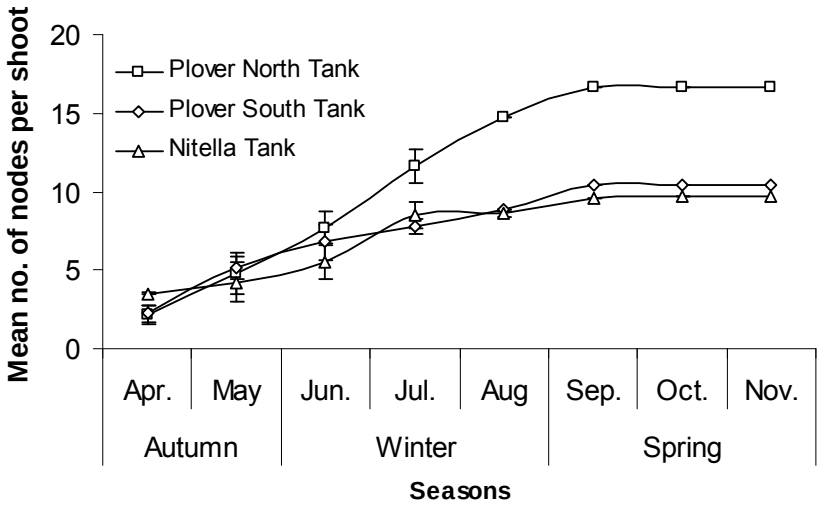

Figure 3.17 Mean number of nodes per shoot of *N. congesta* cultured in the laboratory in 2004.

N. congesta meadows in the Plover North Tank had the highest number of nodes.

Table 3.4 One-way ANOVA comparing mean height of shoots, mean number of nodes and mean number of branches of *N. congesta* cultured in the laboratory in 2004, α = 0.05, n = 20.

	Plover South Tank	Plover North Tank	Nitella Tank		
	Mean±sd	Mean±sd	Mean±sd	F	***P***
Mean height	54.53±17.33	98.56±40.85	41.76±24.82	1.82	0.19
Mean number of nodes	8.57±2.09	12.69±4.84	7.84±2.50	4.21	**0.03**
Mean number of branches	0.84±0.69	1.37±1.12	0.75±0.56	1.16	0.34

Mean number of nodes per shoot was significantly different but not mean height and mean number of branches (see Appendix 1).

3.6.6 Growth measurement of *N. congesta* in the field

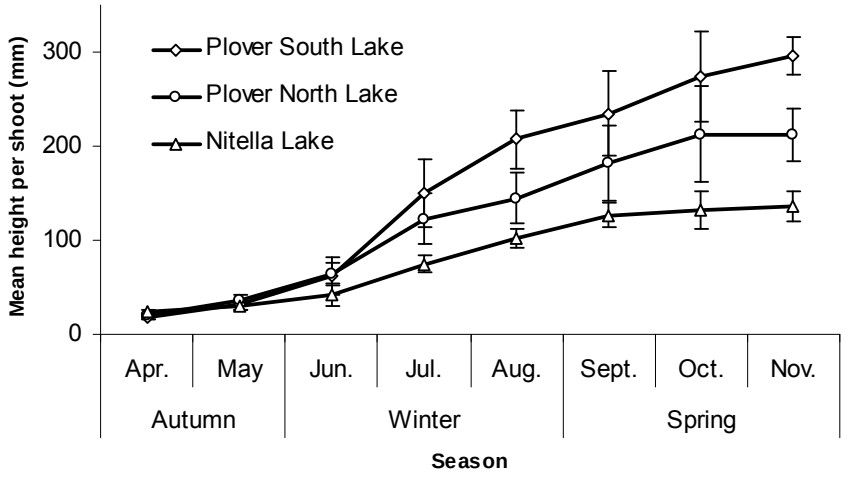

Figure 3.18 Mean height of shoot of *N. congesta* observed in the field in 2004.

Plover South lake had the highest growth rate

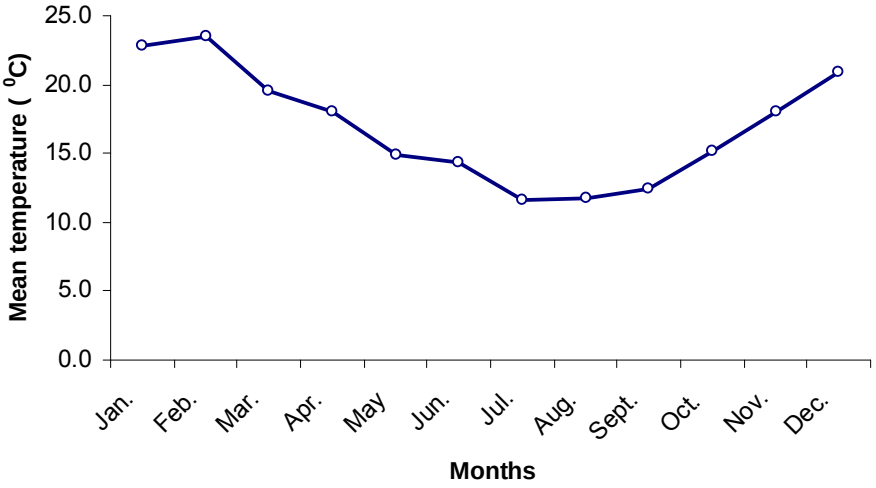

Figure 3.19 Mean temperature (^{0}C) in field in 2004.

High growth rate in winter (Fig. 3.18) corresponds with lowest temperature (Fig. 3.19). As temperature increases in spring, growth rate decreases initiating the production of reproductive bodies (antheridia and oogonia).

Table 3.5 Field growth rate (mm day^{-1}) of *N. congesta* in Plover South, Plover North and Nitella Lakes in 2004, n = 25.

	Plover South Lake	Plover North Lake	Nitella Lake
Autumn	0.52±0.30	0.61±0.39	0.50±0.13
Winter	1.92±0.89	1.17±0.62	0.79±0.33
Spring	0.95±0.29	0.74±0.65	0.36±0.36

Highest growth rate was observed in winter in all three lakes and among the three lakes, the highest growth rate was observed in Plover South lake.

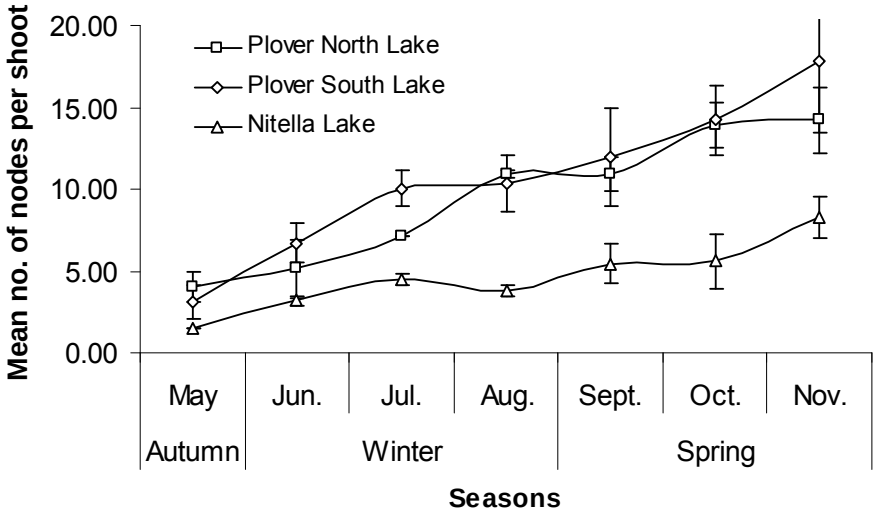

Figure 3.20 Mean number of nodes per shoot of *N. congesta* observed in the field in 2004.

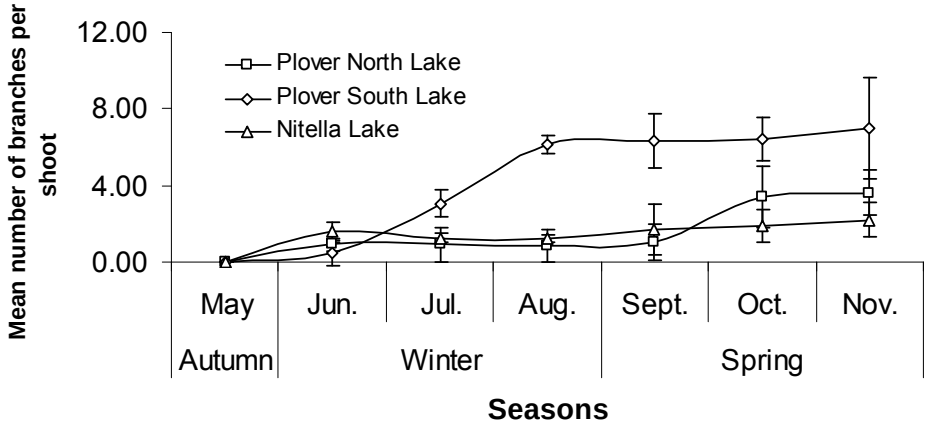

Figure 3.21 Mean number of branches per shoot of *N. congesta* observed in the field in 2004.

"Branches" refer to new shoots from a whorl of branchlets, with nodes and internodes. The highest number of branches per shoot was in Plover South lake.

Table 3.6 One-way ANOVA comparing mean height, mean number of nodes and mean number of branches per shoot of *N. congesta* in the field in 2004, α = 0.05, n = 25.

	Plover South Lake	Plover North Lake	Nitella Lake		
	Mean±sd	Mean±sd	Mean±sd	F	***P***
Mean height	159.20±110.47	124.38±77.18	83.86±47.02	1.69	0.21
Mean number of nodes	10.59±4.80	9.47±4.09	4.61±2.14	4.78	**0.02***
Mean number of branches	4.21±3.00	1.53±1.39	1.40±0.70	4.60	**0.02***

Mean number of nodes and mean number of branches were significantly different (see Appendix 2).

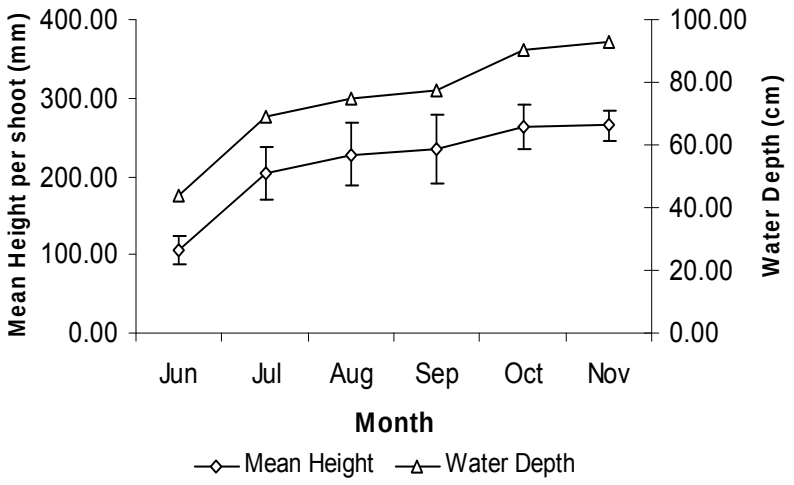

Figure 3.22 Mean height per shoot of *N. congesta* in Plover South Lake Site 1 (deep) in 2005.

Regression analysis of water depth and mean height per shoot of *Nitella congesta* in Plover South Lake Site 1 is significant at the 5% significance level. F = 148.13, R Square = 97.4%, P = 0.00 (see Appendix 3).

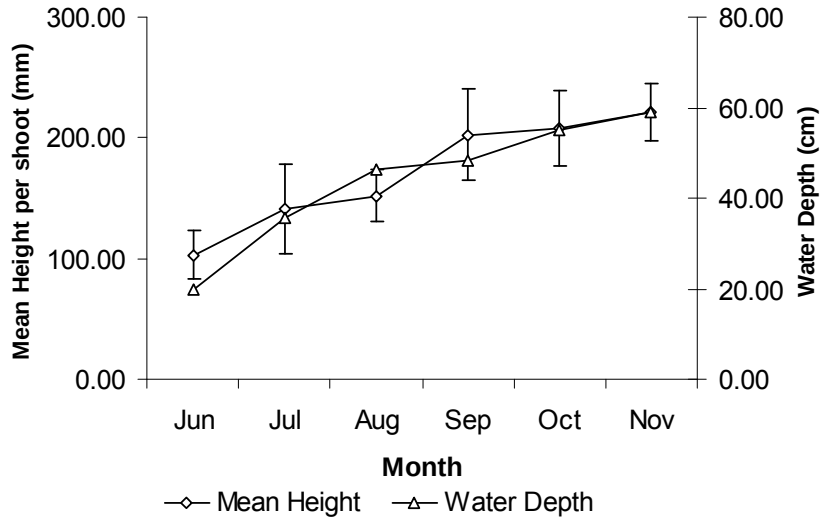

Figure 3.23 Mean height per shoot of *N. congesta* in Plover South Lake Site 2 (shallow) in 2005.

Regression analysis of water depth and mean height per shoot of *Nitella congesta* in Plover South Lake Site 2 is significant at the 5% significance level. F = 35.86, R Square = 90.0%, P = 0.00 (see Appendix 4).

Water depth

The ecology of *N. congesta* was studied in relation to the water depth and water quality of the lakes. The table below shows the observed parameters of depth, salinity and pH compared to parameters reported in the literature.

Table 3.7 The ecological parameters of *N. congesta*.

Ecological parameter	Referenced	Observed
Growth depth	0.0 - 1.0 m (García 2002)	0.0 - 2.50 m
Salinity	0.5 - 3.0g/L (García 2002)	0.1 – 0.4g/L
pH	6.0 – 7.6 (García 1999)	6.0 – 9.0

3.6.7 Life cycle of *N. congesta* in the laboratory

Charophyte life history classification is referred to as either monocarpy or polycarpy based on the reproductive patterns of the alga. Polycarpic charophytes reproduce several times in a life time where as monocarpic ones reproduce once and die. Monocarpics are divided into those that reproduce at an early stage and grow for a long time and those that grow vegetatively for some time before undergoing reproduction and then die (Harper 1977; Casanova 1993). In this study, *N. congesta* exhibited the latter type of monocarpy in the field and same as verified in the laboratory.

N. congesta was observed to be dioecious. The male sex bodies (antheridia) were observed to have developed earlier than the female sex bodies (oogonia). Antheridia were produced mid-September (early spring) and after about two weeks, the oogonia were produced. After a few days, no more male sex bodies could be seen. Only the female shoots with fertilized oospores could be seen. Table 3.8 shows results of male and female shoots counted.

Table 3.8 Percentage of male and female shoots of *N. congesta* counted in the laboratory.

	Plover North Tank	Plover South Tank	Nitella Tank
Male	56	45	52
Female	44	55	48
Ratio	1.27	0.82	1.08

Ratio of male to female shoots in all three aquaria is approximately 1:1.

3.6.8 Life cycle of *N. congesta* in the field

In the field study, *N. congesta* again exhibited monocarpy growing vegetatively for some time before undergoing reproduction. *N. congesta* was dioecious. The production of sex bodies were observed to be; late October to early November (late spring). Male fruiting bodies were produced a couple of weeks earlier than female fruiting bodies. No more male sex bodies could be seen after a few days; only the female shoots with fertilized oospores could be seen. Table 3.9 shows results of male and female shoots counted.

Table 3.9 Percentage of male and female shoots of *N. congesta* counted in the field.

	Plover North Lake	Plover South Lake	Nitella Lake
Male	49	54	51
Female	51	46	49
Ratio	0.96	1.17	1.04

Ratio of male to female shoots in all three lakes is approximately 1:1.

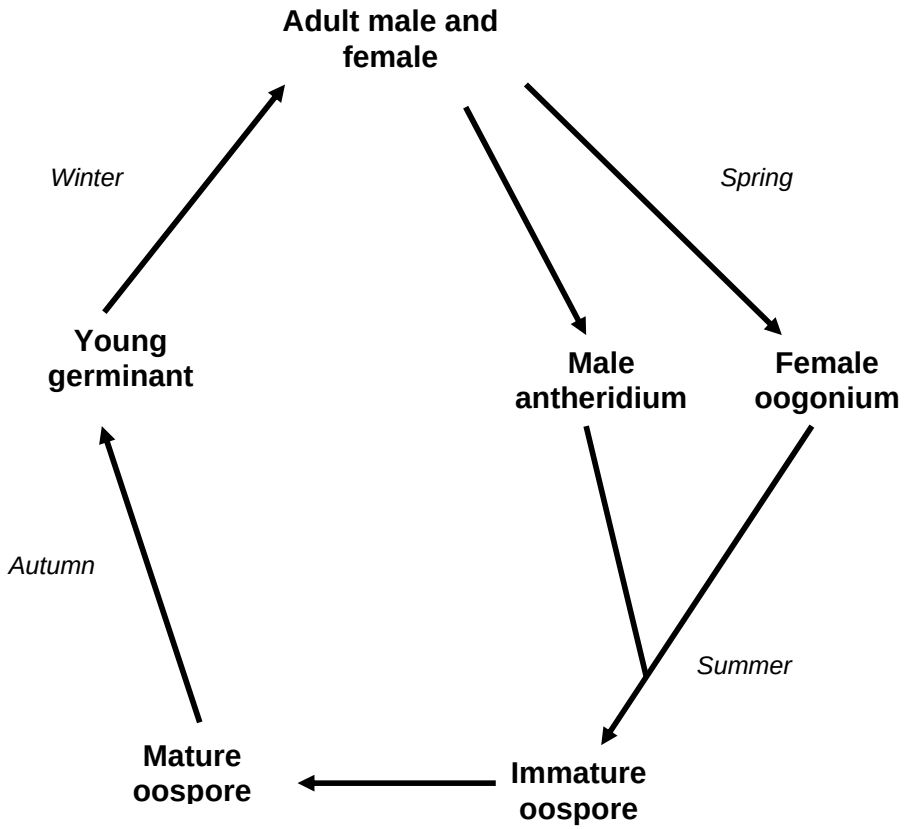

Figure 3.24 Schematic representation of the life cycle of *N. congesta*.

Nitella congesta is dioecious, thus producing separate sex bodies on separate individuals. The male individuals produced orange coloured antheridia which were borne at the tips of the nodes in an octoscutate arrangement. This occurred within a period of 2-4 weeks after which no more antheridia were seen. The female fruiting bodies, the oogonia which were initially somewhat gold coloured, were observed to have developed a couple of weeks after the development of the antheridia. Three to five weeks after the production of the oogonia, the fruiting bodies produced oospores. The oospores reached maturity with an entire change in colour to dark brown during summer. Sporelings of *N. congesta* germinate in late autumn on the onset of rains. Growth is slow during this time until winter where *N. congesta* grows vegetatively and growth rate increases. There is average rapid increase in height of shoots as well as number of nodes and increase in

internode distance. This is observed through to early spring. During spring, the charophyte undergoes sexual reproduction beginning with the production of sex bodies on different individuals. The male individuals produce the orange coloured male sex bodies (antheridia) and female individuals produce the somewhat yellow coloured female sex bodies (oogonia). The sperms of the antheridia fertilize the eggs of the oogonia forming the zygotes (immature oospores). The zygotes undergo maturity as temperature rises during late spring and early summer. At this stage, the oospores become darker and shrink in size (mature oospore). Oospores are then released into the sediment as the standing water dry out. Therefore, the life cycle is adapted to the hydrological regime of the lakes. The same scenario is observed in the laboratory if water in the aquaria is left to evaporate. In this case, *N. congesta* acts as an annual. However, if availability of water is maintained, *N. congesta* acts as a perennial resulting in continuous vegetative growth and reproducing asexually by bubils.

3.7 Discussion

3.7.1 Identification of *N. congesta*

Nitella sp. identified as *N. congesta* resembles *Nitella hyalina* and *Nitella lhotzkyi* in morphological features. This species in Capel Wetlands was initially identified as *N. hyalina* in various previous studies (John and Gayton 1994, Ward *et al*. 1997 and John 2003) prior to this project. Both species produce mucilage covering the apical mersitems and closely resembles *N. congesta* in morphology. However, *N. hyalina* was found to be monoecious while *N. congesta* is dioecious (Wood and Imahori 1965). In comparison with *N. lhotzkyi*, the branchlets at nodes on the axis of *N. congesta* are spheroid and compacted and heteroclemous up to 20 in number. In *N. lhlotzkyi*, the branchlets are however found to be of 2 kinds in each whorl (Wood and Imahori 1965). Normal branchlets are 7-11 in a whorl with only 1-2 furcation and the nodes do have accessory branchlets (Wood and Imahori 1965). Therefore based on observed morphological features compared with literature, the species in this project was identified to be within the concept of *Nitella congesta* (R. Br.) A. Br. However, the lack of consistent presence of

accessory branchlets appears to separate Capel Wetland populations from those described by Wood and Imahori (1965), van Raam (1995) and García (1999). Whether the variability in the accessory branchlets is a plastic character confined to the Capel population is unknown.

3.7.2 Oospore bank

Seed banks are known to function as a reservoir for floristic diversity (Casanova and Brock 1990) and this is an important attribute for a successful revegetation of degraded aquatic habitat. Different macrophyte species may have a variety of germination and recruitment strategies (Casanova and Brock 1996) so that a high degree of seed bank diversity would enhance the probability of an emergence response under a wider range of conditions. The conservation of seed bank preserves attributes of longevity, numerous small seed, large buried seed reserves and year round seed bank presence that are observed for other submerged spore banks (Casanova and Brock 1990; de Winton *et al.* 2004) which characterize a persistent seed bank. The smaller size of *Nitella* oospores may have allowed more to be produced for the same resources (Casanova and Brock 1990) resulting in a greater numerical representation in the oospore bank especially in Nitella lake. It is likely that the permanent nature of Plover North and Plover South lakes contributed to *N. congesta* undergoing vegetative reproduction in preference to sexual reproduction. The lower oospore bank of these lake sediments might be explained by the *N. congesta* meadows undergoing vegetative reproduction with low production of fertilized oospores.

3.7.3 Germination of *N. congesta*

Charophyte oospores are borne on branchlets and upon full ripening, they fall to the sediment where they often remain dormant (de Winton and Clayton 1996; de Winton *et al.* 2000). Oospores of different charophyte species respond to different triggers (Bonis and Grillas 2002) some of which are desiccation (Casanova and Brock 1996), pre-dehiscence growth inhibitors (Sabbatini *et al.* 1987) and seasonality (Sokol and Stross 1986). Although

germination of the oospores of *N. congesta* was successful in the aquaria trials, percentage germination was low. Germination patterns of some charophytes have been identified to follow three strategies or stages; early (germinate rapidly in response to environmental conditions, followed by low germination), late (initial germination low, germinate after specific environmental conditions and finally, continuous germination (where there is a relatively even germination rate) (Casanova and Brock 1999; Haukos and Smith 2001). The second pattern of germination was observed in the case of *N. congesta* in all the trials. The multiplicity of seed bank generations and the complexity of the environmental events that break their dormancy contribute to the ecosystem's potential to recover after a drying event (Brock *et al.* 2003). In this study, the oospores of *N. congesta* were sun dried for 56 days but the percentage germination was quite low < 50% in three lake sediments. This may be due to prolonged dormancy of the oospores or other environmental factors. Drying may have had positive impact on breaking the dormancy of the oospores (Stross 1989; Casanova and Brock 1996; de Winton *et al.* 2004). Also environmental conditions may have favoured the germination of oospores. The high percentage of viable oospores in all sediments indicates that the low rate of germination might be due to lack of favourable environmental factors.

It has been documented that Characean oospores exhibit both primary and secondary dormancy (Stross 1989; Casanova and Brock 1996), but the requirements for breaking each type of dormancy differ depending on the species and the geographic location in which they occur (Casanova and Brock 1996).

3.7.4 Growth of *N. congesta*

Charophytes have a range of growth strategies (Blindow 1992b; Titus *et al.* 2004). Perennial growths have been reported in deep lakes, while in temporary water bodies, growth may be seasonal (Pentecost *et al.* 2006). New individuals do develop from the internodes of decaying individuals beneath the sediment surface or from germinating oospores. The vegetative

growth rate appeared to be seasonal; highest in winter. The young germinants from late autumn or early winter being a period of rapid growth during whereby average rainfall is highest thus increasing water level of lakes and temperature low (not too low but sufficient enough to stimulate growth;11.0 – 20.0 ^{0}C). The thalli appeared very healthy green and covered with a considerable amount of mucilage. The rapid growth that occurred during winter ensured allocation of resources acquired from the sediment directed toward the production of fruiting bodies in late spring (Casanova 1994).

Charophyte growth rate differ depending upon depth (Andrews *et al.* 1984b), substratum particles (Andrews *et al.* 1984a) and to some extent, nutrient status (phosphorus concentration) (Blindow 1988; Kufel and Kufel 2002). Water level plays a significant role in the survival and reproduction in charophytes. In the laboratory, *Nitella congesta* showed initiation of fruiting bodies when the water levels in the aquaria were no more increased but left to decrease considerably. The initiation of fruiting bodies started between late August and the end of September (late winter to early spring) may be attributed to increase in temperature eventually resulting in the drying out of the temporary lakes. Therefore algal resources were channeled to the production of fruiting bodies in sexual reproduction on the onset of increasing temperature. In the laboratory, growth of *N. congesta* in Plover North tank was highest. This could be explained that sediment from Plover North lake had more nutrients than Plover South and Nitella lake. *N. congesta* was initially found in Nitella lake and later oospores transferred to other lakes. However, its establishment in Plover North was only successful after a series of trials. Thus the nutrient in the sediment may not have been used up hence much was available during growth.

The sex ratio of male to female shoots of *N. congesta* in the field and that cultured in the laboratory, was approximately 1:1. Sex allocation in charophytes is complicated and is influenced by geographical and environmental factors (Cox 1981; Lloyd 1982). The male shoots became the most abundant followed by the female shoots becoming the most abundant

later. This protandrous phenomenon has been explained as a temporal niche separating sexes (Cox 1981). The production of antheridia before that of oogonia presupposes that it may be less expensive to produce antheridia than oogonia (Casanova 1994). Therefore assuming that the production of antheridia required fewer resources than that for oogonia, antheridia were produced earlier than oogonia. This hypothesis can be supported by the production of two oogonia on female branchlet nodes where as four antheridia can be produced at a similar position on male shoots (Casanova 1994). More so, antheridia were observed to be larger in size than oogonia and this is explained that allocation of nuclear material to the antheridia is greater than vegetative parts or oogonia (Casanova 1994). Charophytes reproduce predominantly by asexual means, for example, by the generation of secondary protonema from the bulbils in permanent water bodies. In this case, the plant energy is allocated toward vegetative growth instead of sexual reproduction. In ephemeral water bodies on the other hand, charophytes reproduce by sexual means whereby drought resistant oospores are produced. Thus more resources are allocated towards oospore production rather than vegetative growth (Casanova 1994).

The life cycle of *N. congesta* demonstrated that it is adapted to the hydrological regime of the lakes which depended on the rainfall pattern. In the field, the production and release of oospores was triggered to coincide with the summer drawdown, depositing the oospores in the sediment to ensure succession. On the onset of rains during late autumn/early winter, there is a mass germination of viable oospores. During this time there is decrease in temperature and photoperiod which resulted in higher vegetative growth. During spring when temperature begins to rise, algal resources are channeled into sexual reproduction.

In conclusion, the growth, life cycle pattern and other ecological characteristics possessed by *N. congesta* makes it an appropriate tool for rehabilitation of the ephemeral lakes at the Capel Wetland Centre. The next chapter looks at the ability of *N. congesta* to serve as an agent of phycoremediation.

Summary

The morphology, oospore bank, germination growth and life cycle of *Nitella congesta* were investigated both in the laboratory and in the field. Oospore bank studies showed an oospore density average of approximately 2 g^{-1}dry weight of sediment. Germination trials showed low rate of germination and low germination percentage, though oospore viability was very high. Shoots of *N. congesta* in the field were found to be of higher mean total length, mean number of branches and mean number of nodes. Mean growth rate was also found to be higher in the field than in the laboratory. However, the seasonal pattern of growth rate and life cycle proved to be identical in both field and laboratory shoots.

A positive regression ($P = 0.00$, $\alpha = 0.05$) was observed between water depth and mean height of *N. congesta*. Similar life cycle stages were observed in both the laboratory and field, with a little delay in completing the life cycle in the field related to water depth. Production of sex bodies is delayed by higher depth of water. There were significant differences observed in the growth of *N. congesta* among the three lakes in the filed. Culture studied in the laboratory reflected the same trend depending upon where culture came from.

4 HYPERACCUMULATION AND PHYCOREMEDIATION OF HEAVY METALS BY *N. congesta*.

4.0 Introduction

Contamination of the aquatic environment by heavy metals has attracted much attention due to their high toxicity to aquatic plants and animals as well as their long retention in the environment (Ait Ali *et al.* 2004; Mishra and Tripathi 2008). The presence of high concentrations of metals such as Cd, Pb, Cr, Zn in soil have potential toxic effects on growth and metabolism of plants (Shah and Dubey 1998; Agrawal and Sharma 2006). Plant species which are endemic to metalliferous soils tend to be tolerant to metal toxicity. This has led to the discovery of some plants with adaptive mechanisms that enable them to withstand the toxicity of such metals. They are being used for the remediation of contaminated soils (Blaylock 2000; Glick 2003). The remediation of heavily metal-contaminated soils often involves expensive excavation and removal of soil to landfills. Some plants have the ability to process very high concentration of organic compounds without any significant toxic effects with some of these chemicals being transformed into less toxic metabolites (Gardea-Torresdey 2003).

Successful implementation of phytoremediation in the field depends on a significant quantity of metal being removed from the soil through plant uptake to decrease effectively the soil metal concentration (Blaylock 2000). For effective phytoremediation, several conditions must be met including metal availability in the soil for root uptake. Also the plant must be adapted to various environmental conditions which may be present in the contaminated soils (Blaylock 2000).

The term hyperaccumulator has been used to describe a plant that has the capacity to accumulate trace metals at a tissue concentration approximately 100 times more than that of “normal” plants species (Baker and Brooks 1989). About 450 species of metal-hyperaccumulating plants have been reported (Reeves and Adigüzel 2004), out of which about 320 species are nickel hyperaccumulators. On the other hand, only 18 species of Zn-hyperaccumulating plants have been reported (Baker and Brooks 1989). The Zn-hyperaccumulating ability of *Sedum alfredii* Hance has been identified recently in China (Yang *et al.* 2002).

Zinc toxicity in crops occurs less frequently than Zn deficiency (Broadley *et al.* 2006) though it occurs in soils contaminated by mining, smelting and other anthropogenic activities especially in low-pH soils (Chaney 1993). Symptoms of toxicity are evident at concentrations ≥ 300 mg kg^{-1} with some plants showing toxicity symptoms at ≤ 100 mg kg^{-1} (Chaney 1993; Marschner 1995). Symptoms of toxicity include stunted growth and decreased yield (Chaney 1993). Thus a high soil or water concentration of zinc requires a drastic reduction of zinc concentration for successful plant growth. Many higher plant species have evolved over the decade as zinc hyperaccumulators and have been used extensively to achieve significant results.

Baker (1981) proposed two strategies of metal tolerance by plants; (1) exclusion, whereby plants avoid excessive uptake and transport of metals, and (2) accumulation and sequestration, whereby plants take up large amounts of metal and transfer the metal to the shoots for accumulation. Metal removal by wetland plants can be greatly enhanced by selecting the appropriate plants species (Fritioff and Greger 2003). Chaney *et al.* (1997) proposed the following characteristics to be possessed by a plant suitable for phytoremediation; ability to accumulate metals preferably in the above ground part, tolerance to metal concentration accumulated, fast growth and high biomass, widespread and easily harvestable with highly branched root system. *N. congesta* has a considerable biomass, fast growing, easy to propagate and can act as an annual as well as perennial depending on environmental factors in an aquatic environment.

In aquatic ecosystems, aquatic plants play critical functional roles such as taking up, storing, release and decomposition of nutrients (Fairchild *et al.* 1998). Some of these plants possess the ability to remove and possibly metabolise toxic chemicals thus preventing the impact on them and other biota in the ecosystem (Karen *et al.* 1998). Some aquatic macrophytes which include large algae and water plants have gained enormous attention due to their ability to sequester heavy metals (Lee *et al.* 1998).

Several aquatic macrophytes have the potential to accumulate heavy metals inside their body and many have been used for heavy metal removal from a variety of sources (Maine *et al.* 2001; Axtell *et al.* 2003; Mishra, Upadyaya *et al.* 2008). Aquatic macrophytes are thought to remove metals by three methods; (a) metals are restricted from entering the plant and are attached to the cell wall (b) metals are accumulated in the root, but translocation to the shoot is constrained (c) hyperaccumulation, metals are concentrated in the plants parts (Mishra and Tripathi 2008)

However, little is known about algae such as charophytes as zinc hyperaccumulators, though many species of algae have been reported to accumulate metals from their aqueous environment (Hassett *et al.* 1981). *Nitella* species were found to accumulate Cd (Hassett *et al.* 1981). Since metal concentrations in water often lie below the detection limit of instruments (Claveri *et al.* 1995), there has been growing interest in the use of bioaccumulators to monitor water quality by environmental agencies (Nimis *et al.* 2002). Hernández and Olguín (2002) suggested the use of algae enriched in polysaccharides, may be used as an efficient biosorbent for heavy metals. *Nitella congesta* with its profuse polysaccharide ensheathment might be an ideal alga to investigate the process of hyperaccumulation in its mucilage.

The uptake of metal ions by algae is a physiologically controlled process (Wilde and Benemann 1993). Some trace metals are required as trace nutrients by microalgae since they form part of the active sites of essential enzymes (Wilde and Benemann 1993). Bioremoval in natural or uncontrolled situations involves both passive and active transport mechanisms beginning with the diffusion of the metal ion to the surface of the microbial cell. Thus once the metal ion has diffused to the cell surface, it will bind to sites located on the cell surface which have an affinity for the metal (Wilde and Benemann 1993). Basic physiological parameters play an important role in the mobility and bioremoval of metallic elements, especially the group containing Co, Cu, Hg, Ag and U whose mobility is related to their water solubility (Volesky 1990). In the study by Hassett *et al.* (1981), twice as much as Cd was

accumulated by a *Nitella* sp. cultured in soft water compared to the same alga cultured in hard water. Thus many factors may be responsible for the effective and efficient accumulation of a metal by algae.

4.1 Phytoremediation by wetland plants

Macrophytes can absorb pollutants in their tissue and provide a surface and an environment for microorganisms to grow (Vymazal 2002). Macrophytes transport a high percentage of the oxygen available in the rhizosphere, which stimulates both aerobic decomposition of organic matter and the growth of nitrifying bacteria (Brix 1997; Scholz 2006). By contrast to microorganisms, macrophytes play only a secondary role in the degradation of organic matters in wetland systems (Stottmeister *et al.* 2003). They are therefore an indispensable part in the long-term functioning of wetlands (Scholz 2006).

Wetland plants also take up heavy metals from the environment but tend mainly to accumulate them in below ground tissues (Stoltz and Greger 2002; Weis and Weis 2004). Wetland plants differ in the extent to which they can accumulate metals. Some wetland plants have been reported to accumulate specific metals such as *Salvina natans* for Hg (Sen and Mondal 1987) and giant duck weed (*Lemna polyrrhiza*) for Zn (Sharma and Gaur 1995). Other wetland plants were found to accumulate appreciable amounts of a suite of metals including Cu, Cr, Fe, Mn, Cd and Pb. Examples of such wetland plants include coontail (*Ceratophyllum demersum* L.), giant duckweed (*Spirodela polyrrhiza* (L.) Schleid.), bacopa (*Bacopa monnieri* (L.) Pennell) and wild rice (*Hygrorrhiza aristata*) (Rai *et al.* 1995). However, in the same study, channel grass (*Vallisneria spiralis* L.) and alligator weed (*Alternathera sessils*) were found to accumulate the same metals in very lower concentrations (Rai *et al.* 1995). Table 4.1 shows a list of wetland plants used in phytoremediation and their respective sources.

Table 4.1 Aquatic plants that have been used in phytoremediation.

COMMON NAME	SCIENTIFIC NAME	REFERENCE
Algal bloom	*Microcystis sp.*	(Rai and Tripathi 2007)
Balrush/Cattail	*Typha latifolia, Typha domingensis*	(Manios *et al.* 2003; Ciria *et al.* 2005; Hadad *et al.* 2006)
Duckweed	*Lemna minor*	(DeBusk *et al.* 1996; Zayed *et al.* 1998; Wang *et al.* 2002)
Fuzzy water clover	*Marsilea dromondii*	(Qian *et al.* 1999)
Irish leaved rush	*Juncus xihoides*	(Qian *et al.* 1999)
Parrot's feather	*Myriophyllum spicatum*	(Lesage *et al.* 1991; Kamal *et al.* 2004)
Pond weed/ Curly leaf pond weed	*Potamogeton natans Potamogeton crispus*	(Ali *et al.* 1999; Fritioff and Greger 2006)
Poplar trees	*Populus deltoids*	(Southichak *et al.* 2006)
Rabbitfoot grass	*Polypogon monspeliensis*	(de Souza *et al.* 1999)
Reed	*Phragmites australis, Phragmites karka*	(Bragato *et al.* 2006; Aslam *et al.* 2007; Vymazal *et al.* 2007)
Reed canary grass	*Phalaris arundinacea*	(Vymazal *et al.* 2007)
Salt marsh bulbrush	*Scirpus robustus*	(de Souza *et al.* 1999)
Smart weed	*Polygonum hydropiper*	(Qian *et al.* 1999)
Smooth cordgrass	*Spartina alterniflora*	(Qian *et al.* 1999)
Taro	*Colocasia esculenta*	(Skinner *et al.* 2007)
Umbrella plant	*Cyperus alternifolius*	(Qian *et al.* 1999)
Water fern, Water velvet	*Azolla caroliniana, Azolla pinnata*	(Bennicelli *et al.* 2004)
Water hyacinth	*Eichhornia crassipes*	(Vesk *et al.* 1999; Zhu *et al.* 1999; Skinner *et al.* 2007)
Water lettuce	*Pistia stratiotes*	(Qian *et al.* 1999; Skinner *et al.* 2007)
Water zinnia	*Wedelia trilobata*	(Qian *et al.* 1999)
Zebra rush	*Scirpus tabernaemontani*	(Skinner *et al.* 2007)

Adopted after Rai (2008)

4.2 Phycoremediation by *N. congesta*

The two essential processes required for the remediation of mine void-pit lakes are enhancement of water quality and habitat of aquatic biota (John 2003). Among factors appropriate for the development of functional wetlands is the presence of macrophytes. An ideal macrophyte, for the creation of wetlands should have attributes such as the ability to provide habitats and food for macroinvertebrates, fish and waterfowl (Søndergaard *et al.* 1997).

John (2000) introduced the term Phycoremediation defining it as the remediation of aquatic environments by using algae resulting in the enhancement of water quality as well as reduction in heavy metals which leads to the sustainable development of aquatic systems. Numerous literature on accumulation of metals by algae have dealt with seaweeds. However a filamentous algae, *Mougeotia* sp. was discovered to accumulate iron in 25% of its dry weight (John 2000). Another algae, a charophyte, *Nitella congesta* (which was mistakenly reported as *Nitella hyalina*) was found to be hyperaccumulator of Ca, Mg, Mn, Fe, Cu and Zn as well N and P (John 2000).

Studies of metal accumulation by *Nitella congesta* have shown that metals are accumulated in the thallus and mucilage (John 2000). However, the mechanism of accumulation is unknown. The mucilage of *Nitella congesta* is composed of a mucopolysaccharide containing alkaline phosphate (John and Gayton 1994). Metal tolerance in plants can be achieved by sequestration of the metals in tissues or cellular compartments that are insensitive to the metals (Weis and Weis 2004).

The solubility and transport of many heavy metals into roots is enhanced in acidic soils, which creates special toxicity problems (Meagher 2000). Pollutants can be remediated in plants through several natural biophysical and biochemical processes such as absorption, transport and translocation; hyperaccumulation; or transformation and mineralization (Meagher 2000). The most significant single parameter influencing the biosorption process is pH. However apart from the most comprehensive effort by Hassett *et al.* (1981) on this subject, there has not been much literature defining the optimum pH for the uptake of metal ions by algae (Wilde and Benemann 1993). Hassett *et al.* (1981) used numerous algal strains using a novel microplate technique with the conclusion that the influence of pH on metal accumulation by algae is species specific. The optimal pH for bioremoval by algae is mainly dependent on the type(s) of algae (Wilde and Benemann 1993). Some metal ions are preferentially bound at high pH, some at low pH (Wilde and Benemann 1993) and others over a broad pH range (Bedell and

Darnall 1990). The use of immersed plants for phytoremediation has an advantage since all parts are in contact with water body. In an ideal situation, hyperaccumulators should be native plants or plants with a high adaptability to the environment at the polluted site (Kubota and Takenaka 2003). *N. congesta* in this case can be considered as a native alga adapted to the local conditions and was discovered to have colonized some of the lakes more than a decade ago.

The effects of heavy metals on aquatic plants have been observed for more than three-quarters of a century (Whitton 2003). A number of studies have reported accumulation of heavy metals (which are non-essential for metabolism or potentially toxic) by aquatic plants in concentrations much higher than in surrounding aqueous environment (Whitton 2003). The high concentration of heavy metals such as Ca, Mg, Mn, Fe, Zn and Al in the lakes of Capel Wetlands is of concern because of their effect on the aquatic ecosystem as well as the biological organisms in these ecosystems. Therefore, there was the need for an efficient and cost-effective approach to the remediation of the soil and water media in order to bring the concentration of the above mentioned metals to an acceptable level. The objective of this part of the study is to investigate the ability of *N. congesta* to accumulate metals from contaminated sediment and water.

4.3 Materials and methods

4.3.1 Metal accumulation by *N. congesta* in the field

Water and sediment samples were collected from from Nitella, Plover North and Plover South lakes on 17th June, 2004 and sent to a NATA accredited laboratory, SGS Laboratory, Perth, Western Australia for chemical analysis of the following; Fe, Ca, Mg, Mn, Al, Cu and Zn. Samples of *Nitella congesta* found in these lakes (up to 50g) were collected (on 16th November 2004) at random at the end of the vegetative growth cycle (at the onset of first fruiting bodies). The mucilage was separated from the thallus by hand into a clean beaker. Water quality parameters were measured *in situ* with a hand held

TPS Water quality meter. Unpublished data of metal accumulation by *N. congesta* in Nitella lake in 2003 was obtained from Jacob John and analysed.

About 50g of mucilage was obtained from the *N. congesta* meadows in Nitella lake was obtained after separating it from the thallus. This was sent to a NATA accredited laboratory, ULTRACE Analytical Laboratories, 58 Sorborne Cresent, Canning Vale, Western Australia for chemical analysis in 2004.

About 38g of mucilage was obtained from the *N. congesta* meadows in Nitella lake out of which 10g was freeze dried and analysed for elemental contents by Energy Dispersive Spectroscopy (EDS) using a Phillip's XL 30 scanning electron microscope in 2007.

4.3.2 Zn accumulation by *N. congesta* cultured in the laboratory

Samples of *N. congesta* were collected from the culture aquarium tanks in the laboratory. Only the shoot tips (distal 10 cm) were used for the investigation cultures (Urbaniak 2006). Samples of the fresh-weight shoot tips (up to 20g) was weighed and thoroughly washed with deionised water. The shoot tips were then placed in three different concentrations of 20 mg/L, 30mg/L and 100 mg/L $ZnCl_2$ in four replicates plus control setups. The pH values were adjusted to 6.0±0.2 by using 0.1mmol L^{-1} NaOH (Qui *et al.* 2006). The setups were left under natural conditions in the greenhouse at the Department of Environmental and Aquatic Sciences for 96 hours. The algae were washed thoroughly and dried at 60^{o}C to a constant weight. Samples of the thallus (about 500mg) were digested with nitric acid using a Milestone microwave. Zinc and other trace metals were measured by ICP-AES by a NATA accredited CSBP Soil and Plant Analysis Services, Perth, Western Australia.

The bioconcentration factor (BCF) was calculated as follows;

BCF = [metal μg $g^{-1}]_{(shoot)}$ / [metal mg $L^{-1}]_{(metal\ solution)}$ (Chen *et al.* 1998; Ait Ali *et al.* 2002; Ait Ali *et al.* 2004).

The total metal accumulation rate, expressed as μg g^{-1} DW day^{-1}, was calculated as follows;

Accumulation rate = $\left(\frac{([\text{Metal}]_{\text{shoot}} \times \text{DW}_{\text{shoot}})}{4}\right) \times \text{DW}_{\text{shoot}}$ Adopted from Ait Ali *et al.* (2004). '4' in the equation is the number of days of 'growth'.

The results were analysed using one-way ANOVA and the Turkey HSD test to compare the means of the treatments at 5% significance.

4.4 Results

4.4.1 Metal accumulation by *N. congesta* in the field

Graph was plotted from unpublished data of metal accumulation by *N. congesta* in Nitella Lake obtained from John in 2003. Concentrations of Mg, Mn, Zn and Fe in mucilage were higher than that of thallus of *N. congesta* from Nitella Lake. Concentration of Ca was however higher in thallus than mucilage (Fig. 4.1).

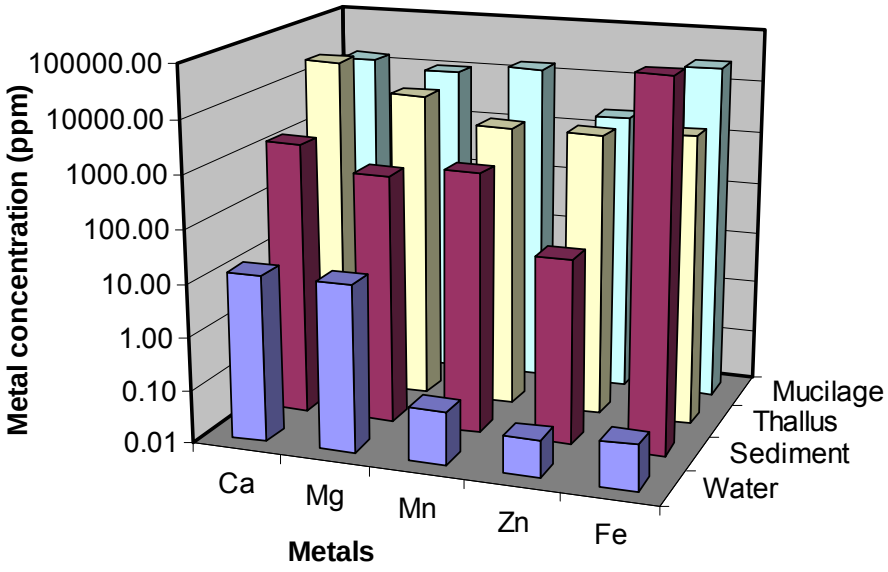

Figure 4.1 Concentration of metals (in water and sediment) and accumulation by *N.congesta* from Nitella Lake (thallus and mucilage) in 2003.

The lake water showed the lowest concentration of the metals (Ca, Mg, Mn, Zn and Fe) (Fig.4.1).

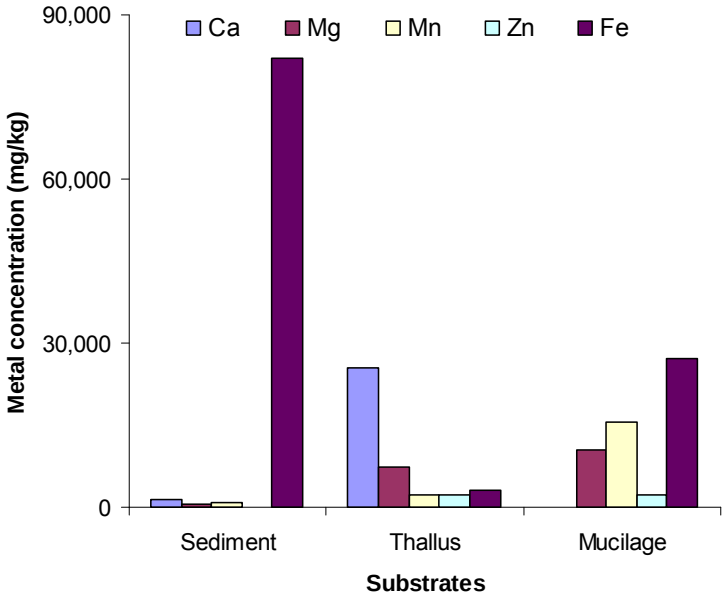

Figure 4.2 Concentration of metals in sediment of Nitella lake and thallus mucilage of *N. congesta* meadows analysed in 2003. Sediment was analysed before germination of oospores of *N. congesta*; thallus and mucilage were analysed prior to fructification of *N. congesta* meadows. (Data obtained from unplished data by J. John).

The concentration of iron in the mucilage of *N. congesta* from Nitella lake analysed was higher than that of the thallus, while the thallus had the highest concentration of calcium (Fig. 4.2).

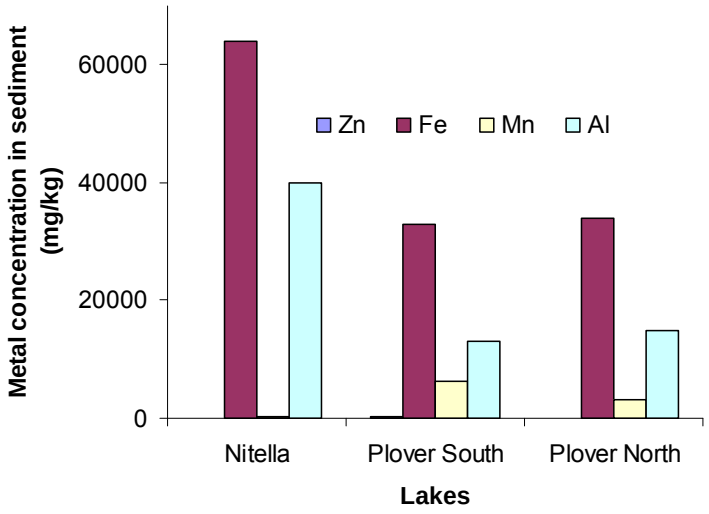

Figure 4.3 Metal concentration in lake sediment from three lakes at Capel Wetland Centre sampled in June 2004 during growth of *N. congesta* meadows in the field.

The sediment of Nitella lake had the highest concentration of iron and aluminium but no manganese. The concentration of zinc was also very low in all lake sediments (Fig. 4.3).

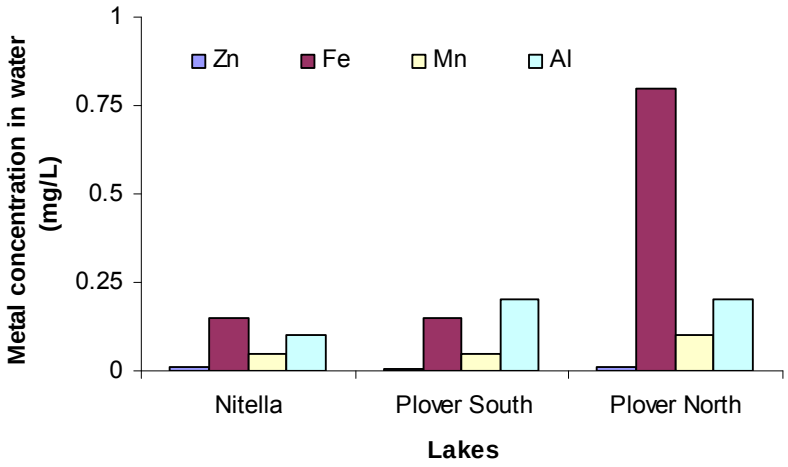

Figure 4.4 Metal concentration in lake water from three lakes at Capel Wetland Centre sampled in June 2004 during growth of *N. congesta* in the field.

Plover North lake had the highest concentration of iron and manganese while the concentration of zinc in all the three lakes (water) was very low (Fig. 4.4) as in lake sediment (Fig. 4.3). (Data received on 2nd August 2004).

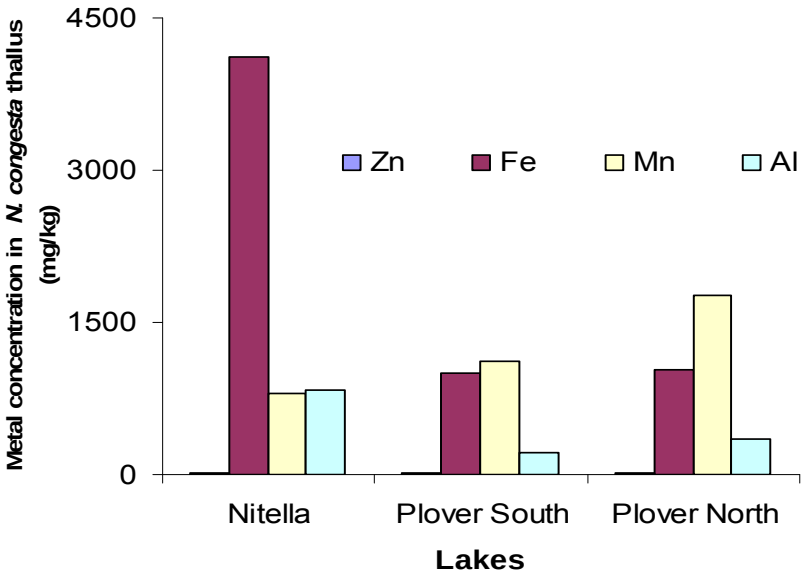

Figure 4.5 Concentration of metals accumulated by *N. congesta* thallus sampled in November 2004 in the field prior to fructification of *N. congesta* meadows.

N. congesta thallus from Nitella lake accumulated the highest concentration of iron and aluminium. However, *N. congesta* thallus from Plover north and Plover south lakes accumulated more manganese (Fig. 4.5). (Data received on 23rd November 2004).

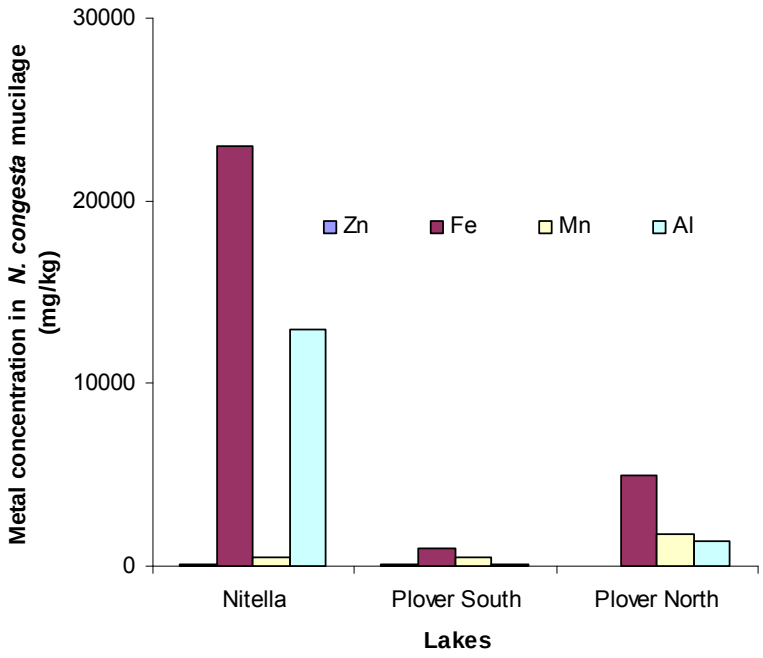

Figure 4.6 Concentration of metals accumulated by *N. congesta* mucilage sampled in November 2004 in the field prior to fructification of *N. congesta* meadows.

Mucilage of *N. congesta* from Nitella lake showed highest concentration of iron and aluminium (Fig. 4.6). The mucilage of *N. congesta* meadows from Nitella lake accumulated very high concentrations of iron (23000 mg/kg) and aluminium (13000 mg/kg). The results obtained show that *N. congesta* can accumulate metals in high concentrations by the mucilage.

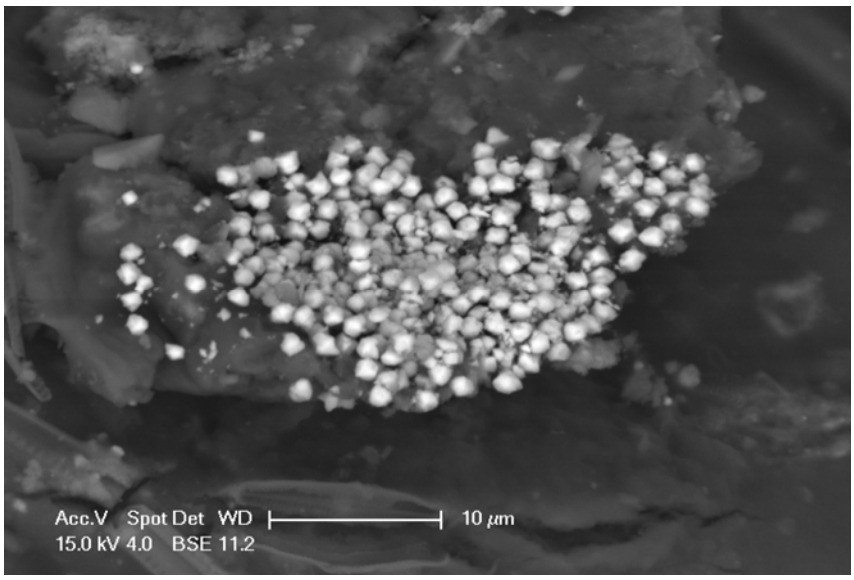

Figure 4.7 "Bright crystals" formed in the mucilage of *N. congesta* analysed in 2007. It is suspected to be formed from Fe and S (as FeS_x).

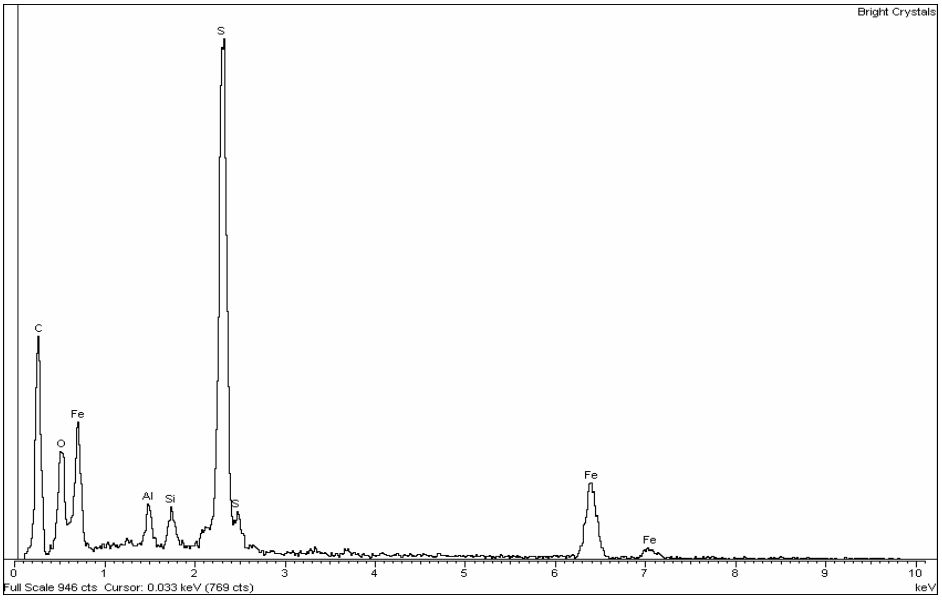

Figure 4.8 EDS spectrum of *N. congesta* mucilage showing accumulation of metals.

Peaks of sulphur and iron show high concentrations of these metals thus confirming possible presence of FeS_x as crystals above.

4.4.2 Zn accumulation by *N. congesta* in culture experiments

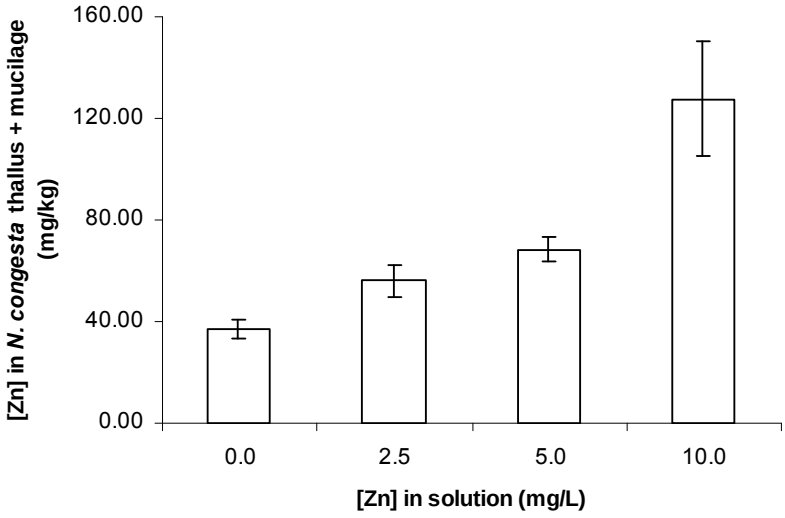

Figure 4.9 Zn accumulation by *N. congesta* cultured in the laboratory.

Table 4.2 Zn concentration in solution and shoot of *N. congesta*.

Sample	$[Zn]_{solution}$ (mg/L)	$[Zn]_{shoot}$ (mg/kg)	BCF	AR
HA1	10.0	114.35	11.44	66.05
HA2		103.95	10.40	74.22
HA3		139.08	13.91	76.16
HA4		153.50	15.35	116.18
HB1	5.0	61.53	12.31	26.00
HB2		71.56	14.31	38.13
HB3		67.82	13.56	40.21
HB4		72.94	14.59	33.73
H1	2.5	63.64	25.46	28.14
H2		58.59	23.44	30.37
H3		48.61	19.44	24.50
H4		52.98	21.19	22.02
C1	NIL	36.78	ND	
C2		41.87		
C3		36.12		
C4		33.17		

*ND denotes “not determined”

BCF denotes Bioconcentration Factor

AR denotes Accumulation Rate (μg g^{-1} DW day^{-1})

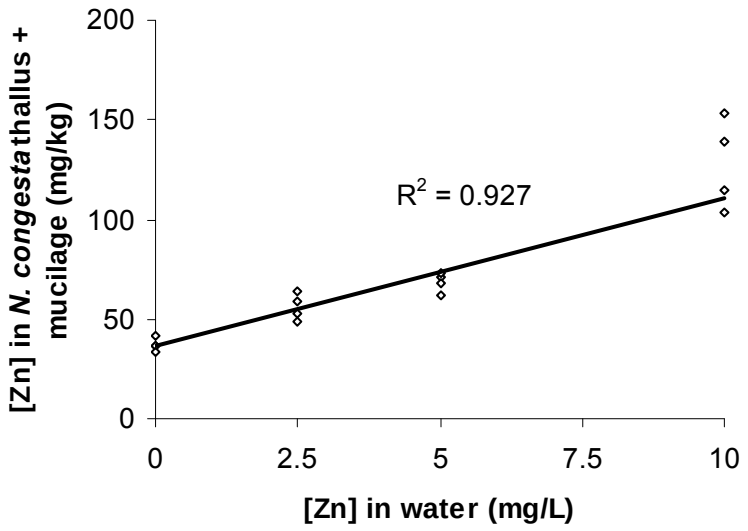

Figure 4.10 Zn accumulation by *N. congesta* showing regression.

In the laboratory experiment, the concentration of zinc accumulated by *N. congesta* was obtained from tissue and mucilage increased progressively at zinc concentrations of 2.5mg/L, 5.0mg/L and 10.0 mg/L. The maximum shoot zinc concentrations were determined at the treatment with 10.0 mg/L zinc with mean Bioconcentration Factor (BCF) of 12.77±2.26. However, at the metal concentrations of 2.5mg/L and 5.0mg/L, the bioconcentration factors were 22.38±2.62 and 13.69±1.0 respectively. Thus the bioconcentration factors at the lower metal concentrations were higher than the bioconcentration factor at the highest metal concentration.

4.5 Discussion

The thallus of *N. congesta* from the field was found to contain high concentrations of calcium, while no calcium but high iron concentration was found in the mucilage. The thallus of *N. congesta* accumulated calcium in high concentration than the mucilage (Fig. 4.2). This could be explained that the *N. congesta* depended on calcium ions for survival. In a study by Davenport *et al.* (1996), it was discovered that the presence of calcium ions controlled the influx of sodium ions thus controlling turgor. However, the exact mechanism involved in that process is unclear. Therefore, the

accumulation of high concentration of calcium by *N. congesta* may be for maintaining turgor pressure of the thallus.

Among the three lakes that were sampled, mucilage of *N. congesta* from Nitella lake had the highest concentration of iron (Fig. 4.6). This may be due to the high concentration of iron in the sediment of the lake (Fig 4.3) since *N. congesta* thallus from the same lake also showed highest concentration of iron (Fig. 4.5). The concentration of manganese in lake water was very low (Fig. 4.4) as compared to that in lake sediment (Fig. 4.3). The concentration of manganese in the *N. congesta* thallus from the lakes (Fig. 4.5) were found to be higher than in the lake water (Fig. 4.4). This could be attributed to accumulation over time. That is from 17th June 2004 (when the sediment and water samples were analysed) to 16th November 2004 (when charophyte samples were analysed).

The EDS spectra of the mucilage show concentration of some metals such as iron and aluminium. The iron and sulphur peaks in the spectra (Fig. 4.8) may have corresponded to the “Bright Crystals” in Figure 4.7. These crystals could be Fe_2S resulting from the reaction between Fe and S which are in high concentrations in the sediment of Nitella lake where *N. congesta* meadows were sampled from. Nevertheless, the assumption is that the majority of sulphur content of a lake would be in the form of sulphate anion as a common feature of ASS (Appleyard *et al.* 2004; Hinwood *et al.* 2006)

In an earlier study by John in 2003, among metals analysed, it was found that the highest concentration of calcium was in the thallus of *N. congesta* (Fig.4.1 and Fig. 4.2). The rest of the metals were of very high concentrations in the mucilage. Therefore, it may be that the mucilage of *N. congesta* have high affinity for these metals. The mucopolysaccharides may have been negatively charged sites which have the ability to attract the positively charged metal ions. Also as a non-living material, the mechanism of metal ions accumulation by the mucilage could be adsorption.

The uptake of metal ions by algae is predominantly a physiologically controlled process (Wilde and Benemann 1993). Examples include the accumulation of the radionuclide technetium by the marine brown algae *Fucus serratus* (Van der Ben *et al.* 1990) and bioaccumulation of tin by estuarine macroalgae (Wright and Weber 1991; PawlikSkowronska *et al.* 1997). Binding of metals to surfaces and extracellular ligands are important as well as the release of exudates which may be esasential in decreasing the toxic effects to the algae (Xue and Sigg 1990; Claessens and Van Cappellen 2007). Microalgae can bind (and detoxify) metal ions through complexation with polyphosphate bodies (Jensen *et al.* 1982; Siderius *et al.* 1996; Mehta and Gaur 1999).

The mucilage of *N. congesta* was observed to be disappearing from the basal part of the thallus with that of apical meristems intact. Thus the mucilage may have acted as a protective shield from the chemical effects of very high concentrations of metals ions after serving as a reservoir for the metals. The removal of metals in natural or uncontrolled situations typically involves a combination of both active and passive transport mechanisms starting with the diffusion of the metal ion to the surface of a microbial cell (Wilde and Benemann 1993) in this case, the mucilage of *N. congesta*.
The metal ions then diffuse to the cell surface, and bind to sites on the cell surface which exhibit some chemical affinity for the metal (Wilde and Benemann 1993).

The threshold for Zn hyperaccumulation was found to be 10000 mg kg^{-1} dry weight in the shoots (Baker and Brooks 1989; Reeves and Baker 2000). However it was showed that a zinc concentration ≥ 3000 mg kg^{-1} dry weight can be considered a reasonable concentration for Zn hyperaccumulation since most plants contain 50 – 500 mg kg^{-1} Zn even when they are grown on zinc soils (Reeves and Baker 2000). Moreover, Baker *et al.* (1994) reported that it was not possible for *Thlaspi caerulescens* to accumulate up to 10000 mg kg^{-1} Zn in its shoots unless it was grown on considerably highly contaminated soils.

Although the Zn concentration obtained in this experiment was 127.72 ±22.64 mg kg^{-1} dry weight, which is below threshold for Zn hyperaccumulation, a BCF > 10 at all three metal concentrations can be considered very high; a characteristic of hyperaccumulation. In most experiments, suspected hyperaccumulator plants are cultivated for several days sometimes during the entire life histories of the plants. Therefore with a period of only 96 hours and showing BCF >10, *N. congesta* could be said to be a hyperaccumulator of zinc. Additionally, the relationship between the zinc concentration in solution and the zinc concentration accumulated was linear (R^2 = 0.927). One-way ANOVA and the Tukey HSD test comparing the means of treatments was significant at 5% significance (F= 41.270, P = 0.000) (Appendix 5).

The hyperaccumulation of metals by plants is somewhat an intriguing phenomenon involving the following steps; (a) transport of metals across the plasma membrane of root cells, (b) xylem loading and translocation, and (c) detoxification and sequestration of metals at the whole plant and cellular levels (Lombi, Terall *et al.* 2002). In this project, only the distal apical portion of *N. congesta* was used as mucilage at this portion was intact. The accumulation rate of *N. congesta* at the different concentrations was also found to be high in all cases. The 10 mg/L Zn solution was found to be the highest with a mean value of 83.15±22.45 µg g^{-1} DW day^{-1}. The hyperaccumulation of zinc in the laboratory experiment yielded higher rates supporting the assertion that under controlled greenhouse conditions, phytoremediation is generally more effective than under field conditions (Banuelos 2000). The physiology of zinc accumulation in hyperaccumulator plants, is not well understood (Lasat and Kochian 2000). More so, little information exists concerning the mechanism of hyperaccumulation of metals especially by aquatic macrophytes such as *N. congesta*.

There has been conflicting reports concerning the functions of the presence of ‘plaques’. Some have opined that the presence of plaque reduce the uptake of metals by plants whiles others are of the contrary (Weis and Weis 2004). For example, the presence of concretions was perceived to reduce

the accumulation of manganese by *Phragmites australis* (Batty *et al.* 2000) as well as accumulation of zinc by *Aster tripolium* (Otte *et al.* 1989). Thus the plaque might have acted as a barrier.

In *Typha latifolia* (cattail) however, it was observed that the presence of an iron plaque did not reduce the accumulation of toxic metals (Ye *et al.* 1998) whiles it enhanced uptake of zinc by rice (*Oryza sativa*) (Zhang *et al.* 1998). In the study of hyperaccumulation of metals by *N. congesta*, it was found out that the mucilage, though a non-living material did not act as a barrier to the hyperaccumulation but rather it acted to enhance the accumulation of the metals. The mucilage of *N. congesta* therefore served as reservoir for the hyperaccumulated metals. However the mechanism of metal accumulation or/and the enhancement of metal accumulation was not determined.

The lower efficiency of *N. congesta* in the accumulation of zinc from the solution in the laboratory study, compared to the accumulation of other metals in the field can be explained as follows: The accumulation of metals by submerged macrophytes can be enhanced by harvesting the algae periodically. This keeps the algal population in a continuous growth stage, which maximizes the rate of accumulation (Oron 1990). However, due to the short duration of the laboratory experiment, this was not done. It could also be attributed to the toxicity of zinc.

In conclusion, *N. congesta* is a hyperaccumulator of metals and provides a new specie of alga for the remediation of contaminated soil and water. Its presence and successful establishment in the lakes of Capel Wetlands Centre will therefore ensure the efficient and cost effective removal of the metals associated with sand mining that have been deposited in the lake sediment and water during the sand mining era.

Summary

The metal accumulating ability of *Nitella congesta* from Nitella Lake was investigated. Accumulation of Ca, Fe, Zn, Al, Mg and Mn by *N. congesta* from Nitella lake was investigated from field samples. It was found that the mucilage of *N. congesta* contributed to the accumulation of metals thus serving as a reservoir for iron and aluminium above the threshold of 10,000 mg/kg.

Laboratory experiment to study the hyperaccumulation of zinc by *N. congesta* yielded a threshold of 127.72±22.64 mg/kg dry weight. However, the average bioconcentration factor calculated was > 10 which is a characteristic of hyperaccumulators. One-way ANOVA & Tukey HSD test comparing the mean metal concentration of treatments showed that the mean difference is significant at 5% level (P = 0.000).

5 *Nitella congesta* AND MACROINVERTEBRATE DIVERSITY AND ABUNDANCE IN CAPEL WETLANDS

5.0 Introduction

There has been an increased emphasis on invertebrate communities in the monitoring of wetlands. This is because invertebrates are known to play important role in the rates of nutrient cycling, primary productivity, decomposition, and the flux of energy between trophic levels in the wetland ecosystems (Batzer and Wissinger 1996; Brady *et al.* 2002).

Macroinvertebrates inhabit freshwater habitats for part or all of their life cycle and widely used in wetland ecosystem management as biomonitors. They make up part of the grazing and detrital food chains by feeding on algae, other invertebrates and dead matter (Davis and Christidis 1997). Despite well documented evidence on the use of macroinvertebrates as biomonitors in river and stream assessment (Rosenberg and Resh 1993; Metcalf-Smith 1996), their use in wetland biomonitoring, restoration and management is minimal. In a baseline survey conducted by Cale and Edward (1987), 77 invertebrate taxa were recognized within the lakes of Capel Wetlands Centre. The results showed that the lakes of the Capel Wetlands Centre had relatively high species richness in comparison with surrounding natural wetlands. This was attributed to the presence of fringing macrophytes in the lakes. It was concluded that the presence of macrophytes increased species richness by 25 to 50%.

5.1 Macroinvertebrates as indicators

The choice of biological indicators should depend largely on the purpose of monitoring and the availability of the indicators in the environment. Macroinvertebrates are one of the most commonly used taxonomic groups for stream bioassessment in Australia (Norris and Norris 1995). A number of reasons make these group of organisms crucially important in the functioning of wetlands, making them surrogates for the health of the wetland ecosystems (Balcombe *et al.* 2005). Macroinvertebrate communities serve as indicators of water quality and therefore their conservation should be of prime importance due to their sensitivity to changes in water quality such as eutrophication and pollution (Chessman *et al.* 2002). Their species abundance and diversity place them in a vital position as excellent

biomonitors as their presence or absence give a good indication of the disturbance of a site (Davis and Christidis 1997).

Macroinvertebrates form very important links in the energy system and element cycles by providing food source for fish as well as birds while acting as detritus decomposers (van den Berg *et al.* 1997; Brady *et al.* 2002). They are abundant, readily surveyed and taxonomically rich (Dodson 2001), hence the use of invertebrates to quantify water quality in wetland ecosystems by many researchers (Wallace *et al.* 1996). They also contribute to wetland function by assisting in nutrient cycling (Merrit *et al.* 1984), making them contribute indirectly to the transfer of nutrients from the sediments, detritus and water column to higher trophic organisms, with the resultant impact on wildlife species that feed on them (Balcombe *et al.* 2005). Macroinvertebrates are also used in the assessment of avian productivity since numerous birds particularly waterfowls depend on them for food (De Szalay and Resh 1996; Anderson and Smith 1998) as observed many times at the Capel Wetlands Centre.

The advantages of using macroinvertebrates as indicators include: (1) they are ecologically important and their distribution reflect changes in environmental conditions; (2) they are ubiquitous, accessible and able to collect easily; (3) they are significantly sedentary and amenable to spatial analyses. Using macroinvertebrates as indicators however have disadvantages that include: (1) it requires special expertise; (2) data accumulates slowly, though sampling is easy; (3) it is perceived as costly; (4) quantitative sampling is difficult due to the high numbers of samples; (5) macroinvertebrates may not respond to all impacts such as the effect of herbicides and their distribution can be affected by factors other than water quality (Rosenberg and Resh 1993; Stewart and Loar 1993).

5.2 The relationship between invertebrates and macrophytes

Aquatic plants play an important role in the structuring of freshwater communities. They influence the interactions between predators while acting

as food source (Lauridsen *et al.* 1998). The abundance and distribution of freshwater macrophytes are dependent not only on abiotic factors such as depth, pH, light, sediment type and water chemistry but also dependent on biotic factors such as herbivorous grazing (Sheldon 1987). Macrophyte biomass, productivity and relative species abundance can be altered by invertebrate grazing, thus suggesting that the potential impact of grazers in aquatic ecosystems is large (Lodge 1991).

Invertebrate grazing of macrophyte biomass have both negative and positive effects. On the other hand, invertebrate grazing on some species of algae allows a resultant increase in abundance of other species. For example, primary production of epiphyton and increased algal species richness can be enhanced by grazing. On the whole, invertebrate grazers have a profound effect on submerged macrophyte biomass, productivity and species composition, although the degree of influence is dependent on different anti-grazing strategies of different species (Ostrofsky and Zettler 1986; Lodge 1991).

The relationship between macrophytes and grazers appears to be mutualistic. In such an association, while the macrophyte benefit from the removal of epiphyton and increased nutrient turnover, the grazers (e.g. snails) obtain a surface for grazing and ovipositioning as well as shelter. Macrophytes tend to provide protection for macroinvertebrates from predation (Benke 1976; Benke *et al.* 2001). However, deleterious effects of grazers can be combated macrophytes by the excretion of antibiotics and chelatants (chelators which tend to make nutrients unavailable to epiphytes) (Brönmark 1985; Jones *et al.* 1999; Lau and Lane 2001; Pinowska 2002). Charophytes are of immense importance to the aquatic ecosystem as they tend to harbour a diverse number of invertebrates across the expansive area they cover (Hutchinson 1975). Studies by van den Berg *et al.* (1997) showed that the biomass and seasonal duration of charophytes determine the abundance and species composition of macroinvertebrates.

The objective of this part of the study was to test the hypothesis that *Nitella congesta* enhances the habitat of macroinvertebrates, thereby increasing their diversity.

5.3 Materials and methods

5.3.1 Macroinvertebrates species diversity and abundance

Two sites were selected in Nitella lake where *Nitella congesta* was predominant. The first site (N1) (33^{o} 36.07 S, 115^{o} 30.15 E) was located in the south western end of the Capel Wetlands Centre, and was dominated by well established *Nitella congesta*. The second site (N2) (33^{o} 36.06 S, 115^{o} 30.16 E) was located in the northern end of the wetland and virtually devoid of *N. congesta*.

Two sites were also selected in Higgins lake, the newest artificial lake in the wetland. It was not formed as result of mining and was devoid of *Nitella congesta*. The first site (H1) (33^{o} 34.47 S, 115^{o} 31.17 E) was close to the main road that seem to divide the wetland. Site 2 (33^{o} 34.40 S, 115^{o} 31.22 E) was close to the Bussell Highway. Sampling was done only once in Higgins Lake in 2004 and 2005.

Macroinvertebrates were sampled on two occasions in Nitella Lake at five weeks interval in 2004 and 2005 thus allowing charophytes to re-grow and support more invertebrates in between sampling in each instance. A cylinder made of metal mesh (1m in diameter) was lined with a flexi glass lining to prevent loss of captured invertebrates moving across. This was then placed at the charophyte dominated site; with ease because the water was exceptionally clear thus ensuring nearly a 100% visibility. The diameter of the mesh cylinder was made large enough to enclose a large area of *Nitella congesta* meadows.

Macroinvertebrate samples were collected by the use of a net (Laasonen *et al.* 1998; Wood *et al.* 2000) (net frame of 40 $\times$ 25 cm, mesh size 250 µm) within the cylinder enclosure. Sediment from both the charophyte dominated

and control sites were collected into different 20 L buckets. The samples were sieved with a 250 µm mesh wooden framed sieve and all macroinvertebrates collected were preserved in jars with 70% ethanol (Linke *et al.* 1999). Strands of *N. congesta* were also taken to the Algology Laboratory at the Department of Environmental and Aquatic Sciences, Curtin University for further washing in order to dislodge any macroinvertebrates attached. A numerical count and species list of the macroinvertebrates collected was made. Identification of macroinvertebrates was done to lowest level possible by the assistance of Erin Thomas. At each sampling, water temperature, pH, conductivity and salinity were measured *in situ* with a portable TPS WP-81 water quality meter.

5.3.2 Data Analysis

Shannon-Weaver diversity index was used to measure the variability of the communities using the number of species present.

$$H' = \sum_{i=1}^{S} P_i \log \left(P_i\right)$$

where H' is the Shannon-Weaver Diversity Index

P_i is the proportion of the total abundance of sample represented by species i,

s is the number of species in the sample.

To determine the evenness of distribution,

$$J = \frac{H'}{H_{max}}$$

J is Evenness Measurement;

H' is obtained from the Shannon-Weaver model;

H_{max} is the maximum value of diversity of a site obtained by:

$$H_{max} = \mathrm{Log}_{10}\ k$$

k is the number of categories or different species

To compare the faunal similarities between the sample sites, Sorenson's Index of Similarity was used;

$$IS_s = \frac{c}{0.5(a+b)}$$

c = number of species common to both sites

a = number of species in site 1

b = number of species in site 2

Cluster analysis was used to group sites. One-way ANOVA was used to compare diversity, evenness and species diversity of macroinvertebrates from the sites in Nitella and Higgins lakes. Student T-Test was used to test hypothesis. All statistical analysis was performed using SPSS Grad Pack 15.0.

5.4 Results

Table 5.1 Species presence and abundance of macroinvertebrates at selected sites in Nitella and Higgins Lakes in 2004 and 2005.

SPECIES	SITE AND DATE OF SAMPLING											
	NITELLA LAKE								HIGGINS LAKE			
	24/09/2004		29/10/2004		23/09/2005		28/10/2005		29/10/2004		28/10/2005	
	N1	N2	N1	N2	N1	N2	N1	N2	H1	H2	H1	H2
COLEOPTERA - Dytiscidae												
Diving beetle larvae	1	2			3		4					
Sternopriscus			1						2	5	1	3
DIPTERA - Chironomidae												
Chironomus occidentalis		1							2		2	
Procladius villosimanus		4		1								
EPHEMEREOPTERA -Caenidae												
Tasmanocoensis sp.	1				2							
GASTROPODA-Lymnaeidae									1	1	3	2
HEMIPTERA												
Corixidae - Waterboat men	1		28		15		15	4				
Nepidae - *Ranatra*	1											
Gelastocoridae - *Nerthra*			1				3					
LEPIDOPTERA												
Caterpillar larvae	1											

Table 5.1 Cont'd

ODONATA												
Libellulidae												
Orthetrum caledonicum	6		4	6	3		4					
Diplacodes bipunctata			2		4		2					
Aeshnoidae												
Adversaechna brevistyla									1	5	5	4
Hemianax papuensis												
Procodrulia affinis			2				2					
Corduliidae												
Procodrulia affinis	1											
Coenagrionidae – Damselfly larvae			1		2		2					
Lestidae – Damselfly larvae			1				2					
Podocopida			8									
NEMATODA - Oligochaeta			5		5		6	2				

Table 5.2 Water quality parameters at the sampling sites in Nitella lake.

	24/09/2004		29/10/2004		23/09/2005		28/10/2005	
	N1	N2	N1	N2	N1	N2	N1	N2
Cond (mS/cm)	0.34	0.34	0.94	0.94	0.56	0.58	1.34	1.36
pH	8.51	8.2	9.57	9.4	8.7	8.42	9.66	9.59
Salinity (ppM)	181	181	480	450	218	222	489	492
Temp (^{o}C)	20	19.6	22	22	18.8	19	24	23.5

Table 5.3 Water quality parameters at the sampling sites in Higgins lake.

	24/09/2004		28/10/2005	
	H1	H2	H1	H2
Cond (mS/cm)	4.55	4.59	3.72	5.01
pH	6.95	6.83	6.55	6.59
Salinity (ppM)	637	541	318	241
Temp (^{o}C)	22.7	22.4	22.7	22.4

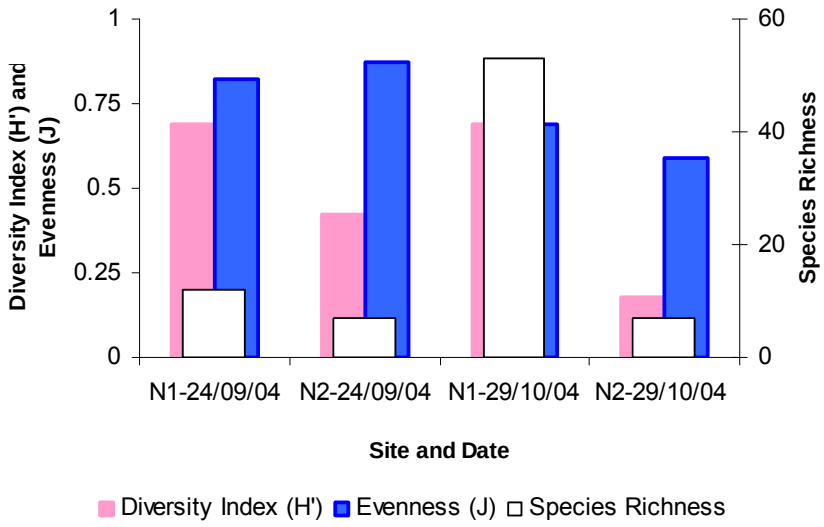

Figure 5.1 Diversity (H'), Evenness (J) and species richness measure of macroinvertebrates in Nitella Lake in 2004.

N1 = Charophyte dominated and N2 = Control sites (devoid of charophytes)

Charophyte dominated sites (N1) showed higher species richness and diversity on both samplings in 2004.

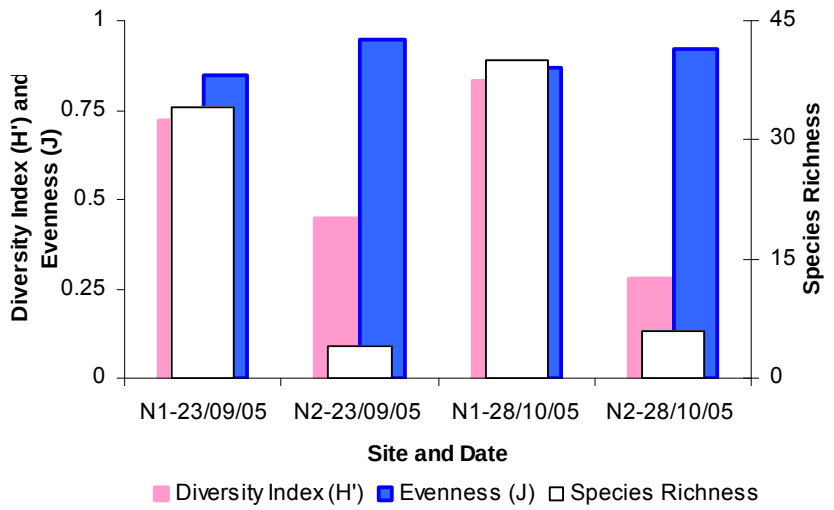

Figure 5.2 Diversity (H'), Evenness (J) and species richness measure of macroinvertebrates in Nitella Lake in 2005.

N1 = Charophyte dominated and N2 = Control sites (devoid of charophytes)

Charophyte dominated sites (N1) showed higher species richness and diversity on both samplings in 2005.

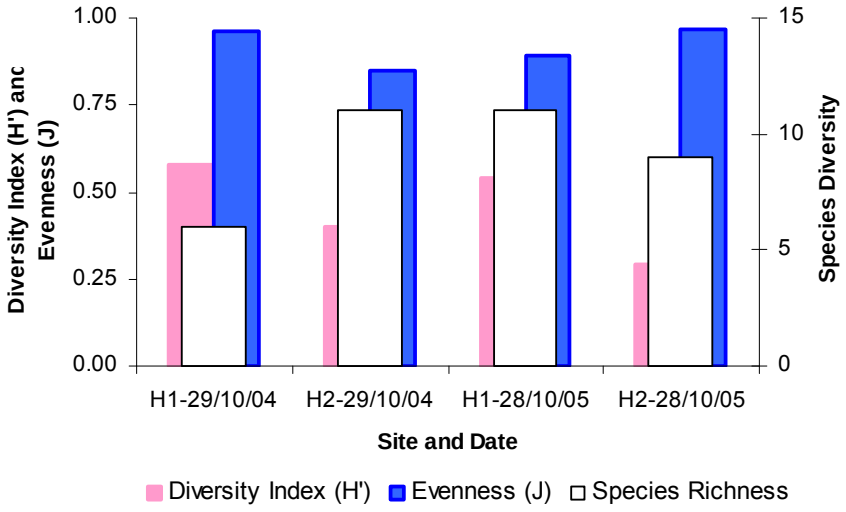

Figure 5.3 Diversity (H'), Evenness (J) and Species richness measure of invertebrates in Higgins Lake in 2004 and 2005.

H1 and H2 = sites in Higgins lake (both sites devoid of charophytes)

Site H2 showed higher species richness in 2004
Site H1 showed higher species richness in 2005

Table 5.4 Similarity Index measurements from sample sites in Nitella and Higgins Lakes.

Lake	Date	Site	IS_s
Nitella	24/09/2004	N1	0.20
		N2	
	29/10/2004	N1	0.17
		N2	
	23/09/2005	N1	0.40
		N2	
	28/10/2005	N1	0.36
		N2	
Higgins	29/10/2004	H1	0.86
		H2	
	28/10/2005	H1	0.86
		H2	

N1 = charophyte dominated site in Nitella lake

N2 = control site (devoid of charophytes) in Nitella lake

H1 and H2 = sites in Higgins Lake; both sites without charophytes

Sites in Nitella lake (N1 and N2) showed low Similarity Index (ISs), < 1. This indicates they shared mostly dissimilar species.

Sites in Higgins lake (H1 and H2) showed high Similarity index (ISs) close to 1. This indicates they shared mostly common species.

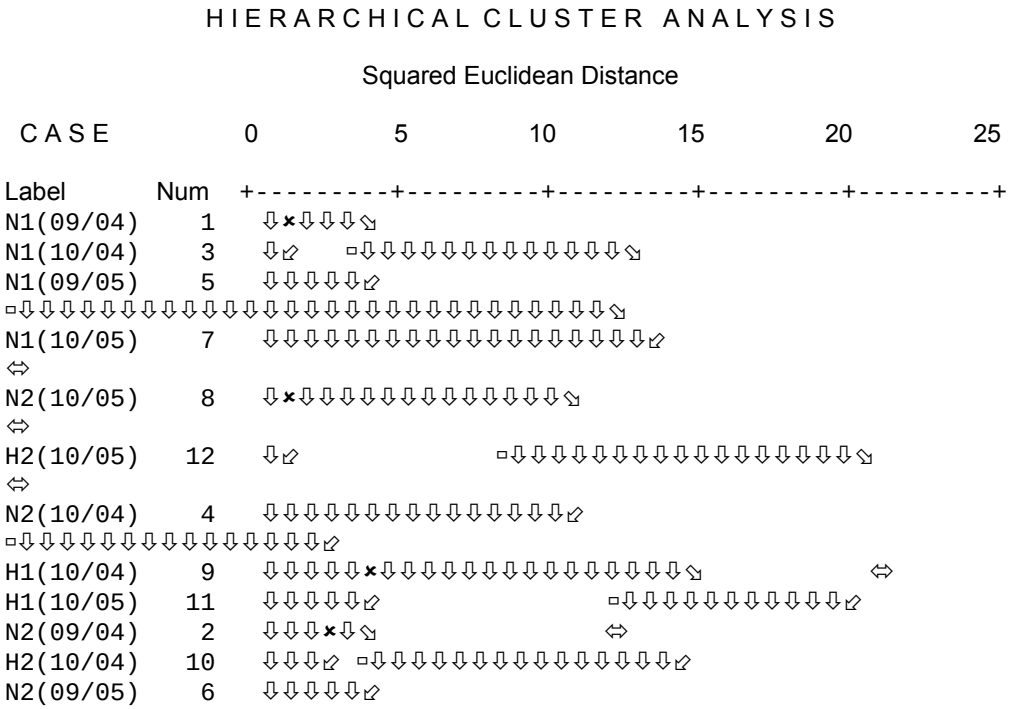

Figure 5.4 Dendogram for diversity in macroinvertebrate communities among sites in Nitella and Higgins Lakes.

N1 = Charophyte dominated site in Nitella lake
N2 = Control sites in Nitella lake (devoid of charophytes)
H1 and H2 = sites in Higgins lake (both sites devoid of charophytes)
All charophyte dominated sites in Nitella lake (N1) are clustered together. Sites 1 in Higgins lake (H1) are clustered together. Control sites in Nitella lake (N2) are clustered together with second sites in Higgins lake (H2).

Table 5.5 One-way ANOVA comparing evenness, species richness and diversity of macroinvertebrates from sites in Nitella and Higgins Lakes at 5% significance level.

	F	*P*
Evenness	5.127	0.033
Species Richness	0.588	0.576
Diversity	13.326	**0.002**

Diversity is significantly different at 5% significance level but not evenness and species richness (Appendix 6).

5.5 Discussion

This part of the study was conducted to investigate the relationship between charophytes and macroinvertebrate abundance and biodiversity. Different macroinvertebrate communities found in different sites can be related to many factors. In this study, macroinvertebrate abundance was high in the *N. congesta* dominated sites in all cases of sampling. This supports the fact that *N. congesta* provide refuge for macroinvertebrates thus shielding them from predation. They also serve as food source for the macroinvertebrates (Perrow *et al.* 1999). The presence of charophytes does alter the structure of macroinvertebrate communities as reported in literature (Middleboe and Markager 1997; Perrow *et al.* 1999).

During the first sampling, the most dominant species on the *N. congesta* dominated site in Nitella Lake was *Orthetrum caledonicum* (Libellulidae,

Odonata) with 50% of the total population. On the control site, the dominant species was *Procladius villosimanus* (Chironomidae) with 57.1% of the total population. The second sampling recorded a shift in species dominance. On the *N. congesta* dominated site, Corixidae (Hemiptera) was the dominant species with 52.8% of the total population. The control site recorded *Orthetrum caledonicum* Libellulidae (Odonata) as the dominant species with 85.7% of the total population. The shift in dominance species may be due to the increase in *N. congesta* meadows per unit area after the first sampling. This may have served as a refuge for the Corixidae (waterboat man) due to predation. Physical conditions such as temperature of the water as well as life cycle stages may also have contributed to the dominance shift.

On both occasions, the control sites had a species abundance of 7 indicating no increase or decrease. There was an increase in species abundance from 12 to 53 on the *N. congesta* dominated sites between the first and second visits; an increase of more than 400%. The increase in species abundance may be attributed to the presence of *N. congesta*. The morphological structure of *N. congesta* with the high number of branchlets may have provided enough room for refuge thus decreasing possible predation by preys. Also other lower level macroinvertebrates may have served as food source.

Sampling in 2005 showed a slightly different trend of species dominance on both visits. On the first sampling in 2005, the most dominant species in the *N. congesta* dominated site was Corixidae (Hemiptera) with 44.1% of the total population. The control site had no macroinvertebrates. During the second visit, both sites were dominated by (Hemiptera) with 37.5 % of the species population in the charophyte dominated site and 66.7% of the population in the control site. However, the species abundance was higher in the charophyte dominated site. The shift in dominance species may be due to the presence of *N. congesta* serving as refuge and food source for the Corixidae (waterboat man).

In all instances, the species richness and the corresponding diversity indices were higher in *N. congesta* dominated sites compared to the control sites (Fig. 5.1 and Fig. 5.2). However, the evenness values for charophyte dominated sites decreased slightly compared to the control sites. This is indicative that the main factor(s) contributing to the diversity index of the control sites is not the number of different species but the equal numbers of species from the various groups. The diversity indices of the *N. congesta* dominated sites were the same for the first samplings in 2004 and 2005. However there was a significant decrease in the evenness in 2004. This indicates that the high diversity measurement of the *N. congesta* dominated site was due to the abundance of the dominant species, the Waterboat men (Corixidae) with 52.8% of the total species richness of 53.

The difference in species richness of the *N. congesta* dominated site was not very much and that of the control site was also nearly equal. On both visits, the Waterboat man (Corixidae) was found to dominate the *N. congesta* dominated site with 44.1% and 37.5% for the first and second visits respectively. Diversity Indices in all cases corresponded to the number of groups or families found in the sites supporting the fact that high species diversity indicates a diversity of habitats and healthy ecosystem.

For example, the Odonata family comprising dragonflies and damselflies which are rarely associated with polluted waters were most abundant in the *N. congesta* dominated site (Googerham and Tsyrlin 2002). This shows that the water quality around the charophyte meadows was better and supported a more diverse and abundant macroinvertebrate community (Davis and Christidis 1997). However, though macrophytes provide protection for macroinvertebrates from predation, predators such as those in the family Odonata were found to be associated with charophyte habitats as well. It can be suggested that such predators may have a significant influence on the structure of macroinvertebrate community biodiversity in such habitats.

The Sorenson's Similarity Index (ISs) (Table 5.2) shows that there is virtually no similarity in species between the *N. congesta* dominated and control sites

on both visits. In 2004, the percentage similarities on both visits were 20% and 17% while in 2005, the percentage similarity on both visits were 40% and 36%. However, similarity of the sites in Higgins lake are the same (86% in both 2004 and 2005) showing shared common species among them. One-way ANOVA comparing evenness, species richness and diversity of the sites shows that diversity is statistically significant as in Table 5.3. Cluster analysis of the diversities showed that sites dominated by *Nitella congesta* were clustered together while sites without charophytes were clustered together as in Fig. 5.4.

Overall, macroinvertebrates were abundant in the *N. congesta* dominated site. This supports the hypothesis that charophytes are associated with increased diversity of invertebrates. A test of the hypothesis using the student t-test showed that there was a significant difference between the macroinvertebrate diversity of the two different sites (i.e. charophyte dominated sites and control sites) ($P = 0.000$, $\alpha = 0.05$). The strong increase in salinity of the charophyte dominated sites N1 and N2 can also be a cause of the difference in macroinvertebrate abundance between the two dates (c. four weeks) (Table 5.2). Moreover, higher salinity might inhibit the growth of *N. congesta*. Even though Higgins Lake recorded only a few species compared to the Nitella lake, there was virtually no dominance by a particular species. The Evenness measure showed how wide-spread the species in Higgins lake were. Also being newly formed, Higgins Lake had the advantage of not being derived from a mine-void. Water quality in this lake is therefore less affected by mining effluent. However, few species were found in this lake, especially in the water column. Most of the macroinvertebrates were found in the sediment.

The similarity between the number of species found in the control sites from all the visits indicates that areas uninhabited by charophytes do not support higher abundance or diversity. Macroinvertebrate species that are open area swimmers will always be found moving between *N. congesta* dominated and uninhabited sites. It can be concluded that this part of the study indicates

N. congesta is associated with higher abundance and diversity of macroinvertebrates in the Capel Wetlands.

Summary

The relationship between the presence and establishment of *Nitella congesta* and the abundance and diversity of macroinvertebrates was studied in two lakes at the Capel Wetlands Centre. Sampling for the analysis of the community of macroinvertebrates was at five weeks interval in 2004 and 2005. Composition of macroinvertebrate fauna was determined at sites differing in *N. congesta* cover. The presence of *N. congesta* was found to be the determinant of the distribution of macroinvertebrate abundance and diversity. Macroinvertebrate abundance and diversity was found to be high at sites where *N. congesta* was established. One-way ANOVA comparing evenness, species richness and diversity of the sampled sites showed that diversity were statistically significant at 5% significance level (P=0.02).

6 EFFECT OF EUTROPHICATION ON THE ESTABLISHMENT OF *N. congesta* AT THE CAPEL WETLANDS CENTRE

6.0 Introduction

Eutrophication is a major problem for many lakes in Western Australia. Oligotrophic lakes are characterized by low nutrient concentrations in the water column, a diverse plant and animal community, a low level of primary biomass and good over all water quality for most uses. In contrast, eutrophic water bodies have high level of productivity and biomass at most trophic levels, frequent occurrences of algal blooms, anoxic bottom waters (hypolimnion) during periods of thermal stratification, often fewer types of plant and animal species, enhanced growth of littoral zone aquatic plants and poor water quality (Ryding and Rast 1989). Increased nutrient loading is attributed to be one of the most important reasons for the switch from a macrophyte-dominated to a non-vegetated turbid state in many shallow lakes (Scheffer and Jeppesen 1998). Therefore one of the most effective long-term strategies for controlling eutrophication of lakes is to reduce the quantity of the nutrients entering the water bodies (Ryding and Rast 1989).

In a natural undisturbed environment, the nutrient supply of a lake will be derived from the drainage of the catchments together with direct rainfall on the lake surface and any internal recycling which may occur from the sediments. Nitrogen and phosphorus are found in soils predominantly as organic compounds in either detritus (humus) or living tissues. Nitrogen concentration in excess causes eutrophication as well. However, eutrophic lakes and reservoirs can be valuable for fisheries as increase in nutrient levels in the water bodies also increases overall fish production (Harper 1992). Increased productivity inherent in eutrophication may be useful in counteracting the negative impacts of acidification of a lake (Ryding and Rast 1989). This is supported by the outcome of a study conducted by Kerekes *et al.* (1984) in which they examined both chemical and biological characteristics of acidified oligotrophic and eutrophic lakes. They found that the eutrophic lake exhibited increased biological activity at different trophic levels.

The disappearance of charophytes from eutrophic waterbodies have been highlighted in literature (Blindow 1992a; Kufel and Kufel 1997; van den Berg

et al. 1998). The lakes at the Capel Wetlands Centre were found to be of low pH, with very high ammonium levels, low phosphorus levels and high concentrations of iron, manganese and sulphates in the early period of rehabilitation (Brooks 1992; Doyle 2000). The low phosphorus levels within the lakes was the result of iron and magnesium compounds within the sediment removing soluble phosphorus from the water body (Chambers and McComb 1996). However the effect of increasing the nutrient concentration in the lakes on the functionality of the wetland ecosystem has not been documented.

6.1 Factors and processes that affect eutrophication

Eutrophication problems are often caused by an excessive input of phosphorus. Phosphorus retention in wetlands is influenced by sorption, precipitation, complexation, sedimentation and resuspension. The importance of the different mechanisms is largely determined by chemical and physical factors such as pH and temperature respectively (Gumbricht 1993). Nevertheless, in lakes which are heavily polluted, other nutrients or factors contribute immensely to limiting algal growth (Ryding and Rast 1989).The basic causes of cultural eutrophication of a water body seem always external to the water body. However, the characteristics of the water body can affect these factors both negatively and positively. The overall productivity of a water body is not exclusively controlled by the external nutrient loading or in-lake nutrient concentrations (Harper 1992). Productivity is indirectly affected by other factors which affect the distribution, availability and utilization of the nutrient inputs (Harper 1992). Nutrient availability and algal productivity of a water body depends to a large extent on the physical and biotic structure of the water body, implying there can be differences in the responses of similar lakes with the same annual nutrient loading. The differences can be attributed to internal nutrient cycling (i.e. food-web structure and sediment regeneration) as well as specific lake basin properties which include morphology and in-lake hydrodynamics (Harper 1992).

6.2 Limiting nutrients

As a control measure for cultural eutrophication of lakes, one of the mechanisms is to reduce the quantity of the nutrient entering the water body (Ryding and Rast 1989). Assuming that algal growth of a water body is not controlled by a non-nutrient factor (e.g. inadequate light, suboptimal temperature etc.), reducing or "limiting" the input of the nutrients inflow should subsequently reduce the algal biomass in the water body. Laboratory and field experiments have demonstrated the major role of phosphorus and nitrogen and in some cases silicon, in influencing the dynamics of algal populations, as well as algal concentrations and their species composition. Limiting the input of these nutrients will therefore serve as an option for the control of eutrophication (Ryding and Rast 1989). In comparison to the rich natural supply of major nutrients, phosphorus is least abundant. However, it is the most common limiting factor in biological productivity and the most significant form of inorganic phosphorus is orthophophorus (PO^{4---})(Wetzel 1983).

Golterman (1975) suggested that the whether or not phosphorus is the limiting nutrient in a given situation is insignificant since phosphorus is the only essential element that can easily be made to limit algal growth. Carbon also normally becomes an algal growth-limiting nutrient only in a situation where the water is saturated with both phosphorus and nitrogen, light availability and temperature are high and the transport of carbon dioxide from the atmosphere to the water column is slow (Ryding and Rast 1989).

6.3 Eutrophication and its causes

Eutrophication affects both physical and chemical environments and can lead to significant changes in the zooplankton and phytoplankton communities' structure (Flores and Barone 1994; Uku and Mavuti 1994). Due to its low solubility and very low concentrations in natural waters, phosphorus limits maximum attainable algal biomass in many inland waters. Thus eutrophication problems are often caused by excessive input of phosphorus (Ryding and Rast 1989).

Attributes likely to be important indicators of the negative impacts of nutrient enrichments of lakes include the development of the toxic algal bloom, increased growth of epiphytic algae, the loss of submerged vegetation and to some extent, the growth of macroalgae (Harper 1992). Macro-nutrients are a major variable controlling algal biomass in lakes. However, it is important to recognize that other variables such as trace elements and organic factors, under some conditions can contribute to the algal biomass in water bodies.

An increase in the nutrient level often stimulates phytoplankton growth, which in turn increases turbidity leading to a reduction of the maximum growth depth of submerged vegetation. Therefore, increased nutrient concentration will reduce the bottom area covered by submerged vegetation until it is absent (Faafeng and Mjelde 1998). However, uncovered sediments in shallow lakes are much more vulnerable to resuspension and more easily give rise to turbid water during periods of wind and wave action than in lakes with plant covered sediments (Faafeng and Mjelde 1998). This was observed in the following laboratory experiments.

There has been an approved proposal on discharging treated wastewater from the local Water Corporation waste water processing plant (with an estimated phosphorus concentration of about 4,000µg/L) into the lakes of Capel Wetlands. This was considered desirable by some stakeholders as additional nutrients would increase the primary productivity of the lakes. Therefore, with *N. congesta* as one of the major submerged macrophytes in the lakes of Capel Wetland Centre, an attempt to render the lakes eutrophic is likely to unleash an adverse effect on its establishment with a resultant effect on the water quality and functionality of the lakes and subsequently biodiversity conservation at the wetlands.

The objective of this part of the study was to evaluate the potential effects of eutrophication of the lakes of the Capel Wetlands Centre on the establishment of *N. congesta* as a functional wetland macrophyte.

6.4 Materials and methods

6.4.1 Effect of eutrophication on the growth of *N. congesta* in the laboratory

Two kilograms of sediment containing oospores of *N. congesta* collected from Nitella Lake was distributed into 12 aquarium tanks of volume approximately 16 L (24cm × 22cm × 30cm) each. The aquarium tanks were filled with 10L of dionised water and divided into 3 groups of 4 treatments each and labeled, C1-C4 (Control), M1-M4 (Mesotrophic) and E1-E4 (Eutrophic). For freshwater, the Organization for Economic Cooperation and Development (OECD) (OECD 1982) cited in (Ryding and Rast 1989) has proposed a simple system of thresholds for the assessment of the eutrophic status of water bodies (Table 6.1). To the mesotrophic and eutrophic tanks, nutrient was added as anhydrous disodium hydrogen phosphate (VI) (Na_2HPO_4) to obtain the total phosphorus concentration of 30 µg PL^{-1} and 100 µg PL^{-1} respectively (OECD 1982; Wetzel 1983). The aquarium tanks were aerated with aquarium pumps and air stones in the Greenhouse at the Department of Environmental and Aquatic Sciences, Curtin University. The aquaria setup was left in natural day length conditions from 17th June 2005 – 30th November 2005 (136 days). Phosphate was added fortnightly. Water level was kept constant throughout the experiment.

The growth of *N. congesta* under the different nutrient concentrations was studied. To measure the growth rate, 20 individual were selected at random upon germination and tagged (see section 3.5.5) and their growth measured weekly. The growth rate for each nutrient status was calculated from the shoot length data using formula 3.1. as in section 3.5.5. The number of branches per shoot and number of nodes were counted along the shoots of the selected individuals. The average internode distance was determined without the internode distances in the apical portion of the shoots. This was because the nodes were somewhat tightly together making measurement of the distance between them difficult. During the production of fruiting bodies, male and female individuals were counted to determine the sex ratio.

At the end of the experiment, all filamentous algae in the mesotrophic and eutrophic setups were collected using a small sweep net into labeled beakers and the fresh weight determined on a top loading Satorious analytical balance. The surrounding water was poured out of the aquarium tanks and the individual *N. congesta* shoots were uprooted into labeled beakers and the fresh weight determined on a top loading Satorious analytical balance.

The filamentous algae and *N. congesta* samples in the beakers were carefully transferred into labeled brown paper bags and oven dried at 40^0C for 48 hours. These were allowed to cool and the weight determined. Drying, cooling and re-weighing were repeated until constant weights were obtained as the dry weights.

Table 6.1OECD boundary values for classification of trophic systems.

Trophic Category	TP	mean Chl	max. Chl	mean Secchi	min Secchi
Ultra-oligotrophic	<4.0	<1.0	<2.5	>12.0	>6.0
Oligotrophic	<10.0	<2.5	<8.0	>6.0	>3.0
Mesotrophic	10-35	2.5-8	8-25	6-3	3-1.5
Eutrophic	35-100	8-25	25-75	3-1.5	1.5-0.7
Hypertrophic	>100	>25	>75	<1.5	<0.7

Adopted from Ryding and Rast (1989)

Explanation of terms:

TP = mean annual in-lake total phosphorus concentration (µg/L)
mean Chl = mean annual chlorophyll a concentration in surface waters (µg/L)
maximum Chl = peak annual chlorophyll a concentration in surface waters (µg/L)
mean Secchi = mean annual Secchi depth transparency (m)
minimum Secchi = minimum annual Secchi depth transparency (m)

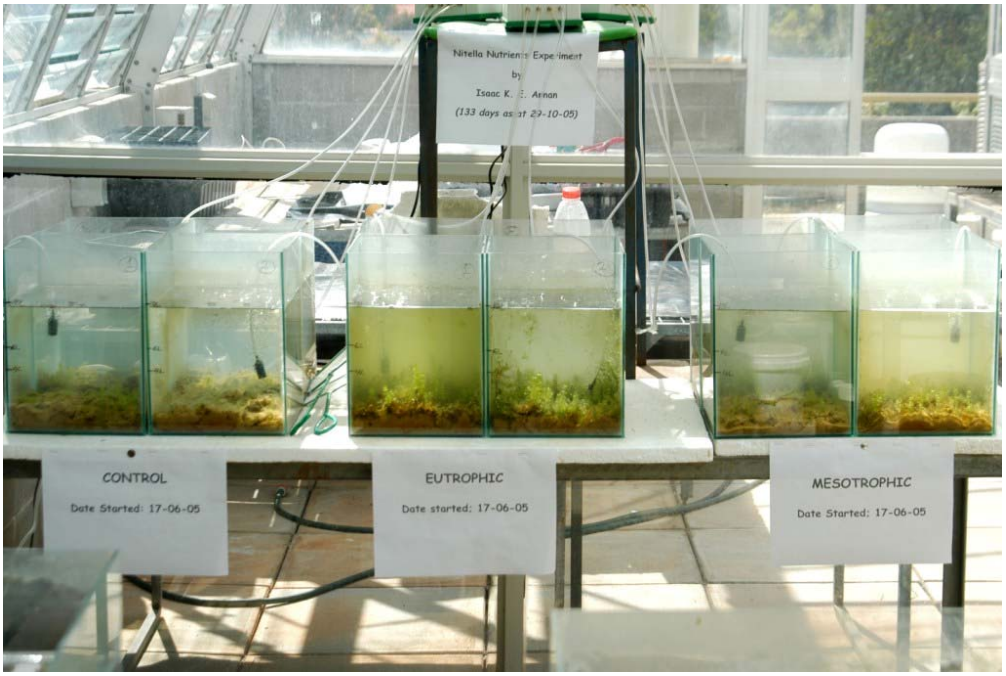

Figure 6.1 Nutrient enrichment experimental set-up.

Four replicates in each treatment of control (oligotrophic), mesotrophic and eutrophic.

Figure 6.2 Nutrient enrichment - the control (oligotrophic) set-up. The control treatment consisted of *N. congesta* culture without phosphorus enrichment (four replicates) for 136 days.

Figure 6.3 Nutrient enrichment - the mesotrophic set-up. The mesotrophic treatment consisted of *N. congesta* culture enriched to a total phosphorus concentration of 30 μgL^{-1} (four replicates) for 136 days.

Figure 6.4 Nutrient enrichment - the eutrophic set-up. The eutrophic treatment consisted of *N. congesta* culture enriched to a total phosphors concentration of 100 μgL^{-1} (four replicates) for 136 days.

6.4.2 Effect of eutrophication on the growth of *N. congesta* in the field

This study was carried out in Nitella Lake. Enclosure cylinders were constructed from Plexiglass sheets with dimensions as shown in Fig.6.5.

15 cm of the total height of the cylinder was demarcated to set as the base and adhered to a 20 L plastic bucket. All possible leakages were tightly sealed. Twelve (12) of such cylinders were constructed and transported to the field. The plastic buckets were positioned in the Nitella Lake and filled with sediment from the lake to the demarcated level. The cylinders were then filled with lake water to make up a volume of approximately 50L each. The cylinders were divided into 3 groups of control, mesotrophic and eutrophic with four replicates each. The mesotrophic and eutrophic treatments were enriched with nutrient as anhydrous disodium hydrogen phosphate (VI) (Na_2HPO_4) to obtain the total phosphorus concentration of 30 µg PL^{-1} and 100 µg PL^{-1} respectively as was done in the laboratory setting. Phosphate was added and set up monitored monthly. A wooden jetty was constructed alongside the cylinders to ensure easy and safe access as shown in the picture, Fig. 6.6.

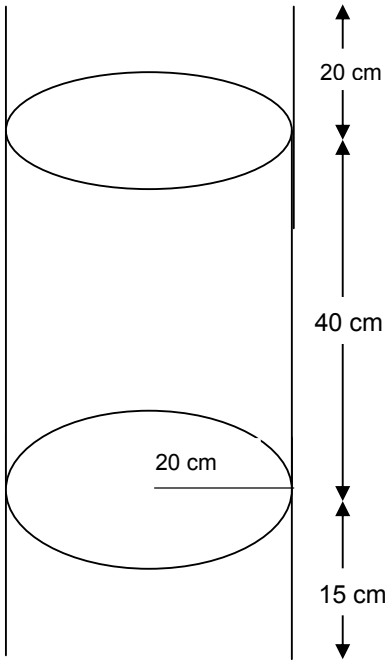

Figure 6.5 Diagram of cylinder used enrichment experiment in the field showing dimensions.

Figure 6.6 Nutrient enrichment experimental set-up in the field in 2007.

6.5 Results

6.5.1 Effect of eutrophication on the growth of *N. congesta* in the laboratory

The germination of *N. congesta* was quite slow comparably in all nutrient statuses. However, upon enrichment by phosphorus, there was sporadic germination in the eutrophic conditions followed by a high growth rate. The growth measurement of *N. congesta* is represented in the Fig. 6.3 – 6.7. The growth rate was in the order, eutrophic (0.81 - 1.00 mm/day) > mesotrophic (0.42 – 0.47 mm/day) > control (0.19 – 0.24 mm/day).

The morphology of the *N. congesta* shoots in the three set ups were observed to be completely different. In the eutrophic treatments, there were profused branching, taller individuals and subsequently longer internode distances (Fig. 6.13; Table 6.2 & 6.3).

Table 6.2 Mean height and internode distance of *N. congesta* at the end of the laboratory experiment.

	Mean height per shoot (mm)	Mean internode distance per shoot (mm)
Control	35.53±3.52	2.84±0.20
Mesotrophic	73.50±3.52	5.90±0.55
Eutrophic	189.75±34.77	21.21±1.16

The highest mean height of the shoots was observed in the eutrophic tanks while the lowest was observed in the control (oligotrophic) tanks.

Table 6.3 Mean number of nodes and branches of *N. congesta* at the end of the laboratory experiment.

	Mean number of nodes per shoot	Mean number of branches per shoot
Control	11.39±0.83	1.04±0.68
Mesotrophic	16.13±0.38	1.10±1.08
Eutrophic	25.61±0.84	3.85±1.23

The highest mean number of nodes and mean number of branches were observed in the eutrophic tanks while the lowest were observed in the control (oligotrophic) tanks. “Branches” here refer to new shoots from a whorl of branchlets, with nodes and internodes.

Table 6.4 One-way ANOVA comparing mean height, number of nodes and internode distance of *N. congesta* individuals in enrichment experiment at 5% significance level, n = 20.

	F	*P*
Mean height	54.395	**0.000**
Mean number of nodes	2.414	0.132
Mean internode distance	81.281	**0.000**

The mean height and mean internode distance were significantly different at 5% significance level (see Appendix 8).

In addition to *N. congesta*, *Chara fibrosa* another species of charophyte was also found to have established in the eutrophic tanks whereas none was found in the mesotrophic and oligotrophic tanks. It was also observed that during the reproductive stage of the life cycle of *N. congesta*, male and female plants were found in the mesotrophic and oligotrophic (control) treatments in the ratio of approximately 1:1. However, there were no fruiting bodies observed in the eutrophic treatments (Fig. 6.12). Species of blue-green algae (*Nostoc* sp.) were found in both the eutrophic and mesotrophic tanks. On the contrary, no blue-green algae were observed to grow in the oligotrophic tanks.

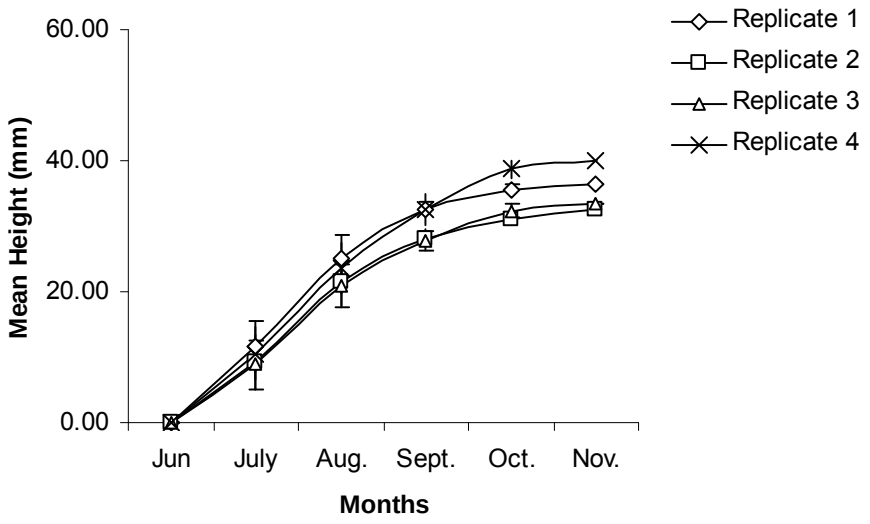

Figure 6.7 Mean height of shoots of *N. congesta* cultured in the control treatment of enrichment experiment in the laboratory. The control treatment consisted of *N. congesta* culture without phosphorus enrichment (four replicates) for 136 days.

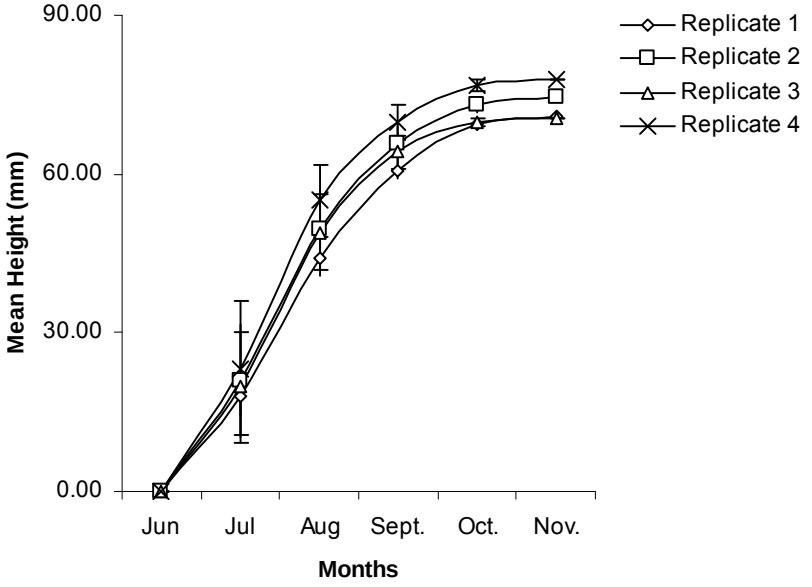

Figure 6.8 Mean height of shoots of *N. congesta* cultured in the mesotrophic treatment experiment in the laboratory. The mesotrophic treatment consisted of *N. congesta* culture enriched to a total phosphorus concentration of 30 μgL^{-1} (four replicates) for 136 days.

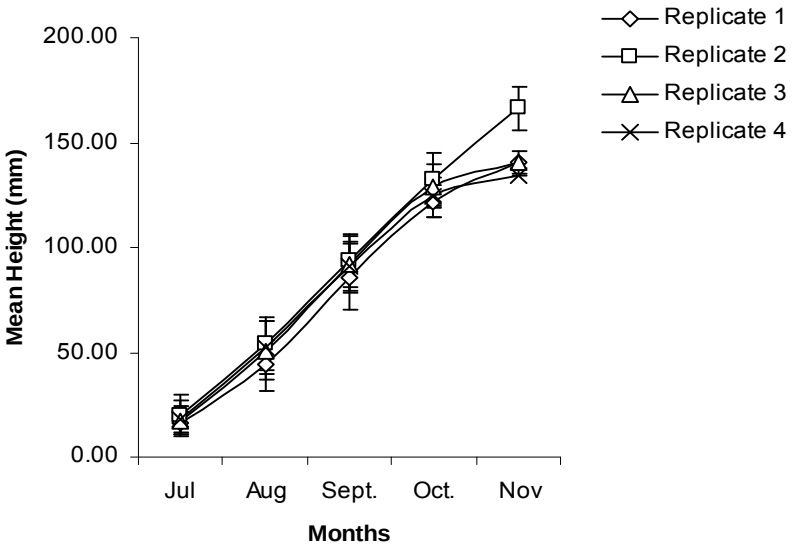

Figure 6.9 Mean height of shoots of *N. congesta* cultured in the eutrophic treatment. The eutrophic treatment consisted of *N. congesta* culture enriched to a total phosphorus concentration of 100 μgL^{-1} (four replicates) for 136 days.

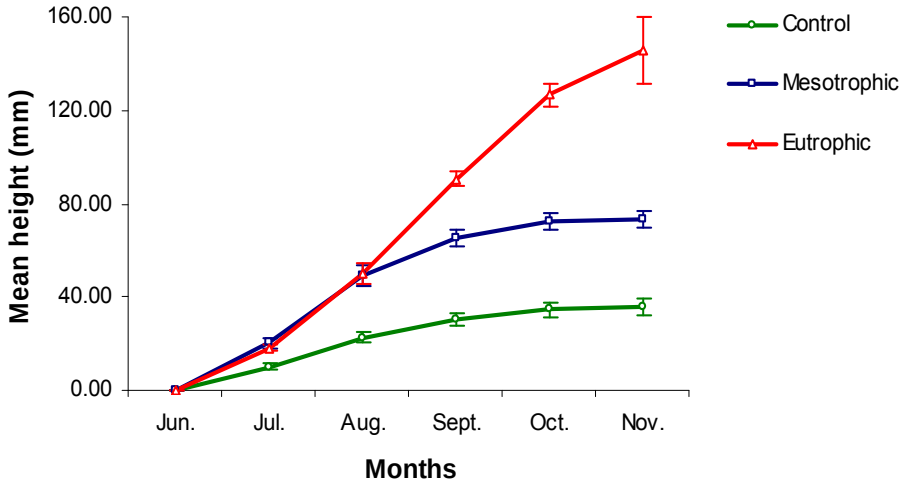

Figure 6.10 Mean height of individuals of *N. congesta* cultured in the enrichment experiment. The control treatment had no phosphorus addition, the mesotrophic treatment was enriched to a total phosphorus concentration of 30 μgL^{-1} and the eutrophic treatment was enriched to a total phosphorus concentration of 100 μgL^{-1}. There were four replicates of each treatment..

N. congesta individuals in the eutrophic set-ups showed the highest height.

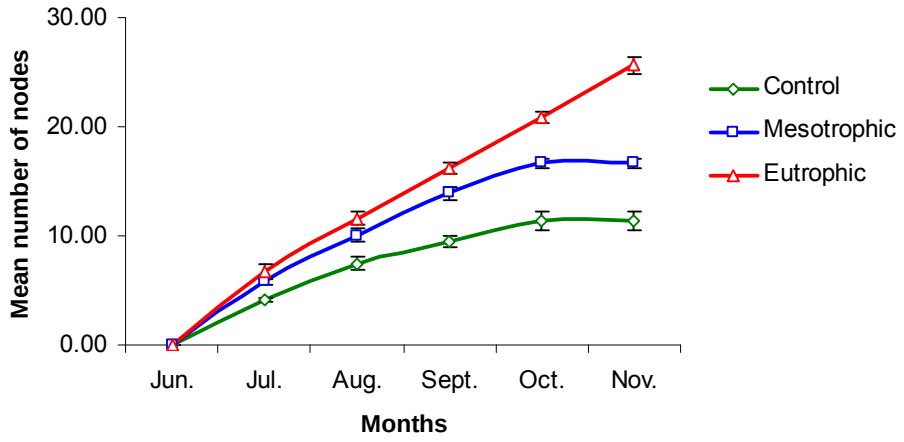

Figure 6.11 Mean number of nodes per individual of *N. congesta* cultured in the enrichment experiment. The control treatment had no phosphorus addition, the mesotrophic treatment was enriched to a total phosphorus concentration of 30 μgL^{-1} and the eutrophic treatment was enriched to a total phosphorus concentration of 100 μgL^{-1} for 136 days. There were four replicates of each treatment.

N. congesta individuals in the eutrophic treatments showed the highest mean number of nodes.

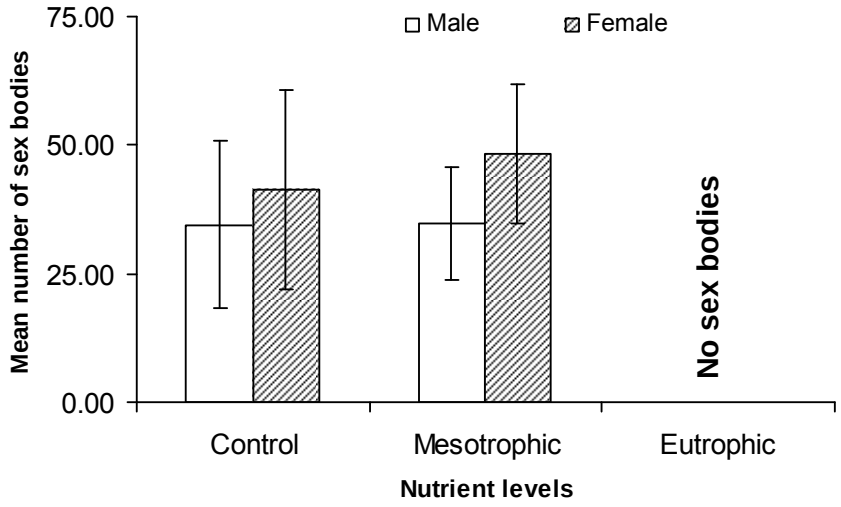

Figure 6.12 Sex ratio of male and female shoots of *N. congesta* based on 100 shoots per replicate counted in the enrichment experiment. The control treatment had no phosphorus addition, the mesotrophic treatment was enriched to a total phosphorus concentration of 30 μgL^{-1} and the eutrophic treatments was enriched to a total phosphorus concentration of 100 μgL^{-1} for 136 days. There were four replicates of each treatment.

No sex bodies were produced by *N. congesta* individuals in the eutrophic set-ups. Sex bodies were produced by *N. congesta* individuals in the mesotrophic and control treatments in the ratio of approximately 1:1 (see Appendix 9).

Figure 6.13 *N. congesta* shoots from the eutrophic, mesotrophic and oligotrophic (control) treatments. The control treatment had no phosphorus addition, the mesotrophic treatment was enriched to a total phosphorus concentration of 30 μgL^{-1} and the eutrophic treatment was enriched to a total phosphorus concentration of 100 μgL^{-1} for 136days. There were four replicates of each treatment.

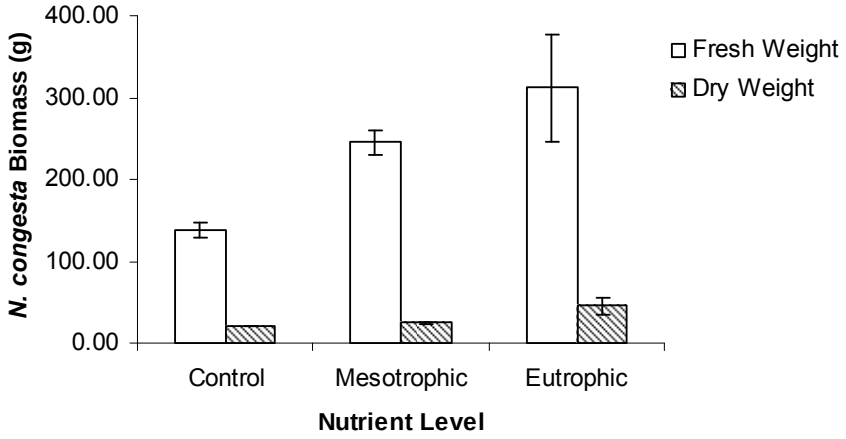

Figure 6.14 *N. congesta* biomass in the three nutrient levels; control, mesotrophic and eutrophic treatments. The control treatment had no phosphorus addition, the mesotrophic treatment was enriched to a total phosphorus concentration of 30 μgL^{-1} and the eutrophic treatment was enriched to a total phosphorus concentration of 100 μgL^{-1} for 136 days. There were four replicates of each treatment.

Highest biomass of *N. congesta* was recorded in the eutrophic treatments.

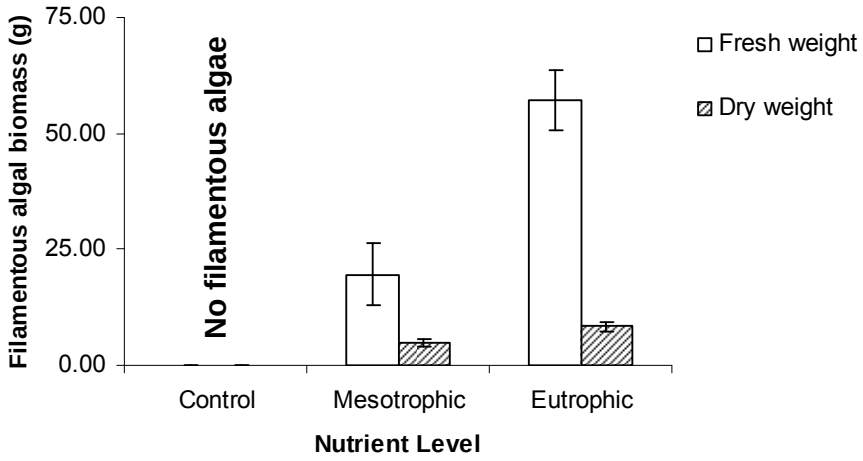

Figure 6.15 Filamentous blue-green algal biomass in the three nutrient levels; control, mesotrophic and eutrophic treatments. The control treatment had no phosphorus addition, the mesotrophic treatment was enriched to a total phosphorus concentration of 30 μgL^{-1} and the eutrophic treatment was enriched to a total phosphorus concentration of 100 μgL^{-1} for 136 days. There were four replicates of each treatment.

Higher biomass of filamentous blue-green algae was recorded in the eutrophic treatments. There was no filamentous blue-green algae recorded in the control or oligotrophic treatments.

6.5.2 Effect of eutrophication on the growth of *N. congesta* in the field

The experiment in the field yielded no response after two successive attempts in 2006 and 2007 since there was no successful germination of *N. congesta* even though in there were lots of blue-green algae observed in the eutrophic enclosures. The acidity of the lake water in the enclosures was observed to be increasing corresponding to the acidity of the rest of the lake. The experiment was therefore discontinued. This prompted a thorough investigation into the causes of the acidity and its subsequent effects on the establishment of *N. congesta* in the lakes of the wetlands which is discussed in a later chapter.

6.6 Discussion

Nitella congesta showed considerable variations in its morphology and life cycle in the different nutrient statuses. The water levels were maintained constant throughout the entire experiment making sure that *N. congesta* shoots were completely submerged. The different nutrient statuses influenced the life cycle of *N. congesta*. Plants in the mesotrophic and control setups therefore acted as annuals by producing oospores in order to ensure species succession. However, *N. congesta* plants in the eutrophic nutrient status acted as perennials locating more plant resources to vegetative growth (Casanova 1994) resulting in longer total length and internode distances, more number of nodes and branches. Therefore, the loss of charophytes in high eutrophic conditions can be attributed in part to the lack of production of sexual reproductive bodies. In this case, the charophytes will thrive alright but the chain of succession is broken due to the non-production of oospores.

Eutrophication also increases turbidity of the water body whiles it reduces water clarity. In the experiment, the clarity of water was very low with high turbidity in the mesotrophic and eutrophic treatments. In the eutrophic

treatments, it got to a point where *N. congesta* apparently became invisible due to high turbidity caused by filamentous algal bloom. This may have resulted in the reduction of light irradiance (van den Berg *et al.* 1998; Schwarz *et al.* 1999).

Eutrophication has been combated by adopting a number of lake restoration techniques (Meijer *et al.* 1999; Gulati and Van Donk 2002; Lathrop *et al.* 2002). However, whether these methods have yielded successful results or not depended on many factors. Among which have shown permanent effects, is the reduction of the external nutrient loading to acceptable levels (Benndorf *et al.* 2002; Mehner *et al.* 2002). However even after nutrient loading has been taken care of internal feedback loops such as nutrient release from the sediment and very long water residence times still encourage and support phytoplankton production (Hilt *et al.* 2006). Cooke *et al.* (2005) proposed additional internal measures that can contribute to the shortening of the relaxation time after external phosphorus load reduction. The initial selection of an appropriate internal restoration measure requires much knowledge of the lake as well as the type of vegetation to be restored (Hilt *et al.* 2006).

In general, all internal measures that contributes to the improving the light conditions of the lake end up with resultant positive effects on the development of macrophytes (Hilt *et al.* 2006). There are other measures however, which may end up with negative results. Among these are sediment removal and coverage, inactivation of phosphorus and oxidative measures (Hilt *et al.* 2006). To effectively decrease total phosphorus in shallow lakes, the use of phosphorus inactivation in sediments by alum, iron or lime has been suggested (Welch and Cooke 1999; Deppe and Benndorf 2002; Cooke *et al.* 2005).

Even though analysis of sediment from the lakes show high concentrations of aluminium and iron (Fig. 4.2), experimental studies on the effect of alum or iron additions towards the development of macrophytes are lacking. It is therefore obvious that long-term stability of the clear water state cannot only be expected above certain critical nutrient levels, which depend on lake size

and depth. However, since the field experiment was not successful due to acidification, much inference could not be draw from the results in terms of eutrophication in the field situation.

In conclusion, in order for the lakes at Capel Wetland Centre to establish good ecological state the rehabilitation should be considered as a very important issue. Therefore any attempt to release treated effluent into the lakes should ensure a low external nutrient loading preferably below the OECD nutrient threshold values for freshwater bodies.

The acidity of the lakes at Capel Wetlands Centre was found to be on the rise with its associated lack of oospore germination and subsequent establishment of *N. congesta* meadows. This therefore called for a look into the causes and effects of the acidification of the lakes which has been dealt with in the next chapter.

Summary

The disappearance of submerged macrophytes in shallow lakes is one of the most critical problems caused by eutrophication. In the light of an approved proposal to discharge waste water into the lakes of the Capel Wetlands Centre, the impact of increased phosphorus concentration on the establishment of *N. congesta* as a suitable macrophyte was studied in an experiment. *N. congesta* was cultured in aquarium tanks in eutrophic and mesotrophic media alongside control and its growth was monitored. Phosphorus was added in the form of anhydrous disodium hydrogen phosphate (VI) (Na_2HPO_4).

It was observed that eutrophication had an impact on growth, morphology and life cycle. In addition to showing profused vegetative growth, *N. congesta* produced no fruiting bodies in the eutrophic medium whereas an approximate ratio of 1:1 male to female sex bodies was recorded in the mesotrophic and control experimental setup but with reduced vegetative growth. Higher biomass of *N. congesta* and other filamentous algal was recorded in the

eutrophic treatments. Filamentous algal biomass was found to correspond with the nutrient concentration and the biomass of *N. congesta*.

One-way ANOVA showed that height, number of nodes and internode distance were found to be different at 5% significance level, P = 0.000.

7 CLIMATE CHANGE AND ACIDIFICATION OF THE CAPEL WETLANDS: IMPACT ON *Nitella congesta*

7.0 Introduction

The Earth's climate involves a continuous change on a range of timescales as a result of "internal" and "external" factors. The internal factors are natural, emanating from complex interactions within the climate system. The external factors can be natural as well as anthropogenic. Natural external factors include the Earth's rotations, variations in radiant energy from the Sun, volcanic eruptions. Anthropogenic external factors include human activities such as release of atmospheric particles, modification of ecosystems and changes in atmospheric composition (CSIRO and Bureau of Meteorology 2007).

The world in general and Australia in particular is experiencing rapid climate change. Temperatures in Australia on average have risen by about 1^{o}C with its subsequent rise in the occurrence of heat waves relative to cold days (CSIRO and Bureau of Meteorology 2007). Climate change has also affected Australian rainfall patterns with the northwest experiencing an average increase in rainfall in the last 50 years. Conversely, the frequency of rainfall has declined in the Eastern and the far southwest part of Australia (CSIRO and Bureau of Meteorology 2007). Over the past century, Australian surface temperatures are reported to have warmed significantly with anthropogenic causes responsible for the warming since the middle of the 20th century. Anthropogenic warming has also been linked to the droughts that have accompanied higher temperatures, decrease in snow cover and the respective increase and decrease in warmer days/nights and cooler days/nights respectively (CSIRO 2007).

It has been predicted that climate change and pollution are world-wide problems that will affect all lakes, irrespective of the size (Beeton 2002). Freshwater ecosystems are among the many important reference sites for the increasing research in global environmental change. The lake environment is sensitive to climate change with the ability to respond directly by thermal stratification, ice-cover and catchments hydrology or indirectly through changes in nutrient availability, alkalinity generation and more

importantly pH (Battarbee 2000; Smol and Cumming 2000). Acidity caused by exposure of sulphidic sediment is another cause.

7.1 Acidification of freshwater bodies

Acidification of freshwater bodies had been an unidentified problem for many years (Psenner 1994). It has become an important issue and has recently attracted the attention of environmental scientists, industrialists and governments. Acidification of surface water as a result of the deposition of strong acid has had adverse impacts on the biological communities (Battarbee and Charles 1986; Muniz 1991). Groundwater acidification is responsible for the leaching of a number of metal ions into lakes (Farmer 1990). Areas of good rainfall and waterlogged soil profiles especially where the groundwater system is nearing a weathering bedrock are mostly sites of groundwater acidification (Hunt and Patterson 2004). Seepage of groundwater into surface waters generally occur along lakeshores with unconsolidated deposits (Sebestyen and Schneider 2004). Thus inflow rates of very high magnitude may eventually alter sediment pore water chemistry leading to the mobilization of iron and sulphur from sediments thus enhancing acidification (Blodau 2004). Freshwater acidification as well as eutrophication has the negative effects of changing species composition, extinction of sensitive species, toxicity for flora and fauna and the release of cyanobacterial toxins in eutrophic lakes Psenner (1994). The effects of freshwater acidification are evident in all trophic levels as well as biota like algae (Flower and Battarbee 1983; Bennion *et al.* 2004), macrophytes (Farmer 1990), macroinvertebrates (Økland and Økland 1986; Havens 1993), fish (Duis 2001) and birds (Ormerod and Tyler 1991; Jenkins and Ormerod 1996). Farmer (1990) also indicated that macrophyte communities are negatively affected by biotic changes as well as their responses to changes due to acidification.

Acidification of freshwater bodies can be triggered by a number of processes such as the release of organic acids from incomplete mineralization, deposition and accumulation of organic matter, natural mineral weathering in

weakly buffered catchments and acidic atmospheric deposition (Schnoor and Stumm 1985). Acidification of lakes in post-mining landscapes is basically caused by Acid Mine Drainage (AMD) apparently after the oxidative weathering of metal sulfides (Bonnissel-Gissinger *et al.* 1998; Nordstrom 2000). The pollution of surface waters by ADM has become a world wide issue (Younger 1992); not limited to a particular geographical location but basically, every where coal or sulfide ores are mined (Blodau 2006). A review by Nixdorf *et al.* (2001) deals much about plant life in extremely acidic waters. They proposed the following chemical and physical factors that influence the survival and growth of organisms in these ecosystems:

- High concentration of Fe (III),
- Increased solubility of metal ions at low pH which is an important factor in mine waters; ADM sources often have high heavy metal content,
- Phosphorous solubility under low pH,
- However, concentration of phosphorus in acidic waters is low due to co-precipitation with Fe (III) oxyhydroixdes,
- At higher acidity (pH 3 and below), all dissolved inorganic carbon is in the form of dissolved CO_2 with a very low saturation concentration.

Wetlands fed by groundwater can encounter adverse consequences as a result of increased sulphate concentrations. If there is seepage rich in iron, then iron-sulphides including pyrite (FeS_2) will accumulate. Heavy metals will also accumulate as metal sulphides or will probably be absorbed on iron hydroxides which will eventually have serious impacts during dry periods (Lucassen *et al.* 2002). Pyrite and metal-sulphide oxidation leading to acidification and heavy metal mobilization is well known from drained acid mines as well as ecosystems on coastal plains (De Jong *et al.* 1994; Portnoy 1999). Oligotrophic pools contaminated with atmospheric sulphur have also been documented to experience acidification especially when they are poorly buffered to counteract the acid production emanating from pyrite oxidation during dry periods (Van Haesebroeck *et al.* 1997).

Ritsema and Groenenberg (1993) indicated that the presence of (bi)carbonate buffers in sediments can serve to slow down acidification resulting from desiccation. Stepwise production of hydrogen ions that cause acidification as well as the expected buffering activity that should eventually neutralize the acidity is shown below (Lucassen *et al.* 2002);

$$2FeS_{2(s)} + 6NO_{3(aq)} + 2H_2O \rightarrow 2FeOOH_{(s)} + 3N_2 + 4SO^{2}_{4(aq)} + 2H^{+}_{(aq)} \quad (1)$$

$$4FeS_{2(s)} + 15O_{2(g)} + 14H_2O \rightarrow 4Fe(OH)_{3(s)} + 16H^{+}_{(aq)} + 8SO^{2}_{4(aq)} \quad (2)$$

$$Ca^{2+} + 2H^{+} \rightarrow 2H^{+} + Ca^{2+} \quad (3)$$

$$HCO^{-}_{3}(aq) + H^{+}_{(aq)} \rightarrow H_2O + CO_{2(g)} \quad (4)$$

$$CaCO_{3(s)} + 2H^{+}_{(aq)} \rightarrow Ca^{2+}(aq) + H_2O + CO_{2(g)} \quad (5)$$

$$CaMg(CO_3)_{2(s)} + 4H^{+}_{(aq)} \rightarrow Ca^{2+}_{(aq)} + Mg^{2+}_{(aq)} + 2H_2O + 2CO_{2(g)} \quad (6)$$

In Equation (3), the hydrogen ions produced react with the cation exchange buffer of the sediment, the bicarbonate (equation (4)) and carbonate (equation (5)). Thus acidification occurs when the buffering capacity of the sediment is insufficient to compensate for the acids produced by pyrite oxidation (Drever 1997). Ferrolysis is the oxidation and hydrolysis of dissolved Fe^{2+} (Mann 1983) (Equation (7)) and this has been considered as the cause of acidification of groundwater at playa margin discharge zones in the eastern wheatbelt of Western Australia.

$$4Fe^{2+} + 6H_2O + O_2 \rightarrow 4FeOOH + 8H^{+} \text{ (McArthur } \textit{et al.} \text{ 1991)} \quad (7)$$

This results in the precipitation of red/brown insoluble iron oxides at the surface of the soil (Hunt and Patterson 2004). The groundwater in the western wheatbelt areas in Australia is enriched in ferrous iron in close proximity to mafic dykes thus triggering acidity by ferrolysis when discharged at the ground surface (Hunt and Patterson 2004).

7.2 Acid Sulphate Soils

Acid sulphide soils (ASS) is the common name for naturally occurring soil and sediment that contain iron sulphides. In Australia, the ASS that have attracted much attention are those formed in the last 10,000 years (i.e. during the Holocene geological period). ASS occur throughout Australia though their

distribution Australia-wide is unknown (Western Australian Planning Commission, 2003). According to the Western Australia Planning Commission, acid sulfate soils in Western Australia are likely to be found in areas including but not limited to;

- The south west of the State between Perth and Busselton.
- The northern parts of the State's coastline including the Pilbara and Kimberley coastlines.
- The Scott River Plain on the south coast and;
- Parts of the Wheatbelt where land salinisation has taken place.

7.3 Lake acidification and the establishment of *N. congesta*

Nitella sp. is classified as an acid sensitive macrophyte, deriving inorganic carbon from the water column; often absent from acid sites with pH below 5.5 (Maessen *et al.* 1992; Arts 2002). *N. congesta* was observed to have thrived considerably well in the lake water with pH ranges between 7 and 9. However, the onset of acidification with its resultant reduction in pH, had an adverse impact on the establishment of *N. congesta* in the lakes of Capel Wetlands Centre. Acidity of the lakes of Capel Wetlands Centre was found to be increasing from 2005 to 2007 (Fig. 7.4). Capel lies on the Swan Coastal Plain which is made of quaternary deposits, which occurred during the Pleistocene and Recent historical periods of the earth (Davies 2002). Davies (2002) described the sands of Capel region as ancient calcareous dunes probably formed around one million years ago from corals and coralline algae.

Historically, the lakes at Capel Wetlands Centre were found to exhibit variable and often very low pH which had an adverse effect on plants and animals by reducing the availability of inorganic carbon in the water. There was also, low phosphorus levels which limited plant productivity and led to phosphorus limitation, particularly under the high inorganic nitrogen concentrations present then. The pH of the discharge water from the processing plant was buffered which resulted in most of the lakes recording pH value of 6 or above (Davies 2002). Experiments were conducted by

Chambers and McComb (1996) to investigate the effects of various manipulations and results showed that the lake system had poor buffering capacity. Thus, an increase in pH had no effect on nitrogen and phosphorus concentrations indicating that increasing the pH of the Capel Wetlands Centre lakes required addition of a large quantity of alkaline agents over a long period of time. As a result of the input of alkaline agents at the outlet of the plant, the pH did gradually increase to a more or less neutral level after three years (Davies 2002). With the exception of Swamphen lake and Island lake, most of the lakes showed neutrality to high alkalinity at the beginning of the study in 2004. However, the pH of the lakes was decreasing from 2005.

Although this part of the study was initially not pre planned, observations of unsuccessful germination, growth and establishment of *N. congesta* coupled with continuous increase in lake acidity prompted an investigation into the cause(s) of the lake acidity. The objective of this part of the study was to investigate the possible causes of the acidification of the lakes at Capel Wetlands Centre and the impact on *N. congesta*.

7.4 Materials and methods

Water samples from the three lakes selected for the initial study of *N. congesta* growth (Plover North, Plover South and Nitella lakes) were collected on 21st November, 2007 using pretreated laboratory sample containers and sent to a NATA credited laboratory, ALS Laboratory Group, Analytical Chemistry and Testing Services, Environmental Division, 10 Hod Way, Malaga WA 6090, Australia, for the analysis for sulphur, sulphide and sulphate concentrations as well as acidity/alkalinity. Climate data (rainfall and temperature) for Capel/Bunbury region were obtained from the Bureau of Meteorology and analysed. pH measurements of the lakes from 2004 to 2007 were analysed. The pH was measured *in situ* using a hand help pH meter, TPS WP-81 Water quality meter.

7.5 Results

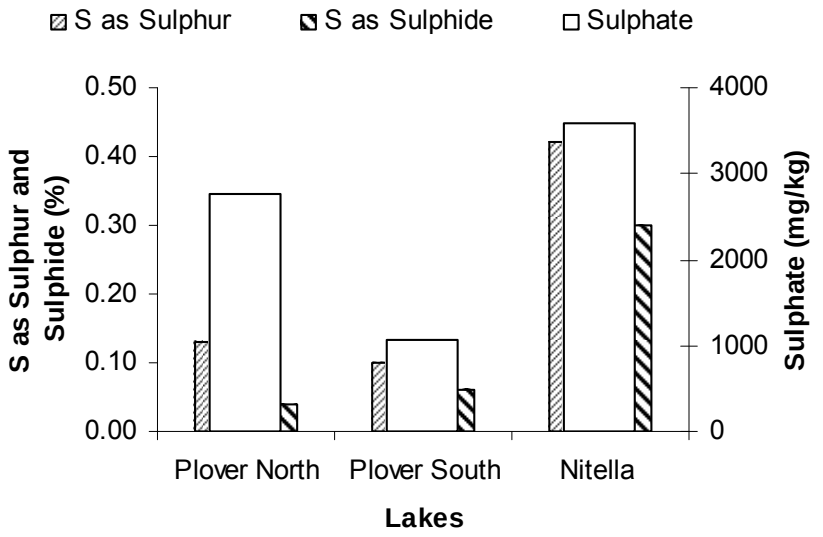

Figure 7.1 Sulphur, sulphide and sulphate concentrations in water from three lakes with *N. congesta* meadows.

Nitella lake had the highest percentage of sulphur, sulphide as well as the highest concentration of sulphate. However, it was not the most acidic lake amongst the three.

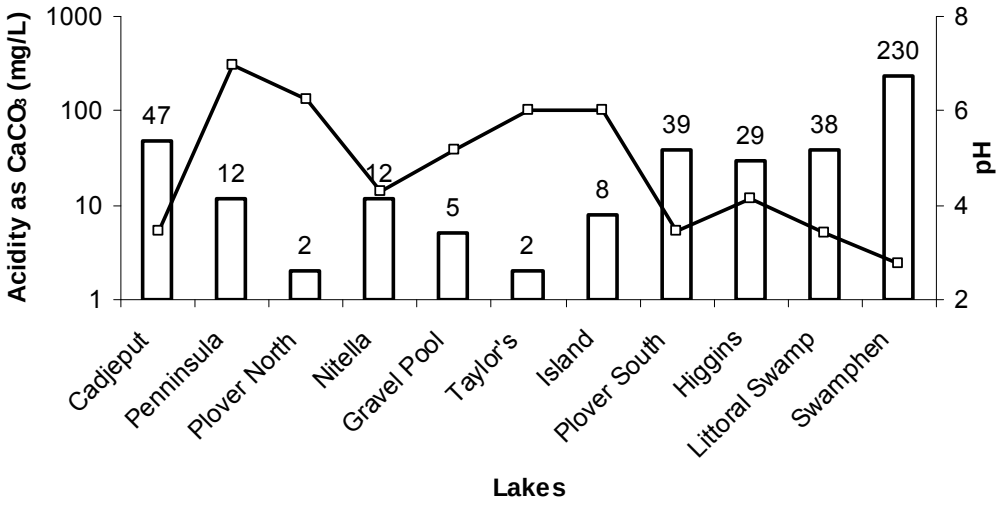

Figure 7.2 Acidity as $CaCO_3$ (mg/L) and pH values of 11 lakes sampled in 2007.

Swamphen lake showed the highest concentration of $CaCO_3$ with the correspondent lowest pH value of 2.77. Among Plover North, Plover South and Nitella lakes, Plover South showed the highest acidity with $CaCO_3$ concentration of 39 mg/L and a corresponding pH value of 3.47. Plover North lake was the most basic amongst the three lakes with a pH value of 6.23.

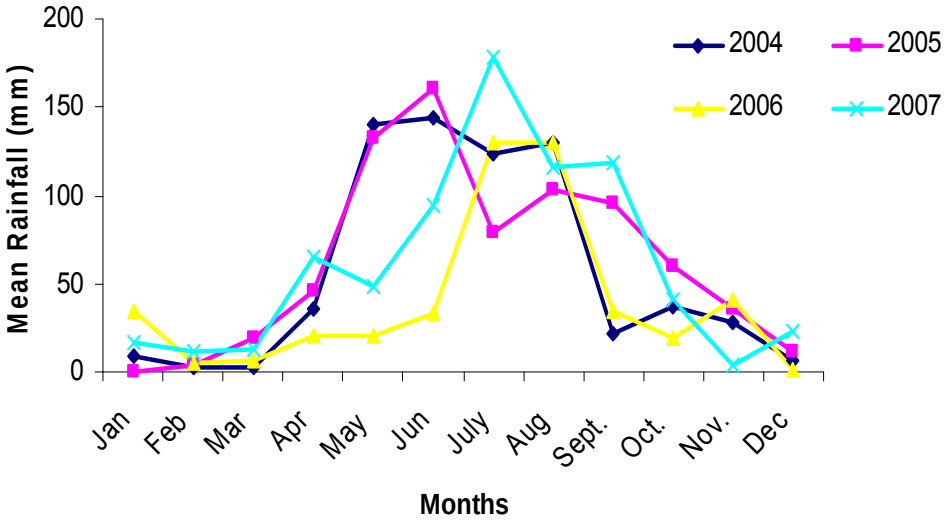

Figure 7.3 Mean monthly rainfall of Capel from 2004 – 2007.

Lowest mean rainfall was recorded in 2006

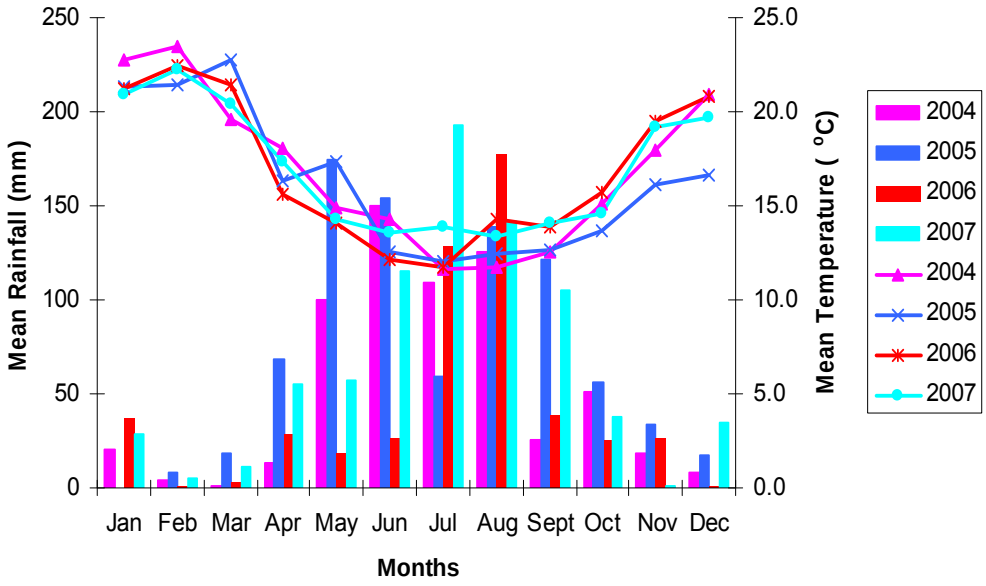

Figure 7.4 Mean monthly rainfall and temperature of Capel from 2004 – 2007.

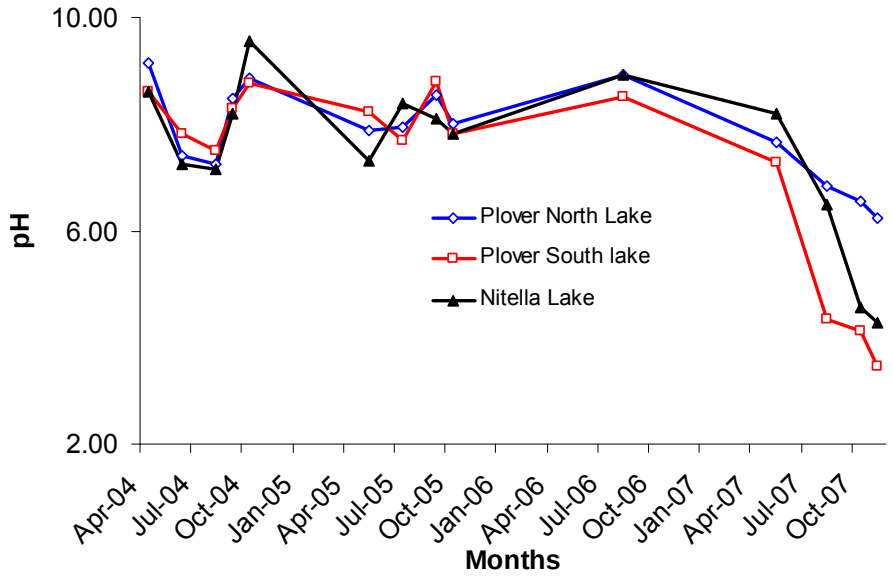

Figure 7.5 pH of three lakes sample from 2004 to 2007.

pH decreased from 2004 to 2007 in all the three lakes sampled. This corresponded with the growth of *N. congesta*. That is at higher pH growth of *N. congesta* could be observed while there was no observable growth as the pH decreased on the onset of acidification. This is represented in the figure below.

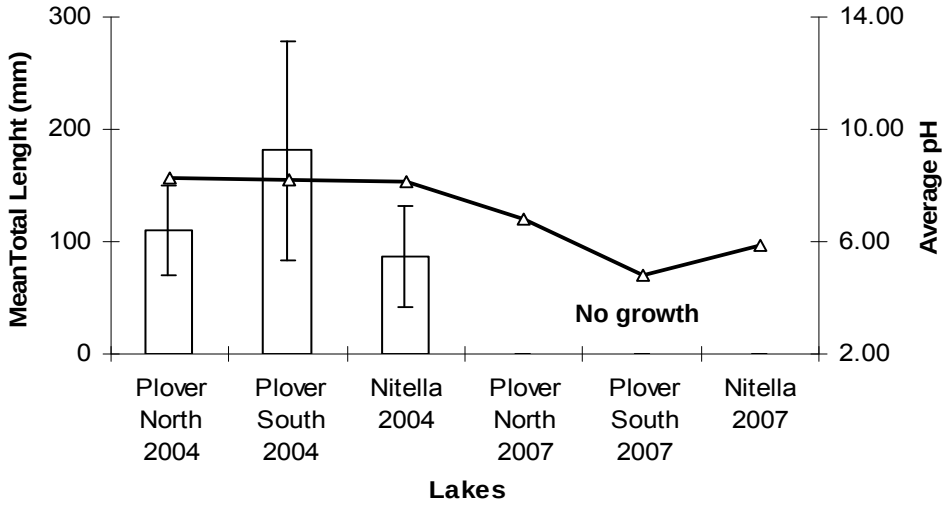

Figure 7.6 Mean total length of *N. congesta* meadows and average pH of lakes in 2004 and 2007 (n = 25). Mean total length was high in 2004 and no growth in 2007 (cf. Fig. 7.5).

Among the three lakes, Plover South lake had the most decrease in pH from an initial 8.61 in 2004 to 3.47 in 2007 whiles Nitella lake recorded a decrease in pH from an initial 8.62 in 2004 to 4.28 in 2007 (Fig. 7.5).

7.6 Discussion

Acidic lakes under all conditions are not productive waters (Blodau 2006). Acidity sequestration occurs under certain conditions. Initially, there should be a concurrent reduction of iron and sulfate. Secondly, the precipitation of iron sulfides should proceed at a very fast rate relative to the oxidation of hydrogen sulfide with iron oxides (Peiffer *et al.* 1992). Blodau (2006) has proposed that a pore water pH of 4.5 to 5 serves as the critical threshold for the accumulation of iron sulphide. Therefore, the decrease in pH of the lakes may have promoted and enhanced the accumulation of iron sulphide.

The relationship between the average pH of the three lakes and the mean total length of *N. congesta* meadows in 2004 and 2007 is shown in Fig. 7.6. This relationship supports the pH sensitivity of *N. congesta*. Thus in 2004 when the average pH range of the lakes was ≥ 6, there was significant normal growth of *N. congesta* with mean total length of 86.86±44.75 to 181.72±92.27 mm. However, in 2007, when the average pH of the lakes studied dropped to < 6, the germination of *N. congesta* was adversely affected resulting in no observable growth. Thus the low pH of the lakes might not have supported the germination of *N. congesta* oospores.

In the growth studies of *N. congesta* (chapter three of this thesis), the highest growth rate was always recorded during winter. Also *N. congesta* germinates with the onset of the rains. Thus a delay or reduction of the onset of the rains may have caused a delayed germination which might have ended up in complete decline of the charophyte. The delayed onset of the rains as well as the prolonged dry period of the lakes (Fig. 7.8) can be attributed to climate variation. Australian rainfall patterns have changed in the last 50 years with the southwest experiencing a decline in rainfall (Bureau of Meteorology, 2007).

The Capel lakes are groundwater dependent after discharging of effluent water ceased. Rainfall has since been the source of groundwater for these lakes. The amount of rainfall recorded in May and June 2004 and 2005 were very high (Fig. 7.3). On the other hand, in 2006 and 2007, the highest amount of rainfall was recorded in July. Thus it could be that the delay of substantial amount of rainfall during the raining season could be a contributing factor to the germination of *N. congesta*. Moreover, the low amount of rain and prolonged drought conditions may have contributed to the drawdown of the water table. The drawdown of water tables and the infiltration of nitrates (NO_3^-) from agricultural land have been documented to favour the oxidation of iron sulphides (FeS_x) in the subsoil resulting in the mobilization of SO_4^{2-} (Lucassen *et al.* 2005). Free sulphide S^{2-} as a product of the reduction of SO_4^{2-}, chemically binds to Fe^{2+} which may be present as a result of lower free Fe concentrations in the aerobic sediment top layer (Lucassen *et al.* 2005). In conclusion, it can be assumed that the acidification of the lakes resulted from the drawdown of the groundwater table during the summer seasons resulting in cracks within the sediments which exposed the lake sediments to extreme heat from the sun subsequently triggering oxidation of FeS_2.

In support of the above points, ASS risk areas sourced from the Western Australia Planning Commission shows that the Capel Wetlands Centre with its lakes, are of soils “moderate to low risk of ASS occurring within 3 m of natural soil surface” (see Appendix 12). The presence of Acid Sulphate Soils, most likely in the form of FeS_2 as the cause of acidification of Swamphen lake with elemental analysis showed high amounts of sulphur, iron and aluminium (Capelli 2006). Swamphen lake was the most acidic lake with acidity as $CaCO_3$ value of 230 mg/L as determined in this study (Fig.7.2). It has been projected that by 2030, there will be up to 40% more droughts by 2070 in eastern Australia and up to 80% more in south-western Australia (CSIRO 2007). The above projections show that the effect of climate change is to be encountered for a very long time and therefore there should be

concerted effort by all stakeholders to put together mitigation measures in order to safe guard its effects on important ecosystems such as wetlands.

Figure 7.7 Nitella Lake in summer in 2006.

Figure 7.8 Nitella Lake in summer with cracks in sediment in summer 2007.

Summary

The causes of the drastic decline of *N. congesta* from Nitella, Plover North and Plover North lakes in 2007 were investigated. The rainfall and temperature pattern of Capel as well as the pH data of the lakes over a four year period (2004 – 2007) were studied. It was concluded that the acidification of the lakes was the cause for decline of charophytes. The drawdown of the ground water table coupled with the prolonged exposure of the sediment of the lakes to extreme dryness might have triggered the oxidation of the sulphide rich sediment. The acidification was interpreted as a consequence of climate change. Before the acidification of the lakes, *N. congesta* germinated and established well in 2004 and 2006. However, with the onset of acidification, there was no germination of *N. congesta* observed. Thus the acidification of the lakes had an adverse impact on the establishment of *N. congesta* in the lakes at Capel Wetlands. This in turn is attributed to decline in rainfall.

8 DIATOM ASSEMBLAGES IN THE MUCILAGE OF *Nitella congesta* AND LAKES: INDICATORS OF ENVIRONMENTAL CHANGE

8.0 Introduction

Diatoms are single celled microscopic algae belonging to the class Bacillariophyceae. They are eukaryotic organisms; each cell has a true membrane-bound nucleus (Medlin *et al.* 1993). They are classified according to the manner of their growth, general shape and the characteristics of the frustules and the raphe or longitudinal slit (Snoeijs 1996). Depending on the morphology, diatoms are described as either centric or pennate. Centric diatoms are radially symmetrical. Pennate diatoms are "boat shaped" with bilateral symmetry (Medlin *et al.* 1993). Diatoms occur in all aquatic environments. They fix major portion of the earth's carbon, generate oxygen and can supply high quality food for animals (Vanni and Findlay 1990). They have a short life cycle and rapidly colonise new habitats (Round 1991). Diatoms can be preserved in the form of permanent slides for microscopic analysis, unaffected for numerous years.

Ecological integrity is measured by using the various responses of biota to environmental changes (Karr 1991). Diatoms have gained attention among biota used based on their position at the base of aquatic food webs and also are among the premier organisms to respond to environmental changes (Lowe and Pan 1996; McCormick and Stevenson 1998). Diatoms have been extensively used in the past couple of decades in freshwater management by the use of several indices and transfer functions to assess acidification (Battarbee *et al.* 1999) and nutrients (Kelly and Whitton 1995). The response of diatoms to environmental changes within a very short time has been extensively studied (Stevenson and Pan 1999). Among the biomonitors for early detection of environmental deterioration and recovery, diatoms may be better than invertebrates and fish (Cattaneo *et al.* 2004). They may be epiphytic (growing on plants), epilithic (growing on rocks, stones or other solid surfaces), epipelic (growing on mud or sediments), epizoic (growing on animals), epipsamic (growing on sand) and epidendric (growing on wood).

Depending on the pH tolerance of diatoms, they are acidobiontic if they occur in pH less than 7 with best development below 5.5, acidophilic if they occur at a pH less than 7, indifferent or circumnuetral if they occur at pH 7, they are

alkalidophilic if they occur at pH greater than 7 and alkalibiontic if they occur at pH less than 7 (John 1993b; van Dam *et al.* 1994). Diatoms are very sensitive to environmental changes such as eutrophication, metal and toxic pollution, salinisation and acidification (Lobo *et al.* 1995; Kelly and Whitton 1998). They are found to be common in the benthos of rivers and streams and in many cases serve as the major constituents of the phytobenthos (John 1998).

8.1 Diatoms as indicators of hyperaccumulation and lake acidification

Diatoms are sensitive to environmental changes such as salinisation, eutrophication, metal and toxic pollution and acidification (Leskinen and Hällfors 1988). Littoral diatoms are critical contributors of the primary production in shallow aquatic ecosystems. They have been successfully used in monitoring disturbances such as eutrophication and acidification (Charles *et al.* 1989; Hall *et al.* 1997). Owing to the direct involvement of diatoms in mineral uptake, they can reflect an increase in salinity more effectively than any other group of organisms and the effects on diatom assemblages have been well investigated (Blinn 1993). They have been used extensively for monitoring acidification affecting many water bodies in countries such as Belgium, Sweden and Finland (Denys and van Straaten 1992).

The mucilage of *N. congesta* was found to have contributed to the diversity and abundance of diatoms in the lakes of Capel Wetland Centre. Diatom communities have exhibited compositional changes as a result of metal contamination in lakes (Cattaneo *et al.* 2004) and rivers (Griffith *et al.* 2002; Hirst *et al.* 2002). Diatoms in the mucilage were therefore used as indicators of change in terms of metal hyperaccumulation by *N. congesta*. It was hypothesized that the species composition of the diatoms on the *N. congesta* habitat (the mucilage) would differ from the species composition of diatoms living in the standing water of the lakes.

8.2 Diatom assemblages: the use of natural artificial substrates

Many diatoms are periphytic and their assemblages can be studied using natural and artificial substrates. Artificial substrates are devices used for studying colonization by periphyton (Aloi 1990). Artificial substrates provide a constant surface area, in order to allow uniform, simple quantitative and replicable sampling. Natural substrates on the other hand vary in surface area, composition, orientations, size, texture and depth (Korte and Blinn 1983; Luttenton and Baisden 2006). Artificial substrates can be used to sample diatoms characteristic of a water body at the time of collection (John 2000).

8.3 Materials and methods

8.3.1 Diatom sampling from mucilage of *N. congesta*

Samples of thallus of *N. congesta* were collected from the three lakes, Nitella, Plover North and Plover South lakes at random sites into plastic buckets and transported the Algology Laboratory of the Department of Environmental Biology, Curtin University. The samples of *N. congesta* were carefully washed and the mucilage carefully removed using the thumb and forefinger into clean beakers. Samples of the mucilage obtained were digested and permanent slides prepared following the method used in section 8.3.3 below.

8.3.2 Diatom sampling from lakes

Sampling of diatoms was done by the use of the JJ Periphytometer (Fig. 8.1) an artificial substrate sampler used for uniform collection of diatom samples designed by John in 1998. Ten (10) glass slides were placed in each sampler and secured by a fishing line. The line was then tied to a 1 meter pole with the sampler end suspended or submerged in the lake water for a period of 14 days. Lake-water pH was measured *in situ* with a handheld pH meter.

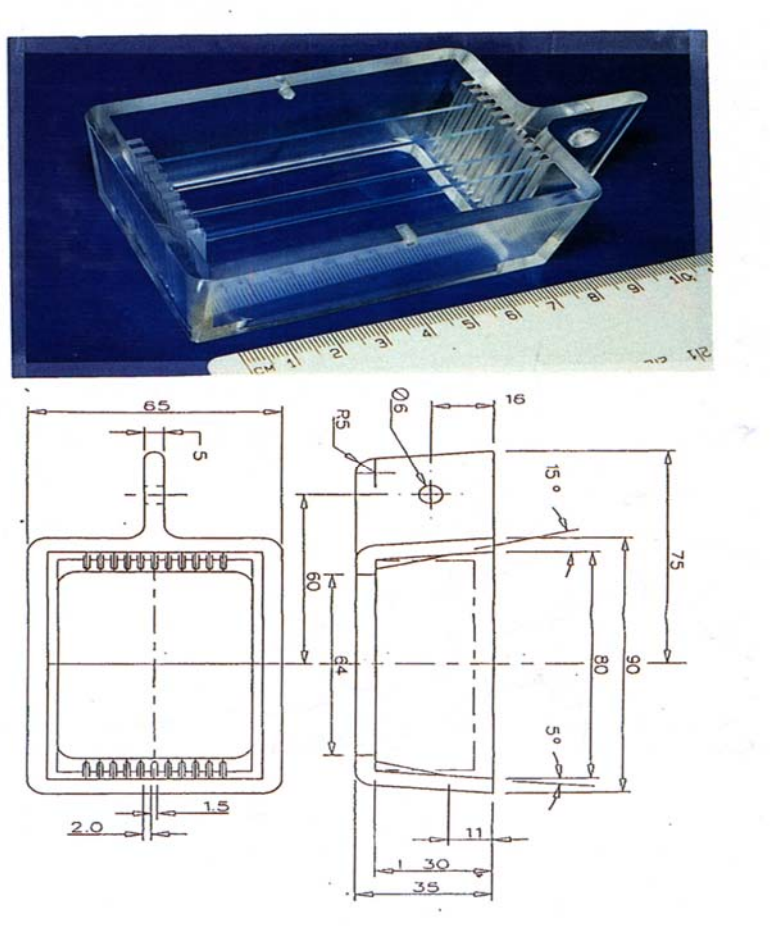

Figure 8.1 JJ Periphytometer - an artificial substrate sampler used for uniform collection of diatom samples.

8.3.3 Preparation of permanent slides

Digestion

The following method of permanent slide preparation followed according to (John 1983). Twenty milliliters (20ml) of each sample was measured into clean labeled 100 ml Pyrex beakers and 20ml of 70% nitric acid was added. The beakers were placed onto hotplate in the fume cupboard and gently boiled. This treatment removes organic material from the samples leaving only the siliceous diatom frustules. The beakers are then allowed to cool and 60 ml of deionised was added and left to stand. The periphyton from the slides in the periphytometer was scrapped into a beaker using a one sided blade and were also digested to get rid of all organic material.

The supernatant was then decanted after a minimum of 3 hours into centrifuge tubes. The tubes were then centrifuged for 5 minutes; 30 seconds on low speed rotation (1500rpm) and the remaining time on high speed rotation (3500rpm). The top aqueous layer was decanted without disturbing the bottom pellet, the tubes was then refilled with deionised water and shaken to resuspend the diatom frustules, and then returned to the centrifuge. This cleaning process was repeated five times in order to remove all traces of nitric acid from the sample.

Mounting

Cover slips (22 × 22 mm) were cleaned by placing them in a small Petri dish with 90% ethanol. The cover slips were removed and dried and placed on the hotplate on low heat. Using the Nichiryo Nichipet digital pipette (5000DG), 900 µL of deionised water was firstly pipetted onto the cover slip and then 100 µL of the cleaned diatoms samples (shaken thoroughly to resuspend the diatom frustules) was added and allowed to evaporate on the hotplate. The cover slip was then inverted onto a slide with a drop of the mounting medium Naphrax (Refractive Index 1.74) which contains the solvent Toluene.

The slide was then placed onto the hotplate at low heat and once the solvent had evaporated, the slide was removed and allowed to cool, hardening the medium. After the first slide for each sample was made, it was examined microscopically to ensure that the frustules evenly dispersed to enable accurate identification, enumeration and photography. If the sample was too dense or sparse, the dilution faction was modified accordingly. Micrographs of species were taken by Olympus DP 70 (Photomicroscope) Digital Camera System. Diatoms and their ecological pH preferences were identified using Lowe (1974), John (1983) and John (1993). Diatoms were identified to species level using specialized literature and by the assistance of J. John, enumerated and results were expressed as relative percentages. Species data obtained were statistically analysed. Species above 1% were used for statistical analysis.

The acidity preference of diatoms was obtained from the literature.

1. acidobiontic diatoms occurring optimally at pH < 5.5
2. acidophilous diatoms occurring mainly at pH < 7
3. circumneutral diatoms occurring at about pH 7
4. indifferent diatoms which have no preference in terms of pH
5. alkaliphilous diatoms occurring mainly at pH > 7
6. alkalibionthic diatoms always occurring at pH > 7.

Shannon-Weaver diversity index was used to measure the variability of the communities using the number of species present.

$$H' = \sum_{i=1}^{S} P_i \log (P_i)$$

where H' is the Diversity Index

P_i is the proportion of the total abundance of sample represented by species i,

s is the number of species in the sample.

8.3.4 Statistical analysis

Species numerical analysis was done if they occurred at abundances of > 1%. Multivariate techniques (cluster analysis and multidimensional scaling) were used to explore the similarities and dissimilarities in the data obtained. Only diatoms with relative percentage above 1% were used in the quantitative and statistical analyses. Statistical package PRIMER version 6 was used for the analysis.

8.4 Results

8.4.1 Diatom species diversity and abundance

Table 8.1 shows diatom species (count and relative abundance) sampled from the lakes and mucilage obtained from *N. congesta* meadows in the lakes. Table 8.2 and Table 8.3 show diatom species (count and relative percentage) sampled from the lakes in 2004 and 2007 respectively.

Table 8.1 Relative percentage of diatoms species in three lakes and mucilage of *N. congesta* from the lakes (only species with relative percentage > 1% were used for quantitative analysis).

SPECIES	LAKE			MUCILAGE		
	Nitella	Plover North	Plover South	Nitella	Plover North	Plover South
	%	%	%	%	%	%
Achnanthidium minutissimum	28.2	7.3	1.7			
Amphora veneta (Kützing)	21.1	5.3	38.8	17.1	1.7	
Anomoeoneis sphaerophora (Kütz.) Pfitzer		2.7				
Brachysira brebissonii	16.9	9.3				
Brachysira vitrea	2.8		5.2	10.3	45.0	
Encynopsis microcephala (Grunow) Krammer	23.9	22.7	10.3	30.8	35.0	
Encyomena minutum		29.3	1.7	8.5	1.7	
Fragilaria capucina		16.0	21.6	5.1		4.3
Frustulia magaliesmontana Cholnoky						6.5
Mastogloia elliptica (Ag.) Cleve.		2.7	6.9			6.5
Mastogloia smithii			3.4	3.4		2.2
Mastogloia tuscula					1.7	
Navicella pusilla			1.7	6.0	5.0	
Navicula cincta					1.7	
Navicula cryptonella			1.7			
Nitzschia microcephala				1.7		
Nitzschia sp.				13.7	5.0	
Rhopalodia novae-zelandie Hustedt			1.7		1.7	78.5
Stauroneis pachycephala Cleve.	7.0	4.7	3.4	1.7	1.7	2.2
Synedra acus			1.7	1.7		

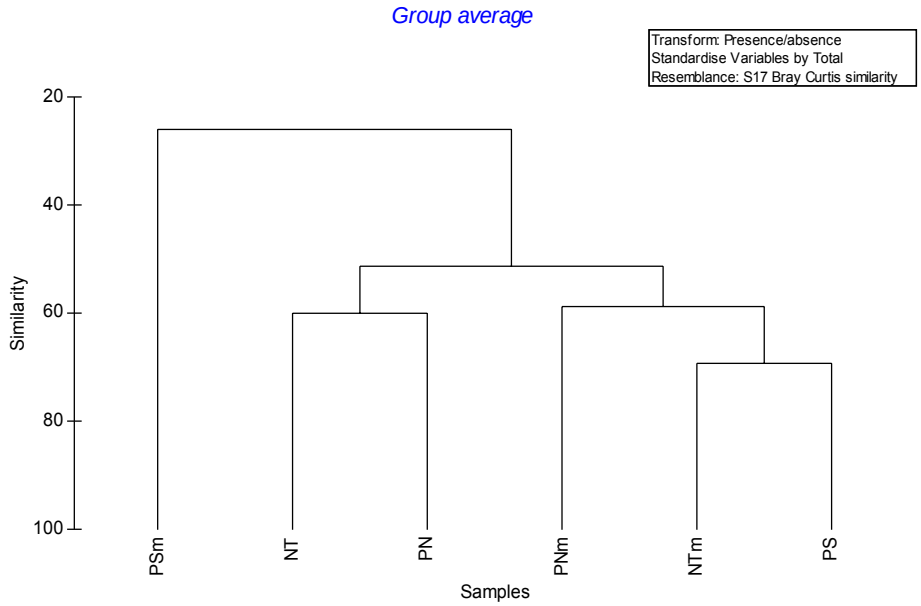

Figure 8.2 Dendrogram of three lakes and mucilage of *N. congesta* from these lakes using group average clustering from Bray-Curtis similarities on transformed abundances.

PSm = Mucilage from Nitella meadows in Plover South lake
PNm = Mucilage from Nitella meadows in Plover North lake
NTm = Mucilage from Nitella meadows in Nitella lake
NT = Nitella lake, PN = Plover North lake, PS = Plover South lake

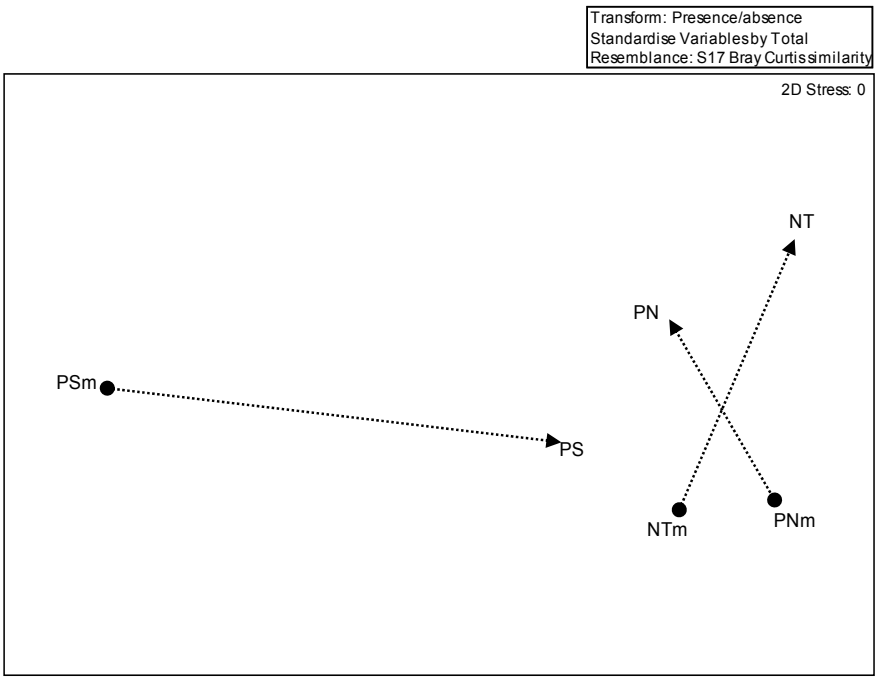

Figure 8.3 MDS ordination of three lakes and mucilage of *N. congesta* from these lakes based on transformed abundances and Bray-Curtis similarities (stress = 0).

PSm = Mucilage from Nitella meadows in Plover South lake
PNm = Mucilage from Nitella meadows in Plover North lake
NTm = Mucilage from Nitella meadows in Nitella lake
NT = Nitella lake
PN = Plover North lake
PS = Plover South lake

Table 8.2 Relative percentage of diatoms species in eight lakes sampled in 2004 (only species with relative percentage > 1% were used for quantitative analysis).

	LAKES							
	NT	PN	PS	TY	BP	GP	BD	TS
SPECIES	%	%	%	%	%	%	%	%
Achnanthidium minutissimum	28.2	7.3	1.7	11.1	3.7	9.4	66.2	40.6
Amphora obtusa					3.7			
Amphora veneta (Kützing)	21.1	5.3	38.8	31.9	7.5	47.2	2.9	5.1
Anomoeoneis sphaerophora (Kütz.) Pfitzer		2.7						
Brachysira brebissonii	16.9	9.3		6.9		4.7	8.8	
Brachysira vitrea	2.8		5.2		1.9			3.6
Ctnephora pulchella				1.4				
Cyclotella meneghiniana Kütz.					3.7			
Cyclotella stelligera Cleve & Grun.					1.9			
Encynopsis microcephala (Grunow) Krammer	23.9	22.7	10.3	8.3	15.0		5.9	8.0
Encyomena minutum		29.3	1.7	5.6		5.5		21.0
Fragilaria capucina		16.0	21.6	18.1	4.7			15.2
Frustulia magaliesmontana Cholnoky					11.2			

Table 8.2 (Continued)

Mastogloia elliptica (Ag.) Cleve.		2.7	6.9	1.4	15.0	3.1		2.2
Mastogloia smithii			3.4		3.7		4.4	
Mastogloia tuscula					20.7			
Navicella pusilla			1.7					1.4
Navicula cryptocephala				2.8				
Navicula cryptonella			1.7					
Navicula gregarium								0.7
Nitzschia microcephala								0.7
Nitzschia obtusa				1.4				
Nitzschia sp.					1.9			
Rhopalodia novae-zelandie Hustedt			1.7			3.1		
Stauroneis pachycephala Cleve.	7.0	4.7	3.4	1.4	5.6	3.1		1.4
Synedra acus			1.7	5.6		23.6	8.8	
Synedra ulna				4.2			2.9	
pH	**9.57**	**8.86**	**8.78**	**7.84**	**8.40**	**7.20**	**8.01**	**7.98**
H'	**0.70**	**0.82**	**0.84**	**0.91**	**1.05**	**0.67**	**0.53**	**0.75**

Lake Identification: Nitella Lake (NT), Plover North (PN), Plover South Lake (PS), Taylor's Lake (TY), Pobble Bonk (PB), Gravel Pool, Boulder's Lake (BD), Tiger Snake Lake (TS).

Table 8.3 Relative percentage of diatoms species in eight lakes sampled in 2007 (only species with relative percentage > 1% were used for quantitative analysis).

SPECIES	LAKES							
	NT	PN	PS	GP	ISD	TS	PBK	PNS
	%	%	%	%	%	%	%	%
Achnanthidium minutissimum		5.1				8.6		
Amphora veneta (Kützing)		5.1			2.8			
Anomoeoneis sphaerophora (Kütz.) Pfitzer		0.6						
Brachysira brebissonii				43.1		7.8	30.3	
Brachysira vitrea	1.8	28.8		44.4	59.7			20.0
Encynopsis microcephala (Grunow) Krammer		1.3						
Encyomena minutum			2.4			3.4		
Entomoneis paludosa					1.4		4.2	
Eunotia binodis				8.3				
Eunotia incisa	3.7							
Fragilaria capucina		42.3	7.9		20.8	8.6	6.7	62.7
Frustulia magaliesmontana Cholnoky	42.2							

Table 8.3 (Continued)

Mastogloia elliptica (Ag.) Cleve.			1.6				3.4	
Mastogloia smithii								2.7
Mastogloia tuscula		1.3	1.6					
Navicula aff. Cari		0.6	4.0					
Navicula aff. festiva	27.5		4.8					
Navicula cryptocephala					1.4			
Navicula rhynchocephala		1.3						
Nitzschia nana		0.6						
Nitzschia obtusa				2.8	1.4			2.7
Nitzschia paleoceae	24.8		77.8		1.4			
Nitzschia sp.						7.8	43.7	
Stauroneis pachycephala Cleve.		11.5		1.4	1.4	3.4	6.7	9.3
Synedra acus		1.3			9.7	60.3	5.0	2.7
Synedra ulna								
pH	**4.00**	**6.25**	**3.47**	**5.19**	**6.02**	**7.47**	**8.16**	**6.95**
H'	**0.19**	**0.69**	**0.39**	**0.47**	**0.55**	**0.63**	**0.64**	**0.49**

Lake Identification: Nitella Lake (NT), Plover North Lake (PN), Plover South Lake (PS), Gravel Pool (GP), Island Lake (ISD), Tiger Snake Lake (TS), Paper Bark Lake (PBK), Penninsula Lake (PNS).

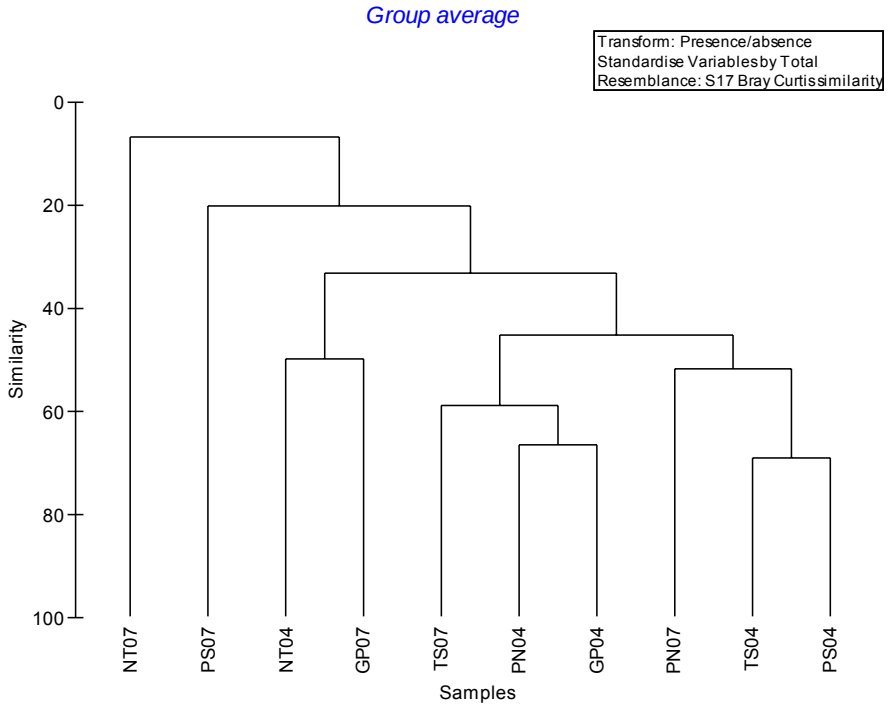

Figure 8.4 Dendrogram of five lakes sampled in 2004 and 2007 using group average clustering from Bray-Curtis similarities on transformed abundances.

NT04 = Nitella lake in 2004
NT07 = Nitella lake in 2007
PS04 = Plover South lake in 2004
PS07 = Plover South lake in 2007
PN04 = Plover North lake in 2004
PN07 = Plover North lake in 2007
GP04 = Gravel Pool in 2004
GP07 = Gravel Pool in 2007
TS04 = Tiger Snake lake in 2004
TS07 = Tiger Snake lake in 2007

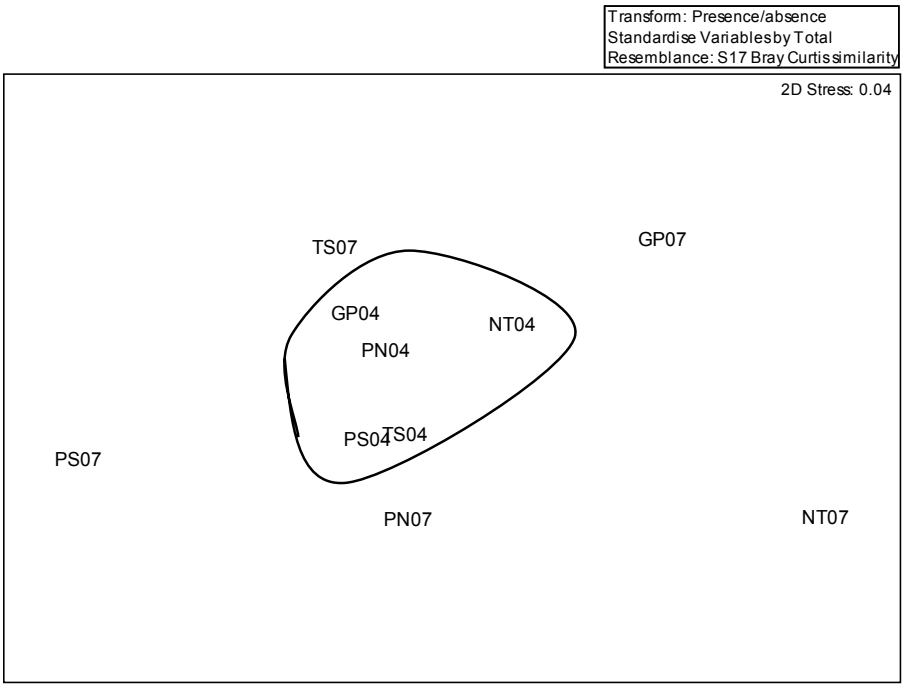

Figure 8.5 MDS ordination of five lakes sampled in 2004 and 2007 based on transformed abundances and Bray-Curtis similarities (stress = 0.04). Lakes sampled together in 2004 when the acidity was low are circled.

NT04 = Nitella lake in 2004
PS04 = Plover South lake in 2004
PN04 = Plover North lake in 2004
GP04 = Gravel Pool in 2004
TS04 = Tiger Snake lake in 2004

NT07 = Nitella lake in 2007
PS07 = Plover South lake in 2007
PN07 = Plover North lake in 2007
GP07 = Gravel Pool in 2007
TS07 = Tiger Snake lake in 2007

Table 8.4 Diatom species, their ecological pH preferences and medium of sampling.

Species	pH preference	Media
Amphora obtusa	Alkaliphilous	Lakes
Amphora veneta (Kützing)	Alkalibiontic	
Anomoeoneis sphaerophora (Kütz.) Pfitzer	Alkaliphilous	
Cyclotella meneghiniana Kütz	Alkaliphilous	
Mastogloia elliptica (Ag.) Cleve.	Alkaliphilous	
Mastogloia smithii	Alkaliphilous	
Mastogloia tuscula	Alkaliphilous	
Navicella pusilla	Alkaliphilous	
Nitzschia sp.	Alkaliphilous	
Rhopalodia novae-zelandie Hustedt	Alkaliphilous	
Stauroneis pachycephala Cleve.	Alkaliphilous	
Nitzschia obtusa	Alkaliphilous	
Brachysira brebissonii	Acidophilous	*N. congesta* mucilage and lakes
Brachysira vitrea	Acidophilous	
Encynopsis microcephala (Grunow) Krammer	Acidophilous	
Encyomena minutum	Acidophilous	
Ctnephora pulchella	Circumneutral	Lakes
Navicula cryptocephala	Circumneutral	
Navicula cryptonella	Circumneutral	
Navicula gregarium	Circumneutral	
Nitzschia microcephala	Circumneutral	
Achnanthidium minutissimum	Circumneutral/ Alkaliphilous	
Fragilaria capucina	Circumneutral/ Alkaliphilous	
Synedra acus	Circumneutral/ Alkaliphilous	
Synedra ulna	Circumneutral/ Alkaliphilous	
Frustulia magaliesmontana Cholnoky	Acidobiontic	

Table 8.5 pH of five lakes sampled 2004 and 2007.

Lake	pH	
	2004	**2007**
Nitella	9.57	4.00
Plover North	8.86	6.25
Plover South	8.78	3.47
Gravel Pool	7.20	5.19
Tiger Snake	7.98	7.47

All the lakes showed a decrease in pH from 2004 to 2007

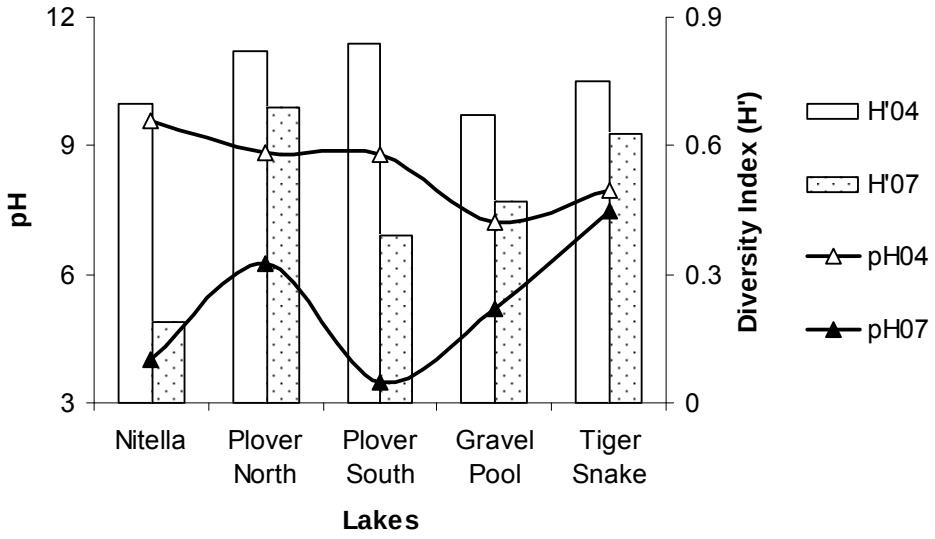

Figure 8.6 pH and diversity index (H') of five lakes sampled in 2004 and 2007

A decrease in pH corresponded to a decrease in species diversity.

8.5 Discussion

8.5.1 Mucilage communities

The diatom assemblages in the mucilage of *N. congesta* from the three different lakes, Nitella lake, Plover North lake and Plover South lake were found to be different from those assemblages in the lakes. *Achnanthidium minutissimum* was present in the three lakes being the most dominant

species in Nitella lake but none was found in the mucilage. *Encynopsis microcephala* was present in all the lakes as well as mucilage from Nitella and Plover North lakes with a higher relative abundance in the latter lake. *Stauroneis pachycephala* was the specie common to both lakes and mucilage.

Cluster analysis (Fig. 8.2) and Multidimensional scaling (MDS) (Fig. 8.3) showed that the diatom communities in the mucilage of *N. congesta* from the lakes were completely different from the diatom communities in the lakes. This may be attributed to some chemical properties of the mucilage that may have attracted common diatom communities in the mucilage. Thus the mucilage served as a microhabitat for these diatom communities probably due to the metals accumulated by the mucilage. All the diatom species sampled from the *N. congesta* mucilage were acidophilous in ecological pH preference (Table 8.4). This may be explained that the metal sequestered by the mucilage may have caused the mucilage to be acid.

8.5.2 Diatom assemblages in 2004 and 2007

The diatom communities used as indicators of acidity showed a shift in dominant species in the lakes in accordance with changes in pH. In 2004 when the acidity of the lakes was low (with an average pH of 8.33 ± 0.73), the dominant species were *Achnanthidium minutissimum* and *Amphora veneta* which were present in all the lakes sampled. *Achnanthidium minutissimum* was the dominant species in Nitella, Boulder, and Tiger Snake lakes. *Amphora veneta* was dominant in Plover South and Taylor's lakes as well as Gravel Pool. *Encynopsis microcephala* was also present in all the lakes sampled except Gravel Pool. The rest of the diatom species were sparsely distributed among the lakes sampled. In 2007 when the acidity of most of the lakes increased (with an average pH of 5.94 ± 1.64) there was no diatom specie which was common to all the lakes. *Fragilaria capucina* was found in most of the lakes (being the dominant specie in Plover North and Penninsula lakes) except Nitella lake and Grave pool. *Stauroneis*

pachycephala was also found in most lakes except Nitella and Plover South lakes.

The acidification of the lakes caused not only a shift in diatom communities but also resulted in low diversity in all the lakes. For example, comparing the five common lakes, sampled in both 2004 and 2007, the diversity indices of the diatom communities decreased with corresponding decrease in pH in all the lakes from 2004 to 2007 (Fig. 8.6). Cluster analysis and MDS (Fig. 8.4 and Fig. 8.5) show that in 2004, there were not much differences in the lakes since the pH of all the lakes sampled was within a range of 7.20 – 9.60. Therefore these lakes seem to be grouped together (circled in Fig.8.5). However in 2007, analyses show that the lakes are completely different and far apart from each other corresponding to significant changes in pH range of 3 to 8.5.

The changes in the most common species clearly shows the response of diatoms to changing lake pH by showing a decrease of alkaliphilous and alkalibiontic species and an increase of acidobiontic and acidophilous species.

Summary

Diatoms were found to be probable indicators of metal accumulation by *N. congesta* and acidification of the lakes of Capel Wetland Centre. They were sampled from the lakes using an artificial substrate over a two weeks period. Diatom assemblages in the lakes were different from those in the mucilage of *Nitella congesta*. The mucilage, with its metal ion concentrations, acted as a microhabitat for the diatoms. The mucilage ensheathing *Nitella congesta* harbour diatoms. As the mucilage contains concentrated levels of ions, it was hypothesized that the diatom assemblages inhabiting the mucilage must be different from those present in the standing water of the lakes.

Diatoms from the microhabitat of the mucilage and in the standing water collected by an artificial substrate were found to be different in community structure. Diatom assemblages before and after acidification of the lakes

were also found to be different from each other. Multi Dimensional Scaling analysis showed that the diatom assemblages before the onset of acidification were different from those assemblages after acidification.

9 CONCLUSION AND RECOMMENDATIONS

This project focused on the charophyte *Nitella congesta*. It was initiated with the objectives enumerated below.

- As a rehabilitation tool it was eminent that its life cycle should conform to the hydrological regime of the lakes. Thus one of the objectives of this project was to study the life cycle pattern of *N. congesta*, in relation to the hydrological regime of the Capel Wetlands, as a suitable macrophyte for rehabilitation.
- The ecological role played by the presence of *N. congesta* in the wetlands was a key factor if it was to be used successfully. Therefore, there was the need to investigate the role of *N. congesta* in the ecological rehabilitation of sand mine wetlands at Capel, by focusing on the limnology, macroinvertebrates and diatom communities associated with the charophyte.
- To achieve an effective rehabilitation of a post mining area, it is very important that any rehabilitation tool should possess the ability to effectively and efficiently accumulate the associated residual heavy metals from the sediment and water. Therefore an investigation was conducted into the ability of *N. congesta* to (hyper)accumulate heavy metals.
- To investigate the impact of eutrophication on the establishment of *N. congesta* in the lakes at the Capel Wetlands Centre with the prior knowledge of an eminent proposal to excessively increase the nutrient concentration of the lakes.
- To investigate the impact of acidification on *N. congesta*.

Charophyte species observed as the dominant macrophyte at Capel Wetlands was identified as *Nitella congesta* with other *Nitella* species such as *Nitella hyalina* and *Nitella lhotzkyi*. However, it was identified based on morphological features and reproductive structures. A morphological feature that posed much difficulty is the presence of the accessory branchlets. Different authors have documented different numbers of accessory branchlets in this species; c 40 (Wood and Imahori 1965; van Raam 1995), c 20 (García 1999). In the population studied, the maximum number of

accessory branchlets observed when present, was 17-20. Thus it may be that the population of *Nitella congesta* at the Capel Wetlands may be different from populations studied elsewhere. DNA analysis and chromosome number count may solve this issue.

Nitella congesta was cultured in the laboratory and growth and life cycle studied in the laboratory and in the field. Differences between growth rates in the laboratory and the field may be attributed to different environmental conditions such as temperature and water depth. Water level and seasonal variations might have contributed to different growth rates in the field and in the laboratory growth of *N. congesta* (Andrews, Box *et al.* 1984b; Casanova 1994). Charophytes are not uniform in their responses to environmental conditions, nor are they restricted to specific patterns of growth dictated by time and season but are adapted to respond to habitat changes in ways that permit persistence in those habitats In this case, different species can respond differently (Casanova 1994). For example inundation of charophytes in different seasons produced different growth patterns for five different charophytes species; *Chara australis*, *Chara muelleri*, *Chara preissii*, *Nitella cristata* var. *ambigua* and *Nitella sonderi* (Casanova and Brock 1999). It has been documented that the growth pattern of individuals of monoecious species differ from that of dioecious species (Casanova and Brock 1999). In this study, *N. congesta* was found to be predominantly annual but showed characteristics of a perennial depending on the availability of water for inundation. However in both laboratory and field populations, highest growth was observed in winter. The life cycle was studied in the laboratory and in the field and the production of fruiting bodies was also found to be related to season and water level changes.

Wetland sediments, in anoxic zone are considered to be sinks for metals and may contain very high concentrations of metals in a reduced state. Metal accumulated by a plant gives a better indication of the fraction of the metal in the environment likely to affect the aquatic ecosystem than do most types of direct chemical analysis (Empain *et al.* 1980). Therefore metal accumulation by *Nitella congesta* in aquatic ecosystems of the wetland can contribute to

the assessment of the level of metal contamination. *N. congesta* was found to accumulate very high concentrations of metals such as iron and aluminium. The mucilage ensheathment had higher concentrations of metals compared to the thallus. However, the mechanisms of metal sequestration by the mucilage and accumulation by the thallus are not yet known. Chemical analysis of the thallus of *N. congesta* from the field shows that *N. congesta* could accumulate metals such Fe and Al beyond the threshold for it to be considered a hyperaccumulator of such metals.

However, in the case of zinc accumulation experiment in the laboratory, though the threshold concentration was not attained, the bioconcentration factor (BFC) and the accumulation rate for the various concentrations were very high. Thus it may be concluded that *N. congesta* is a hyperaccumulator of metals. However, certain biological, chemical and environmental factors may have contributed to the accumulation of zinc below the threshold value.

Nitella congesta was found to provide refuge for macroinvertebrates therefore shielding them from predation (Crowder and Cooper 1982; Hanson 1990). The presence of *N. congesta* enhanced the macroinvertebrate species diversity in the lakes. Therefore, any management plans that would adversely affect the establishment of *N. congesta* the dominant macrophyte in the lakes may have very detrimental effects on the ecological structure of the lakes. This study showed a positive correlation between species richness and percentage cover of submerged vegetation.

The role of nutrients particularly, nitrogen and phosphorus in establishing clear-water conditions and high coverage of submerged macrophytes in shallow lakes are important (Gonzales Sagrario *et al.* 2005). A water body's aging process is accelerated by the enrichment of nutrients, which subsequently leads to faster succession (Sharma 1998). These factors must therefore be taken into consideration in an attempt to reduce the external nutrient loading of the lakes. It is estimated that the phosphorus concentration of the wastewater approved to be discharged into the lakes at Capel Wetlands will be 4mg/L (4000 µg/L). From the laboratory experiments

conducted on the impact of eutrophication on *N. congesta,* a total phosphorus concentration of 100µg/L was enough to prolong the vegetative stage. The successful succession of *N. congesta* as the dominant macrophyte in the lakes of Capel Wetlands depends largely on the oospores deposited in the sediments. Therefore, a continuous cycle of non-production of oospores due to eutrophication will eventually cause the disappearance of *N. congesta* from the wetlands. Reduced phosphorus concentration will lead to minimal concentrations in water bodies (Jeppesen *et al.* 2005).

Eutrophication control strategies should involve the reduction of nutrient loads to the lakes, particularly phosphorus as well as managing the existing high-nutrient state within the lakes in order to minimize the unfavourable biological and chemical effects (Harper 1992). No single approach or control measure will successfully treat all cases of eutrophication (Ryding and Rast 1989). However, depending on the circumstances, the feasible control option in any given situation varies from location to location (Ryding and Rast 1989). Ryding and Rast (1989) suggested the control of external nutrient (especially phosphorus) inputs as the most efficient long-term option for the control of eutrophication of lakes.

Therefore, efforts have been made extensively to reduce the nutrient loading into Capel Wetlands, particularly phosphorus which is predominantly considered to be the main factor controlling primary production in lakes (Gonzales Sagrario *et al.* 2005). Despite these efforts, many lakes' external nutrient loading is still high enough and subsequently impacting on the ecological structure. However, chemical and biological resistance can also impact the ecological structure of lakes even if the external nutrient loading is significantly reduced (Søndergaard and Jeppesen 2007). Therefore, other factors should be considered in the restoration and management of the lakes of Capel Wetland Centre in addition to the reduction of the external nutrient loading. Some of these factors include the lakes' internal nutrient loading as high internal loading of phosphorus from lake sediments has been reported by Phillips *et al.* (2005), Søndergaard *et al.* (2005) and Welch and Cooke (2005) to contribute to the delay in lake recovery after the reduction of the

external loading. In their report on a survey of 35 lakes in Europe and North America, Jeppesen *et al.* (2005) concluded that release of phosphorus endures for 10-15 years after reduction of nutrient loading.

Low phosphate levels found in the selected lakes may be important for the growth, establishment and survival of *N. congesta*. Submerged macrophytes are important for maintaining a clear water state (Jeppesen *et al.* 1997). Previous studies have shown that sensitivity to phosphorus is a major factor that determined the distribution of Characeae in lakes (Hutchinson 1975). Recent studies however, show that there is no limitation to the growth of some species by the increase in phosphorus concentrations (Kufel and Ozimek 1994). In the case of *N. congesta* however, this study showed that increase in nutrient concentration could adversely impact on its establishment.

The future challenge for the rehabilitation of the Capel Wetlands Centre is climate variation in relation to the rainfall pattern and increased temperature. There are possible indications that climate change will continue to pose the risk of acidification of the lakes thus enhancing the absence of the functional macrophytes particularly, *N. congesta*. The complete drying of the sediment by exposure to extreme sunshine during the summer season contributed to the extreme acidity of the lakes. This shows that freshwater sediments may be sensitive to acidification after aeration under field conditions where sulphate concentrations may increase due to the oxidation of iron sulphides. The desiccation and subsequent oxidation of FeS_2 with its consequent acidification of the lakes of Capel Wetlands Centre provides important reference information that should be taken into consideration when any management decisions are to be taken. Under reduced conditions, metal sulphides formed are oxidized due to the desiccation with the metals been bound to insoluble carbonates of hydroxides (Drever 1997). At the drop of pH below 4.5, there is a complete dissolution of all carbonates resulting in the adsorption to Fe oxide as the dominant mechanism (Lucassen *et al.* 2002).

In conclusion, the acidification of the lakes of Capel Wetlands Centre might have been triggered by climate variation, which might have contributed to the decline in rainfall and subsequently the drawdown of the groundwater table and series of prolonged dry periods that exposed the sulphur-rich lake sediments to oxidation. Extreme acidity of lakes can be improved by liming (Mench *et al.* 1994). However, the process of liming may lower the bioavailability of metals and thus extend the time that may be required for phytoremediation (Ernst 2005). Supplying alkaline groundwater can also be a solution to lake acidification (Roelofs *et al.* 2002). This can also lead to increased nitrification rates thus causing a shift in phytoplankton, zooplankton and fish communities. Nevertheless, there is the possibility of eutrophication as a result of high phosphate loading.

In conclusion, *N. congesta* can serve as effective tool for the phycoremediation of the sand mine-void wetlands at Capel, Western Australia due to the reasons presented above.

Recommendations

- Further studies on the taxonomy and morphological features of *N. congesta* population at Capel in comparison with populations elsewhere in Western Australia could be pursued to ascertain any further variations that exist within this species. In this case DNA analysis and chromosome counts could be used to sort out the taxonomy.

- Further studies could analyse the mechanism of metal sequestration by the mucilage and hyperaccumulation by the thallus. Chemical analysis of the mucilage could be done in order to possibly elucidate its molecular structure. This may show probable actively charged sites that may have contributed to the high sequestration of metal ions.

- Should the discharge of wastewater into the lakes begin according to the proposed plan, the health of the charophytes should be monitored

regularly for possible detrimental impacts due to increase in nutrient levels.

- Currently, little is known about the critical chemical processes that take place in the lakes such as organic matter sedimentation and phosphate co-precipitation as well as the response(s) to changes in iron, sulphur and hydrogen ion loading. Therefore, more research is needed to determine chemical interactions and various processes that are involved in the generation of lake acidity.

Glossary

Adopted after Bryant and Stewart (2002), Casanova (1993), García (2001) and García (2002).

Adaxial: Structure facing away from the main axe.

Accessory branchlets: Any extra branchlet at the whorl of branchlet. They can be in 1-2 rows (i.e. *Nitella hyalina*) or undivided (i.e. *Tolypella*).

Adaxial: Structure facing towards the main axe.

Antheridium: Male reproductive structure, globular, can be octoscutate (with 8 shields) or tetrascutate (with 4 shields); with position lateral on the branchlet nodes, rarely at base of branchlet whorls, or terminal as in *Nitella* sp.

Axe: Stem of a charophyte, with nodes (short cells) and internodes (long cells), notional of 'indefinite' growth.

Branch: A new 'plant' growing from a whorl of branchlets, with nodes and internodes.

Branchlets: Lateral ramifications of 'definite' growth, originated from the peripheral axial node cells. In Tribe Chareae they are monopodial; differentiated in segments, end segment and end cell. In Tribe Nitelleae, they are monopodial or divided in ramifications of increasing order; with undifferentiated or differentiated furcated style; the last furcations are called dactyls.

Bract-cells: Unicellular process at the branchlet nodes, differentiated by their position on the branchlet as posteriors (at the back of the branchlet) or anterors (related with the reproductive structures); they can be unilateral or verticillate.

Bracteole: Unicellular process at the branchlet node, generally replacing the antheridia when it is not developed, under the oogonia; originated from the same node cell.

Bulbil: Structure related with the rhizoids as starch storage, globular or stellate, developed from a whorl of branchlets, with the ability to produce new plants.

Convolutions: Number of turns of spiral cells of the oogonium, counted on lateral view.

Corona or terminal corona: 3-4 cells at the end of the branchlet, being the sum of the end cell of the branchlet (short) plus the bract-cells.

Coronula: cells on top of the oogonium, cut at the upper part of the 5 sterile cells surrounding the oospore, which allows the entrance of the spermatozoid. They are formed by 5 cells in 1 tier in Tribe Chareae and 10 cells in two tiers in Tribe Nitelleae. The coronula can be divergent, convergent, spreading; the cell shape can be triangular, elliptical, rounded.

Cortical cells: Cells developed on the axe and branchlets, growing from the peripheral node cells at the whorl of branchlets. They grow up and down from the axe nodes. They are formed by primary cortical cells (cells which divide, producing short and long elements: The spines originate from a division on the short element), and secondary cells, that grow laterally from the short element of the primary cortical cells. Can be triplostichous, diplostichous or haplostichous, aulacanthous, tylacanthous or isostichous.

Dactyls: Ultimate rays of the branchlets in Nitelleae. The dactyls can be an arthrodactylous (only one cell) or arthrodactylous (2 cells: bicellulate or more than 2 cells: pluricellulate); macrodactylous (dactyls long) or brachydacylous (dactyls short). The end cell of a dactyl can be of similar diameter of the penultimate cell and then is allantoid, if it is much narrower is called tapering; end cell confluent (when the same diameter as the penultimate cell) or

mucronate (when are narrower than the penultimate cell). The dactyl apices can be blunt or rounded, apiculate, acuminate or acute.

Dioecious: Male and female reproductive structures are developed on separate thallus.

Diplostephanous: Stipulodes developed in two tiers.

Ecorticate: Lacking cortical cells.

Ectosporostine: External wall of the oospore or ornamented wall, produced from the adaxial wall of the spiral cells after the zygote is produced.

End cell of the branchlet: Last cell cut at the end of the branchlet; it may be reduced, long, acute, allantoid or mucronate.

Flange: Extension of the intercellular crest between striae, usually more developed in Tribe Nitelleae.

Gyrogonite: Calcareous remains produced after fertilization as an intra cellular calcification of the spiral cells and the sterile cell of the oogonium. It is the more common fossil remains. It can be present or may not be developed.

Haplostephanous: Stipulodes in one series, usually the upper tier developed.

Heads: terminal part of the charophytes where the whorls of branchlets are short, usually tuft, probably protecting more effectively the reproductive structures. They are much developed in Tribe Nitelleae.

Heteroclemous: branches where they are differentiated in more than 1 series.

Homoclemous: Branches in one series.

Internodes: Part of the axe between axial nodes. They can be a few mm long to 400 mm long.

Monoecious: Male and female reproductive structures are developed on the same thallus.

Monopodial: When central axe occurs at 2 or more nodes, usually referred to branchlets with a main axe of growth.

Mucus: A jelly-like sudstance produced at the apical shoot of some species of *Nitella*.

Octoscutate: Antheridia with 8 shields.

Oogonium: Female reproductive structure, comprising the pedicel cell, node cell, sterile cells of the oogonia, oosphere/oospore, spiral cells and coronulla.

Oosphere or egg cell: Female reproductive cell that develops inside the oogonium.

Oospore: Zygote surrounded by resistant organic walls called endosporostine and ectosporostine; the external which can be highly ornamented. They can be terete, square/rectangular or compressed in cross section.

Ornamentation: The textual pattern developed on the ectosporostine. It is usually simple in Chareae and *Tolypella* but more complex in *Nitella*.

Peripheral cells: Cells developed in the nodes, around the central cells of the node. They develop at the axial nodes and branchlet nodes. The peripheral cells from the axial nodes give origin to the branchlets, cortical cells, stipulodes: the peripheral cells from the branchlet nodes give origin to the reproductive structures, cortical cells, bract-cells.

Protonema: First product of germination of an oospore, together with the rhizoid. It is phototropic, growing upwards and having chlorophyll.

Rays: Each of the furcations of the branchlets in *Nitella*. Classified from 2^{nd}, 3^{rd}, 4^{th}, 5^{th} …..order. If the central ray is maintained, it is called percurrent.

Rhizoids: These are very thin and transparent ramifications, useful for the charophyte attachment; product of the zygote germination together with the protonema; without chlorophyll and grow downwards.

Sister cell(s) of the oospore or sterile oogonial cell(s): These are 1-3 cells that are not viable; they are produced at the same time of the oosphere. They leave an impression at the base of the oospore and are able to calcify in species developing gyrogonites.

Spermatozoid: Reproductive male cell, able to swim, with a body and two unequal flagella. Its size is around 30 micrometres.

Spiral cells: The 5 sterile cells that turn to the left around the oosphere and sister cell(s) of the oospore, characteristic fixed for all modern genera. Some walls of the oospore and the gyrogonite are developed from them.

Stipulodes: Unicellular processes developed from axial nodal peripheral cells, substending the branchlets. They can develop opposite or alternate in relation with the branchlets.

Striae: Each one of the fossa between two intercellular crests as looking at the oospores in lateral view.

Tetrascutate: Antheridia with 4 shields.

Zygote: Product of fertilization of oospore by the spermatozoid. It is the only 2n structure in charophytes.

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APPENDICES

Appendix 1 ANOVA between height, number of nodes and number of branches of *N. congesta* cultures in the laboratory.

ANOVA

Height

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5371.953	2	2685.977	1.822	.186
Within Groups	30958.564	21	1474.217		
Total	36330.518	23			

ANOVA

Node

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	76.235	2	38.117	2.310	.124
Within Groups	346.498	21	16.500		
Total	422.732	23			

ANOVA

Branches

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.364	2	.682	.962	.398
Within Groups	14.886	21	.709		
Total	16.250	23			

Appendix 2 ANOVA between height, number of nodes and number of branches of *N. congesta* growth studied in the field.

ANOVA

Height

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22894.681	2	11447.341	1.686	.209
Within Groups	142598.967	21	6790.427		
Total	165493.648	23			

ANOVA

Node

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	141.119	2	70.560	4.776	.022
Within Groups	265.926	18	14.774		
Total	407.045	20			

ANOVA

Branches

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	35.023	2	17.511	4.598	.024
Within Groups	68.551	18	3.808		
Total	103.574	20			

Appendix 3 Regression analysis of water depth and mean height per shoot of *N. congesta* in Plover South Lake Site 1.

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Depth[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Height

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.987[a]	.974	.967	10.68638

a. Predictors: (Constant), Depth

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	16915.998	1	16915.998	148.128	.000[a]
	Residual	456.795	4	114.199		
	Total	17372.793	5			

a. Predictors: (Constant), Depth

b. Dependent Variable: Height

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-30.414	20.799		-1.462	.217
	Depth	3.309	.272	.987	12.171	.000

a. Dependent Variable: Height

Appendix 4 Regression analysis of water depth and mean height per shoot of *N. congesta* in Plover South Lake Site 2.

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Depth[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: Height

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.949[a]	.900	.875	16.43425

a. Predictors: (Constant), Depth

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	9685.949	1	9685.949	35.863	.004[a]
	Residual	1080.338	4	270.084		
	Total	10766.287	5			

a. Predictors: (Constant), Depth

b. Dependent Variable: Height

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	35.377	23.668		1.495	.209
	Depth	3.084	.515	.949	5.989	.004

a. Dependent Variable: Height

Appendix 5 One-way ANOVA and Tukey HSD test comparing mean metal concentration of treatments at 5% significance level.

ANOVA

Concentration

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18401.638	3	6133.879	41.270	.000
Within Groups	1783.552	12	148.629		
Total	20185.191	15			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Concentration

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-59.25750*	8.62060	.000	-84.8512	-33.6638
	3.00	12.50750	8.62060	.494	-13.0862	38.1012
	4.00	31.47750*	8.62060	.015	5.8838	57.0712
2.00	1.00	59.25750*	8.62060	.000	33.6638	84.8512
	3.00	71.76500*	8.62060	.000	46.1713	97.3587
	4.00	90.73500*	8.62060	.000	65.1413	116.3287
3.00	1.00	-12.50750	8.62060	.494	-38.1012	13.0862
	2.00	-71.76500*	8.62060	.000	-97.3587	-46.1713
	4.00	18.97000	8.62060	.178	-6.6237	44.5637
4.00	1.00	-31.47750*	8.62060	.015	-57.0712	-5.8838
	2.00	-90.73500*	8.62060	.000	-116.3287	-65.1413
	3.00	-18.97000	8.62060	.178	-44.5637	6.6237

*. The mean difference is significant at the .05 level.

Homogeneous Subsets

Concentration

Tukey HSD[a]

Group	N	Subset for alpha = .05		
		1	2	3
4.00	4	36.9850		
3.00	4	55.9550	55.9550	
1.00	4		68.4625	
2.00	4			127.7200
Sig.		.178	.494	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Appendix 6 One-way ANOVA comparing evenness, species richness and diversity of macroinvertebrates sampled from sites in Nitella and Higgins Lakes

ANOVA

Evenness

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.073	2	.037	5.127	.033
Within Groups	.064	9	.007		
Total	.138	11			

ANOVA

SpeciesRichness

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	333.167	2	166.583	.588	.576
Within Groups	2551.500	9	283.500		
Total	2884.667	11			

ANOVA

Diversity

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.337	2	.169	13.326	.002
Within Groups	.114	9	.013		
Total	.451	11			

Student t-test comparing the biodiversity differences between two sample sites.

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Diversity	10	.5150	.21986	.06953

One-Sample Test

	Test Value = 0					
					95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Diversity	7.407	9	.000	.51500	.3577	.6723

Appendix 7 Proximity Matrix for sites of Nitella and Higgins Lakes

Case	Absolute Squared Euclidean Distance									
	N4	N8	H2	N2	N6	H1	N1	N3	N5	N7
N4	.000	.010	.014	.058	.073	.168	.260	.260	.325	.423
N8	.010	.000	.000	.020	.029	.096	.168	.168	.221	.303
H2	.014	.000	.000	.014	.023	.084	.152	.152	.203	.281
N2	.058	.020	.014	.000	.001	.029	.073	.073	.109	.168
N6	.073	.029	.023	.001	.000	.020	.058	.058	.090	.144
H1	.168	.096	.084	.029	.020	.000	.010	.010	.026	.058
N1	.260	.168	.152	.073	.058	.010	.000	.000	.004	.020
N3	.260	.168	.152	.073	.058	.010	.000	.000	.004	.020
N5	.325	.221	.203	.109	.090	.026	.004	.004	.000	.006
N7	.423	.303	.281	.168	.144	.058	.020	.020	.006	.000

Appendix 8 One-way ANOVA comparing mean height and internode distance of *N. congesta* individuals in the nutrient enrichment experiment at 5% significance level.

ANOVA

Height

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12490.359	2	6245.180	54.395	.000
Within Groups	1033.302	9	114.811		
Total	13523.661	11			

ANOVA

ID

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	774.955	2	387.478	81.281	.000
Within Groups	42.904	9	4.767		
Total	817.860	11			

ID is Internode distance

ANOVA

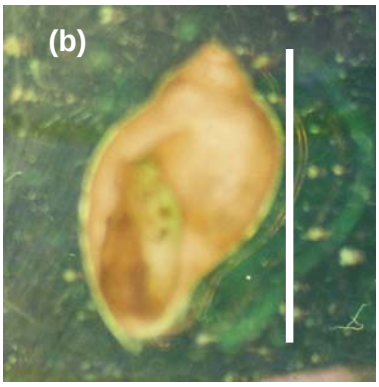
Nodes

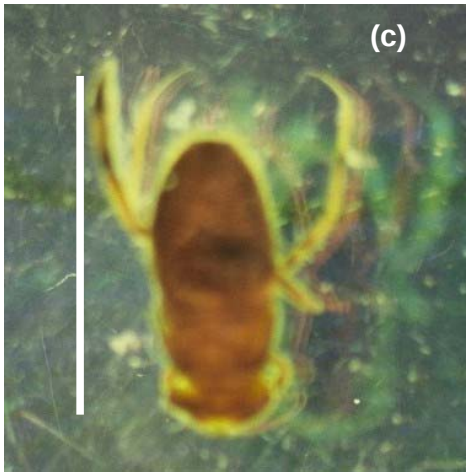
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	138.166	2	69.083	2.414	.132
Within Groups	343.462	12	28.622		
Total	481.628	14			

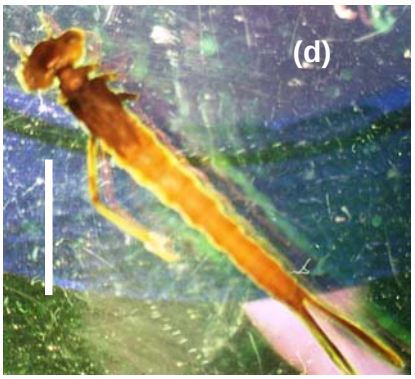
Appendix 9 Sex ratio of male and female plants of *N. congesta* counted in enrichment experiment.

Treatment	Mesotrophic		Eutrophic		Control	
	Male	Female	Male	Female	Male	Female
1	31	36	0	0	18	26
2	52	69	0	0	42	57
3	42	60	0	0	38	36
4	14	28	0	0	40	47
Mean	34.75	48.25	0.00	0.00	34.50	41.50
sd	±16.28	±19.40	±0.00	±0.00	±11.12	±13.43

Appendix 10 Macroinvertebrates identified within Capel Wetlands







(a) *Orthetrum caledonicum* (nymph) Scale bar: 5 mm

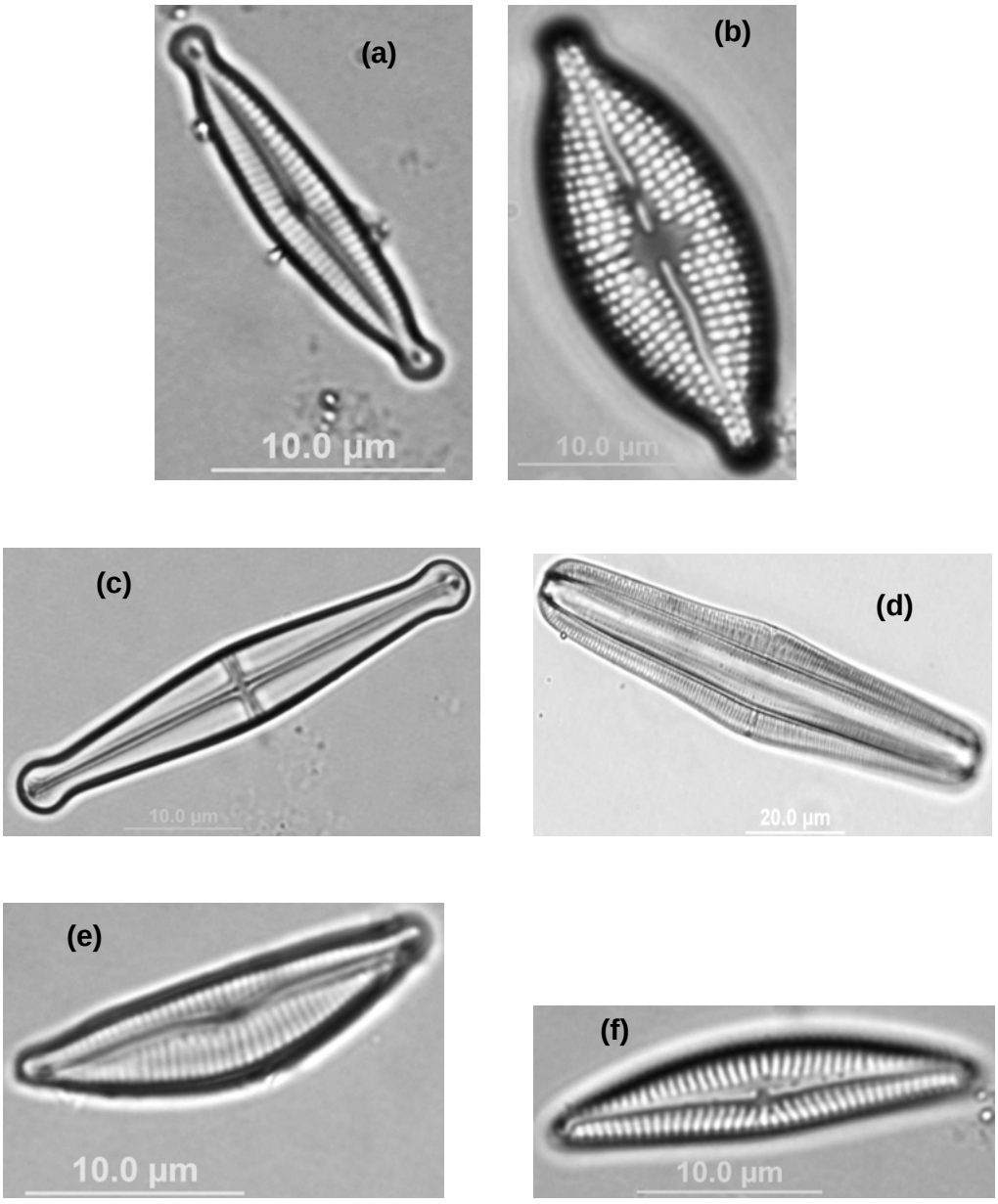
(b) *Lymnaedidae* sp. Scale bar: 8 mm

(c) Corixidae sp. Scale bar : 4 mm

(d) *Orthetrum caledonicum* (larva) Scale bar: 18 mm

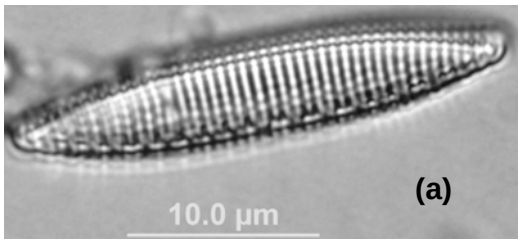
Appendix 11 Diatom species identified in mucilage of *N. congesta* and standing water of lakes at Capel Wetlands

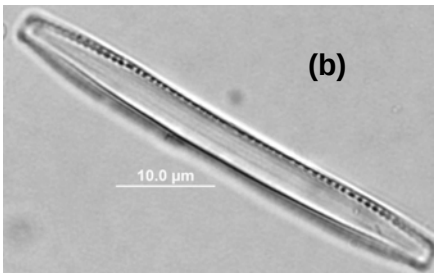
PLATE 1

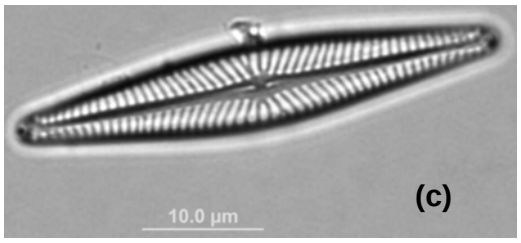


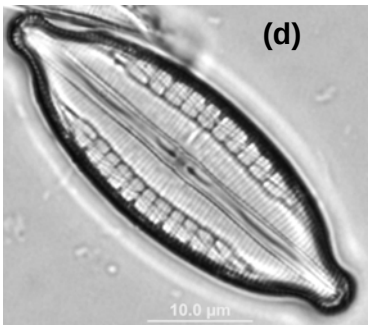
(e) Encynopsis microcephala
(f) Mastogloia tuscula
(g) Stauroneis pachycephala
(h) Rhopalodia novae-zealandae
(i) Encyonema minutum
(j) Navicula pusilla

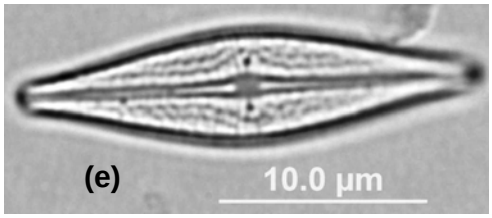
PLATE 2

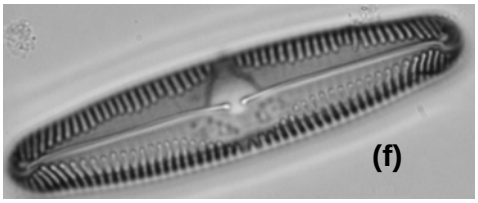


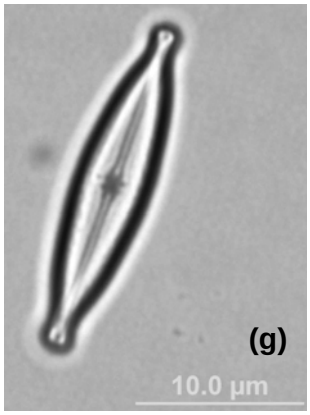






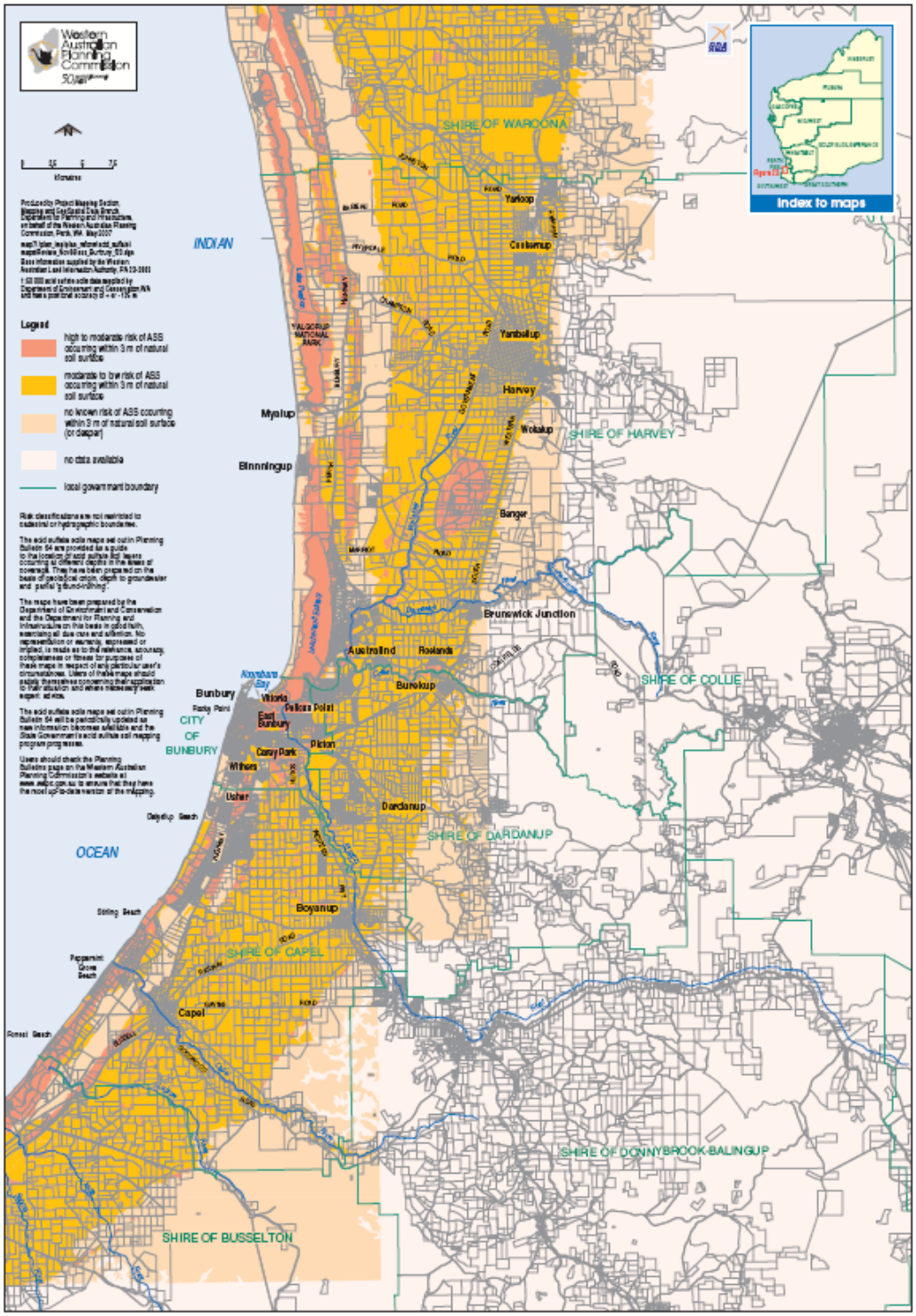






(a) *Nitzschia amphibia (valve)*
(b) *Nitzschia amphibian(girdle view)*
(c) *Navicula sp.*
(d) *Mastogloia elliptica*
(e) *Brachysira brebissonii*
(f) *Pinnularia microstaun*
(g) *Brachysira vitrea*

Appendix 12 Map of Greater Bunbury Region showing acid sulfate soils



Western Australia Planning Commission (2007).