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## **An ecological characterisation of a shallow seasonal claypan wetland, Southwestern Australia**

Nakisa Shahrestani  
*Edith Cowan University*

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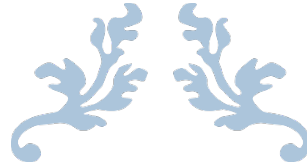
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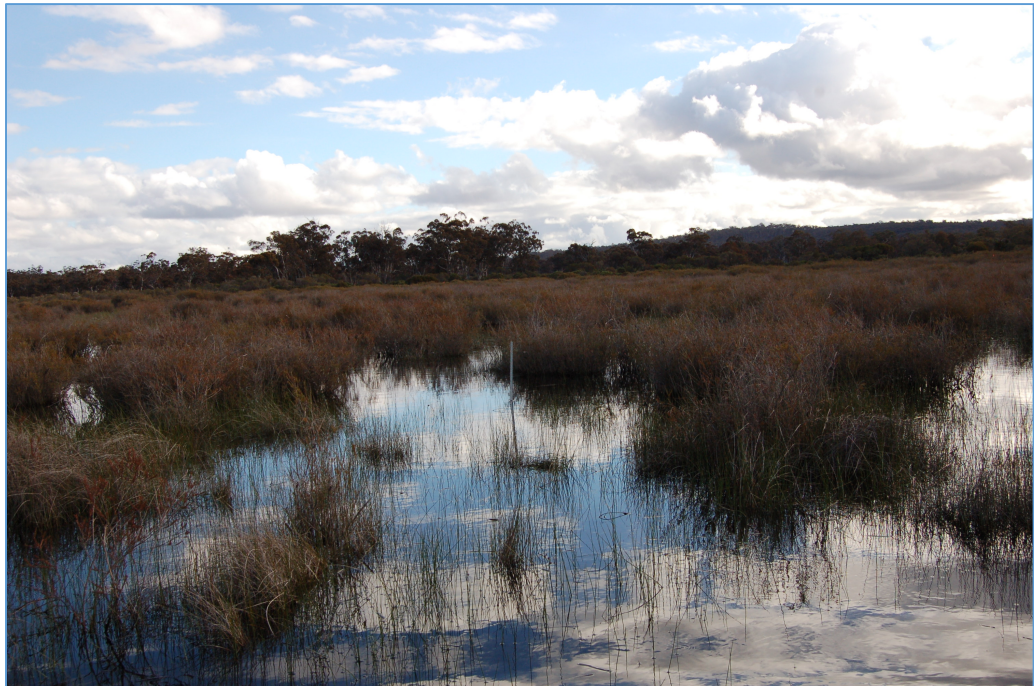


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# AN ECOLOGICAL CHARACTERISATION OF A SHALLOW SEASONAL CLAYPAN WETLAND, SOUTHWESTERN AUSTRALIA

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A thesis submitted in partial fulfilment of the requirements for  
the degree of Master of Science (Environmental Management).

Centre for Ecosystem Management  
School of Science

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# Abstract

Perched, seasonal claypans of southwestern Australia are poorly understood in terms of their ecological character, such as relationship between hydrology and their biota. An example is Little Darkin Swamp, located on the Darling Plateau in southwestern Australia. The overall aim of this thesis was to describe its ecological character, to understand what drives this claypan system and how its ephemeral nature affects wetland processes and functions.

This study first comprised a detailed characterisation of the wetland's attributes, following the geomorphic-hydrological approach proposed by Semeniuk and Semeniuk (2011). This revealed that its hydrology is highly dependent on rainfall, that it is an endorheic system, with a basin that is structurally spatially heterogeneous with distinct vegetation zones, and that surface waters have nutrient levels that are similar to oligotrophic systems. These features make it similar to other claypan wetlands of southwestern Australia and vernal pools of California, USA.

Continuous high-frequency dissolved oxygen data during the hydroperiod showed that there are large temporal and spatial variations in ecosystem metabolism, and that the trophic status of the wetland is finely balanced, fluctuating between auto- and heterotrophy due to its ephemeral nature. Due to its oligotrophic nature, rates of gross primary production (GPP) and respiration (R) were overall low, and the wetland was overall slightly autotrophic over the study period. Furthermore, dual isotope analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of sources and consumers revealed that aquatic macrophytes make a higher contribution to the food web compared to other sampled sources. However, the food web was also supported by sources of carbon that were not sampled, probably filamentous algae and methanotrophic bacteria.

Experimental re-hydration of dried sediments emphasized that the seasonality of the water regime, and the shallow bathymetry of the basin, influences organic matter content, nutrient levels, oxygen consumption, plant growth and macroinvertebrate richness, differently between the centre of the wetland versus the edges. These results confirmed that there are at least two distinct zones in the wetland in terms of biotic response following rewetting, caused by the differences in duration and frequency of inundation of the sediments.

The outcomes of this study showed that the ephemerality (i.e. seasonal drying and wetting) of Little Darkin Swamp drives important internal ecosystem processes, such as ecosystem metabolism, nutrient cycling, and primary production, which in turn determine the trophic status and distribution of biotic communities in the wetland. Therefore, any changes to the

hydrological regime will greatly affect how these system functions and can potentially negatively impact such unique shallow, seasonal perched systems of southwestern Australia.

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# TABLE OF CONTENTS

## **Chapter 1 GENERAL INTRODUCTION**

1.1 The ecological character of wetlands .....	1
1.2 A mystery wetland with potential significance.....	2
1.3 Description of central ecosystem components and processes.....	2
1.4 Clay pans of southwestern Australia .....	4
1.5 This thesis .....	5

## **Chapter 2 WETLAND CHARACTERISATION AND CONDITION**

2.1 Introduction .....	6
2.2 Study site .....	9
2.3 Methods and Materials .....	11
2.3.1 Elevation survey .....	11
2.3.2 Water quality and hydrology.....	12
2.3.3 Soil texture analysis.....	15
2.3.4 Vegetation survey .....	15
2.3.5 Biota .....	16
2.3.5.1 Macroinvertebrates.....	16
2.3.5.2 Plant list.....	16
2.4 Results .....	17
2.4.1 Basin depth and size.....	17
2.4.2 Climate and Hydrology .....	17
2.4.3 Soil and substrate.....	19
2.4.4 Vegetation zones.....	20
2.4.5 Community structures of vegetation zones .....	22
2.4.5 Water quality.....	24
2.4.6 Nutrients, colour and chlorophyll- <i>a</i> .....	27
2.4.7 Biodiversity.....	29
2.4.7.1 Macroinvertebrate richness .....	30
2.4.7.2 Plant diversity and richness.....	31
2.5 Discussion .....	33
Conclusion .....	41

## **Chapter 3 ECOSYSTEM METABOLISM**

3.1 Introduction .....	43
3.2 Methods .....	46

3.2.1 Sampling design.....	46
3.2.2 Physical and chemical characteristics.....	46
3.2.2 Ecosystem metabolism.....	47
3.2.2.1 Field measurements .....	48
3.2.2.2 Data analysis.....	48
3.2.3 Food web sampling and analysis .....	49
3.2.3.1 Laboratory sample preparations .....	50
3.2.3.2 Stable isotope analysis .....	51
3.2.3.3 Data analysis.....	52
<b>3.3 Results .....</b>	<b>52</b>
3.3.1 Physical and chemical characteristics.....	52
3.3.2 Ecosystem metabolism.....	53
3.3.2.1 Temporal dynamics of DO concentration.....	53
3.3.2.2 Temporal dynamics of metabolism .....	54
3.3.2 Carbon sources sustaining the aquatic food web .....	59
3.3.2.1 Trophic levels.....	59
3.3.2.2 Basal carbon sources for the macroinvertebrates .....	61
<b>3.4 Discussion .....</b>	<b>62</b>
Conclusion .....	67
<b>Chapter 4 BIOTIC RESPONSE FOLLOWING SEDIMENT RE-HYDRATION</b>	
<b>4.1 Introduction .....</b>	<b>68</b>
<b>4.2 Methods and Materials .....</b>	<b>70</b>
4.2.1 Study site and sampling design .....	70
4.2.2 Sample collection and preparation .....	71
4.2.3 Experimental set-up .....	72
4.2.4 Physicochemical measurements .....	73
4.2.5 Nutrients, colour, organic matter and biomass production.....	74
4.2.6 Macroinvertebrate sampling.....	75
4.2.7 Analysis.....	75
<b>4.3 Results .....</b>	<b>77</b>
4.3.1 Overall physicochemical response following re-hydration of sediments.....	77
4.3.2 Diurnal physicochemical response without oxygen addition.....	79
4.3.3 Differences in periphyton production, plant growth, nutrients, colour and organic matter .....	80
4.3.4 Overall differences in environmental response between the zones.....	81
4.3.5 Macroinvertebrate emergence following re-hydration .....	84

4.3.6 Influence of environmental parameters on the overall distribution of macroinvertebrate assemblages .....	85
4.4 Discussion .....	<b>87</b>
4.4.1 Conclusion .....	91
<b>Chapter 5 SYNTHESIS</b>	
5.1 Characterisation and classification of Little Darkin Swamp.....	<b>93</b>
5.2 Trophic status and temporal dynamics and spatial variability in metabolism .....	<b>95</b>
5.3 Functional zonation of wetland: Spatial variability in response to sediment re-hydration .	<b>96</b>
5.4 Possible future projections for Little Darkin Swamp.....	<b>97</b>
5.5 Management implications .....	<b>98</b>
<b>Appendix I – Macroinvertebrate richness at claypans of southwestern Australia.....</b>	<b>112</b>
<b>Appendix II – Ecosystem metabolism winter 2015 and food chain .....</b>	<b>113</b>
<b>APPENDIX III- Sediment re-hydration experiment additional data .....</b>	<b>117</b>





# Chapter 1. General Introduction

## 1.1 The ecological character of wetlands

To maintain and protect the values of internationally and nationally important wetlands, an understanding and description of their ecological character is necessary. Ecological character is defined by the Ramsar Convention as *“the combination of the ecosystem components, processes, benefits and services that characterise the wetland at a given point in time”* (Department of the Environment Water Heritage and the Arts, 2008 pp.4). Captured in this definition is the complexity of wetlands as systems and the close relationship between the ecological components, their processes or interactions and the benefits and services they provide. Changes exceeding the natural variations to the ecological character of any wetland suggest that negative impacts are degrading natural processes such as ecological, biological and hydrological functions of the wetland (Department of the Environment Water Heritage and the Arts, 2008). Therefore, a description of the ecological character of a wetland provides a baseline of the condition at a specific point in time, and can be used to assess changes to its condition.

Ecosystem components can be described as physical, chemical and biological components of a wetland, such as physical form, wetland soils, water physicochemistry and biota. Ecosystem processes are the dynamic forces within the wetland, including climate, geomorphology, hydrology, energy and nutrient cycles, physical processes and species interactions (Department of the Environment Water Heritage and the Arts, 2008). The benefits of a wetland are defined as the economic, social and cultural benefits that humans receive. These benefits often rely on underlying ecological components and processes of the wetland, emphasizing their importance. Although a comprehensive ecological character description is the ideal goal, resources often limit the extent of research and data collection possible. Therefore, a strategic selection of the most important components and processes is sometimes necessary when describing ecological character.

In addition to providing a baseline description, an ecological characterisation can also be used by managers to assess likely impacts of threats on a wetland’s ecological character; to assist the development of a management plan; and help evaluate outcomes of monitoring and management efforts. In the process, quantitative benchmarks are established to inform managers of the acceptable variation in ecosystem components, processes, benefits and services without changing the ecological character of the wetland (Department of the Environment Water Heritage and the Arts, 2008). This further aids in identifying indicators and targets for monitoring and management.

Furthermore, Ramsar's ecological character framework can also be used to identify wetlands of international or national importance, by meeting the criteria outlined for either Ramsar wetlands (Ramsar Convention Secretariat, 2013) or the Directory of Important Wetlands in Australia (DIWA) (Environment Australia, 2001). A description of a wetland's ecological characterisation will aid in determining whether it meets these criteria.

## **1.2 A mystery wetland with potential significance**

The Western Australian Department of Parks and Wildlife have recently identified a wetland, namely Little Darkin Swamp in the Wandoo National Park (previously Nature Reserve) on the Darling Escarpment, southwestern Australia. There is currently little information available about the wetland, such as what type of system it is, how it could be classified, or any description of its ecological character. The wetland is broadly characterised as a seasonal clay pan with dense vegetation throughout. Prior to the development of a management plan for this wetland, a description of its ecological character is needed, and thus, the broad aim of this thesis is to provide that ecological characterisation for Little Darkin Swamp. However, a complete ecological character description was out of the scope of this Master's project, and therefore the focus has been placed on investigating in depth particular components and processes which are considered to be most important to the wetland's overall functioning. This includes its ecosystem components, and detailed description of some of the ecological processes such as biotic response to hydrology, energy balance and nutrient dynamics.

## **1.3 Description of central ecosystem components and processes**

One way of giving a comprehensive description of wetland ecosystem components is to adopt the approaches used in a classification system. Systematic identification of the different types of wetlands is important in order to truly capture diversity, rationally make comparisons and enhance management (Semeniuk & Semeniuk, 1997). Classification of wetlands provides a way of understanding their diversity and function, allows comparison within a region, country or worldwide, and provides a rational basis for choosing representative areas for reservation. Many methods have been proposed for wetland categorisation, all of which incorporate defining wetland attributes (e.g. Cowardin et al., 1979; Tiner, 1999; Ramsar Convention Secretariat, 2010; Richardson & Vepraskas, 2001). The classification system proposed by Semeniuk and Semeniuk (2011), namely the geomorphic-hydrologic approach, is widely used in Western Australia. This classification system includes a description of the wetland's hydrological regime, which affects many of the internal ecosystem processes.

The hydrological regime (i.e. magnitude, frequency, duration and timing of inundation) of any wetland controls the biotic communities and ecosystem processes within it (Langhans & Tockner, 2006). Therefore, it is an important ecosystem process that can provide great insight into the ecological character of a wetland. Particularly for seasonal or ephemeral wetlands, drying and rewetting of sediments can influence the water chemistry, which in turn affects the biota of wetlands (Sommer, 2006). Understanding how wetting and drying affects the biota, and how the biota adapt to these conditions (e.g. Strachan, Chester, & Robson, 2015) is important to help predict how changes in hydrology can alter the ecological character of a wetland. In addition, the hydrological regime (and particularly drying and re-wetting) affects internal processes such as nutrient cycling and the flow of materials within the system (Pettit et al., 2011), which can in turn impact a system's metabolism and ultimately trophic status.

Nutrient and energy cycles are important internal ecosystem processes (Boulton et al., 2014), and an understanding of their dynamics is important part of describing the ecological character of a wetland. Nutrients control the rate of vital ecosystem processes such as primary production (Boulton et al., 2014). Light, photosynthesis and nutrient limitation are all linked to support autotrophic (primary producers) and heterotrophic (microbial, invertebrate and vertebrate) production in aquatic systems. The flow of energy and cycling of matter can thus be divided into two pathways: the autotrophic pathway (producer-consumer) and the heterotrophic pathway (detritus-decomposer-consumer) (Boulton et al., 2014). The autotrophic pathway is fuelled by photosynthesis via producers, whereas the heterotrophic pathway is fuelled by detritus and decomposers (Bunn & Davies, 2007). The trophic status of a wetland can be determined by measuring the metabolism of a wetland by comparing the ratio of primary production to respiration (Staehr et al., 2010). This provides important information on the processing of organic matter, nutrient cycles, and energy transfer in aquatic ecosystems.

A description of the ecological character of Little Darkin Swamp will be aided by comparing these ecosystem components and processes to those of close ecological analogues where more detailed information is available. The management and conservation will be facilitated by an understanding of wetland functioning in this context. We propose that Little Darkin Swamp could be a representative of the rare claypan wetlands occurring in southwestern Australia, with analogues such as claypans of the Drummond Nature Reserve in southwestern Australia (Pinder, Quinlan, Cale, & Shiel, 2013) and "vernal pools" of California, US (Keeley & Zedler, 1998).

## 1.4 Claypans of southwestern Australia

The claypans of southwestern Australia have relatively productive soils and many have been cleared and drained after European settlement and used for agriculture, while others have been mined for clay for building materials (Gibson, 2010). There are currently a total of 114 occurrences of these claypan communities listed in 50 separate locations in southwestern Australia, and they cover a total of approximately 909 ha (Brown et al., 2015). The communities are very fragmented and small, with over half of them under 10 ha, and in significant bushland such as Ellenbrook, Forrestdale Lake, Moore River, Byrd Swamp, Austin Bay, Drummond and Kooljerrenup Nature Reserves, Wandoo National Park, and Jandakot Regional Park (Brown et al., 2015).

In 1994, Gibson et al. defined four floristic community types across the Swan Coastal Plain (SCP) occurring on clay substrates, with variation caused by a number of factors such as substrate and rainfall (Brown et al., 2015). These community types were 1) Herb rich saline shrublands in clay pans (SCP community type 8, SCP07), 2) Herb rich shrublands in clay pans (SCP08), 3) Dense shrublands on clay flats (SCP09), and 4) Shrublands on dry clay flats (SCP10a). Some years later, vegetation data for the seasonal clay-based wetlands were analysed by Gibson et al. (2005), and another clay pan type was identified. This fifth community type was called 5) Clay pans with mid dense shrublands of *Melaleuca lateritia* over herbs.

Due to their ongoing loss and conservation significance, all five claypan community types have been ranked as critically endangered communities under the Environment Protection and Biodiversity Conservation Act (EPBC Act) in 2012 (Threatened Species Scientific Committee, 2012). In addition to all being Threatened Ecological Communities (TEC), the five claypan community types have different conservation statuses. Claypan type 5, with mid dense shrublands of *Melaleuca lateritia* over herbs, was included on the Priority Ecological Community list as Priority 1 for WA in 2006 by the Department of Parks and Wildlife (DPaW) (Brown et al., 2015). Priority 1 TEC are defined by DPaW as 'poorly known ecological communities', known from very few occurrences with very restricted distribution ( $\leq 5$  occurrences or total area of  $\leq 100$ ha) which are under threat due to limited extent or located on lands under immediate threat. This means that the communities are well known from one or more localities but do not meet adequate survey requirements, or are not well defined, but are threatened by recognised processes across their range (Department of Environment and Conservation, 2010). Due to their status and current threats, an Interim Recovery Plan (IRP) was developed for 'Claypans of the Swan Coastal Plain' by DPaW in September 2015 (Brown et al., 2015).

## 1.5 This thesis

A lack of recognition of these clay pan wetlands and their value, partly attributed to the lack of knowledge about them, and the threats posed by changing climate and a resultant shift in hydrological conditions, underlies this research. Understanding of the ecological processes and functions of these perched, seasonal clay pans of southwestern Australia will help characterize, classify and manage them. Thus, the overall aim of this thesis is to understand what drives these claypan systems and how their ephemeral nature affects wetland processes and functions.

The focus of this thesis will be Little Darkin Swamp; a claypan wetland located in the Wandoo National Park in wandoo (*Eucalyptus wandoo*) woodland on the Darling escarpment. This wetland has been selected for its unusual character and as a possible representative of the claypan Threatened Ecological Community (TEC) type 'Claypans with mid dense shrublands of *Melaleuca lateritia* over herbs'.

A specific research objective of this project is to spatially and temporally characterise Little Darkin Swamp in terms of descriptors of landform, water and vegetation such as water quality and dynamics, hydrology, fauna and flora communities in relation to ecosystem processes, and to compare these characteristics to other claypan wetlands of southwestern Australia and "vernal pools" of California. In doing so, the research will be able to contribute to the classificatory and inventory status for these types of wetlands. This work will be reported in Chapter 2 of this thesis. A second research objective is to examine the temporal and spatial dynamics of ecosystem metabolism in Little Darkin Swamp, to determine whether the system is autotrophic or heterotrophic, and how this changes over the hydroperiod. In addition, I will examine the sources of carbon fuelling the food web and the trophic status of the biota within the wetland. This will provide information on the internal energy pathways of the wetland and the reveal what type of carbon is fuelling the aquatic food web in the system. This will be the topic of Chapter 3. A final research objective is to characterise the hydrochemical and biotic responses of dry sediments to re-wetting at Little Darkin Swamp, and to determine the specific influence on different inundation zones to re-hydration via a laboratory re-wetting experiment. This will be described in Chapter 4, and will contribute to an understanding of how duration and frequency of inundation (i.e. hydrological regime) impacts the productivity of the wetland.

Together these three pieces of work will provide an ecological characterisation of Little Darkin Swamp to be able to better manage and conserve this type of wetland. This synthesis will be undertaken in the final chapter of the thesis.

# Chapter 2. Wetland characterisation and condition

## 2.1 Introduction

Definition of wetland boundaries based on attributes (landform/geomorphology, hydrology and biotic features and processes) and values are the basis for wetland classification (Semeniuk & Semeniuk, 2011). The geologic/geomorphic and hydrological components of wetlands are less dynamic and changeable in the long term, therefore landform and water are used to classify wetlands at the primary level, while biological attributes are used for further categorisation of wetland types and addition of more detail at higher classification levels. For non-emergent wetlands, which are terrain conforming wetlands occurring in landscapes with sufficient water to maintain them, Semeniuk and Semeniuk (2011) list several wetland components used for classification, and separate these into descriptors of landform, water and vegetation. The descriptors of landform are: cross-sectional shape, size, plan shape, substrate/soils and stratigraphy (lithology), including thickness and layering. The descriptors of water are: water permanence, salinity, consistency of water salinity, turbidity/colour, nutrient-enrichment, other specific hydrochemical features, water source, water depth and rate of movement. Descriptors of vegetation were described by Semeniuk, Semeniuk, Cresswell and Marchant (1990) and are: scale of vegetation complex, areal extent and pattern of distribution of vegetation cover over the wetland, internal organisation in terms of dominant vegetation structure or range of structural types in zones, and details of structure and floristics of vegetation used in combination.

Little Darkin Swamp is a seasonal shallow and vegetated clay-basin, located in the Wandoo National Park of southwestern Australia, and little is known about its wetland attributes. Such ephemeral claypan basins and clay flats of southwestern Australia, collectively known as claypans, are the most diverse wetland type of the region (Sim, 2012c). These wetlands generally contain a large number of local endemic plant species and a large range of geophytes and annual species flowering at different times during the hydroperiod. Claypan wetlands occur in water-gaining parts of the landscape where clay soils form an impervious layer close to the surface causing seasonal pooling of surface and shallow sub-surface water. There is generally no connection to groundwater in these clay pans, and therefore they rely only on rainfall and surface runoff to fill

(Brown et al., 2015). This type of wetland is classified by Keeley and Zedler, (1998 pp.2) as “vernal pools” which are described as *“precipitation-filled seasonal wetlands inundated during periods when temperature is sufficient for plant growth, followed by a brief waterlogged-terrestrial stage and culminating in extremely dry soil conditions of extended duration during the summer period”*. This definition was originally used for vernal pools of California, but many parallels have been drawn with seasonal claypans of Western Australia (Brown et al., 2015). These vernal pools correspond to and are synonymous with other wetlands described worldwide, such as ephemeral wetlands, vernal marshes, buffalo wallows, seasonal pools, and temporary waters.

The ephemeral clay-based wetlands are sources of significant biodiversity with concentrations of threatened and priority species and restricted communities across the Swan Coastal Plain and the surrounding Darling Escarpment in southwestern Australia. Little Darkin Swamp is one of five types of claypans described for the southwest that are distinguished primarily by floristic composition (Gibson et al., 2005). Across these five clay pan communities, 12 declared rare and 42 priority plant taxa have been listed. In addition, three critically endangered fauna species depend on these systems for parts of their life and breeding cycles (Brown et al., 2015).

Keeley and Zedler (1998, pp.2) have characterised vernal pools of California as having four stages in an annual cycle, which includes (i) a wetting phase, (ii) an aquatic or inundation phase, (iii) a waterlogged-terrestrial phase, and (iv) the drought phase. They classify vernal pools as seasonally flooded emergent wetlands of the palustrine system (Cowardin et al., 1979). Three factors, duration, timing and source of inundation, are common across all vernal pools, regardless of their origin, size, shape and species composition. These pools occur in most other Mediterranean climate regions, but according to Keeley and Zedler (1998) are best established in Chile and Western Australia. Alternatively, using the Semeniuk and Semeniuk (2011) wetland classification system, Little Darkin Swamp would be classified as a mesoscale (1000 x 1000m – 100 x 100m), freshwater, clay-based, shrub-covered sumpland which is seasonally inundated. Thus, the question that arises: do the wetland attributes of Little Darkin Swamp conform to claypans and/or vernal pools as described by Gibson et al. (2005) and Keeley and Zedler (1998), and do these attributes fit its classification under the Semeniuk and Semeniuk (2011) wetland classification system?

It is also not known whether the surface water characteristics of vernal pools are comparable to those of claypan wetlands in southwestern Australia, and specifically Little Darkin Swamp. In terms



of specific water quality attributes, vernal pools tend to have low nutrient levels and a water chemistry more similar to oligotrophic lacustrine habitats found at higher elevations and latitudes as a consequence of being rainfall-fed wetlands (Keeley & Zedler, 1998). The water conductivity is proportional to concentrations of the major ions, and is usually at the lower end of the range for aquatic systems. The low nutrient status of these wetlands result in unbuffered water with extreme diurnal changes in pH, dissolved CO<sub>2</sub> and O<sub>2</sub>. The unbuffered water further causes pH to be affected by the carbon dioxide- bicarbonate system, resulting in rises of 2-3 pH units over a few hours due to depletion of CO<sub>2</sub> (Keeley & Zedler, 1998). This makes regional pH comparisons difficult without the inclusion of time of sampling and diurnal time course. The high surface to volume ratio in these shallow basins also results in extreme diurnal changes in temperature (Rains, Fogg, Harter, Dahlgren, & Williamson, 2006).

The ecological functions of the biotic plant and animal assemblages occurring in claypans are mainly driven by wetland hydrology (Brown et al., 2015). The assemblages of flora occurring in a particular location are highly affected by the variations in depth and timing of inundation, which in turn explains the variation in community composition across its extent. For example, geophytes and annual flora that flower successively as the claypans dry, are responsible for the high species richness in these wetlands, rather than the perennial shrubs and herbs (Brown et al., 2015; Gibson et al., 2005). In addition, the aquatic diversity, community structure and ecosystem functioning are controlled to a large extent by the predictability, frequency and duration of inundation. The hydroperiod can directly impact the types of taxa able to inhabit seasonal wetlands due to limitations of the life cycle lengths, as well as by changing the relative importance of biotic interactions that structure community composition (Sim et al., 2013).

These freshwater systems in southwestern Australia are now facing many threats including clearing, grazing, hydrological change, weed invasion, damage by feral animals, altered fire regimes, disease, disturbances due to recreational activities and climate change (Brown et al., 2015). Despite being such valuable and threatened freshwater systems of southwestern Australia, there has been little research on their attributes, and they have not been fully characterised in terms of the descriptors used to classify wetlands. It is therefore increasingly important to establish how these wetlands function, and to monitor their condition in order to identify possible impacts of these threats, to improve management and ultimately prevent further loss of these vulnerable systems.

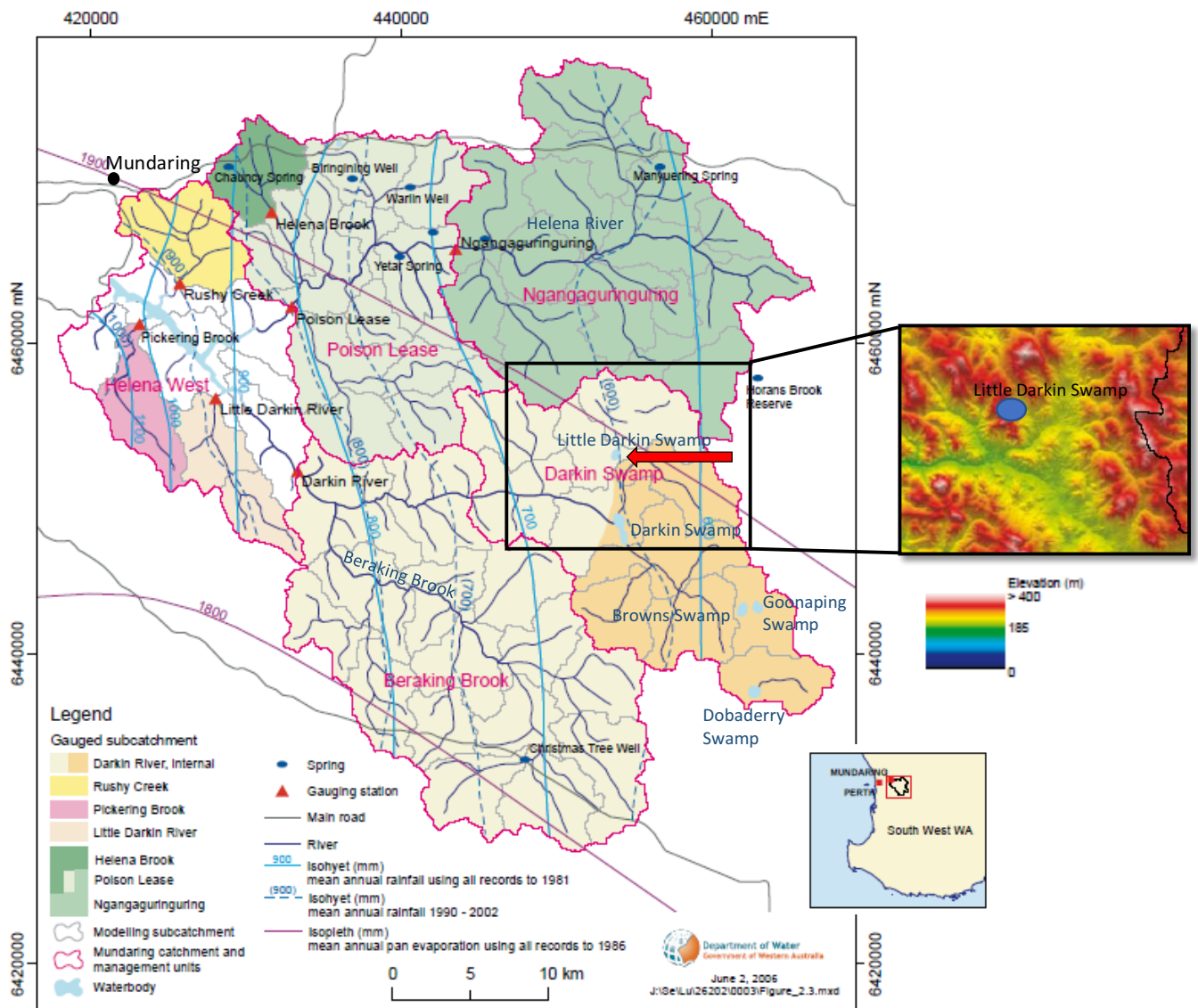
The aim of this chapter is to characterise Little Darkin Swamp, starting with the wetland components used for classification by Semeniuk and Semeniuk (2011). The first objective is to

spatially and temporally characterise Little Darkin Swamp in terms of descriptors of landform, water and vegetation such as water quality and dynamics, hydrology, fauna and flora communities in relation to ecosystem processes. This will also identify potential threats to its ecosystem health and allow for comparisons to vernal pools as described by Keeley and Zedler (1998). The general characterisation of the wetland and its condition will provide baseline information for the subsequent chapters in this thesis. The second objective is to compare Little Darkin Swamp with other claypan wetlands of southwestern Australia to determine if it fits within their classification. The outcomes of these objectives can ultimately contribute to the management and protection of these wetlands in southwestern Australia.

## 2.2 Study site

Little Darkin Swamp is located within the Helena River Catchment situated in the Wandoo National Park southwest of York, southwestern Australia (Figure 2.1). The Helena River, Beraking Brook and the Darkin River are the major streams in the catchment. The catchment consists of five management units, including The Darkin Swamp Catchment Management Unit, which discharges into the headwaters of the Mundaring Weir (Figure 2.1) (Miles, Ghadouani, & Kitsios, 2006). The Darkin Swamp Catchment Management Unit includes five water bodies: Browns Swamp, Goonaping Swamp, Dobaderry Swamp, Little Darkin Swamp, and Darkin Swamp, which is the largest of the five.

Southwestern Australia has a Mediterranean type climate with hot, dry summers and mild, wet winters. The long-term climate of the Helena Catchment is characterised by rainfall from May to October, hot and dry summers with some storm activity (Miles et al., 2006). On average the area receives 1093.7 mm rainfall annually, with most of it occurring between May and September (BOM, Mean annual rainfall 1994-2016, Bickley, WA). Daily temperatures in the region range from a mean maximum of 16°C in July to 34°C in February (Miles et al., 2006). The evapotranspiration is 1900 mm/yr for the whole catchment.



**Figure 2.1.** The location and topography of Little Darkin Swamp within the Darkin Swamp Catchment Management Unit within the larger Mundaring Catchment (Source and adapted from: Smith, Bari, Dixon, & Rowlands, 2007).

There is currently only a small amount of clearing (of native vegetation) in the Darkin Swamp Catchment area. The soils and vegetation consist mainly of lateritic gravel and clay-loam with woodland of *Eucalyptus wandoo*, *E. accedens* and *Corymbia calophylla*. Some sandy areas with *Banksia attenuata* woodland and granite outcrops also occur (George, 2002). The topography of the Darkin Swamp's proximate area is flatter than the rest of the catchment and has poor groundwater drainage (Miles et al., 2006). Little Darkin Swamp is located in an area with higher elevation and further away from the main Darkin River. Both Little Darkin Swamp and Darkin Swamp are located within a paleochannel of the Salt River Paleodrainage (Smith et al., 2007). Little Darkin Swamp has an ovoid shape, with a main circular basin and a shallower narrow tail which

extends outwards and is approximately 750m long and 370m wide across the circular basin, and approximately 160m wide across the tail (Figure 2.2, Google Earth Pro, 11/11/2015).

Little Darkin Swamp appears to be endorheic and water most likely does not flow out of the basin except in a large flood event when the wetland has reached its maximum volume. Under these circumstances the water probably spills out in a south-easterly direction following the tail of the wetland. As there is no drainage point and the clay base impedes infiltration, water loss occurs mostly through evapotranspiration. There is a man-made fire dam built at the north-western edge of Little Darkin Swamp, which holds permanent water and is connected to the wetland in high rainfall years (Figure 2.3). This fire dam is maintained by the WA Department of Parks and Wildlife (DPAW) for firefighting, but also attracts animals to the wetland, including introduced feral pigs and yabbies.

## 2.3 Methods and Materials

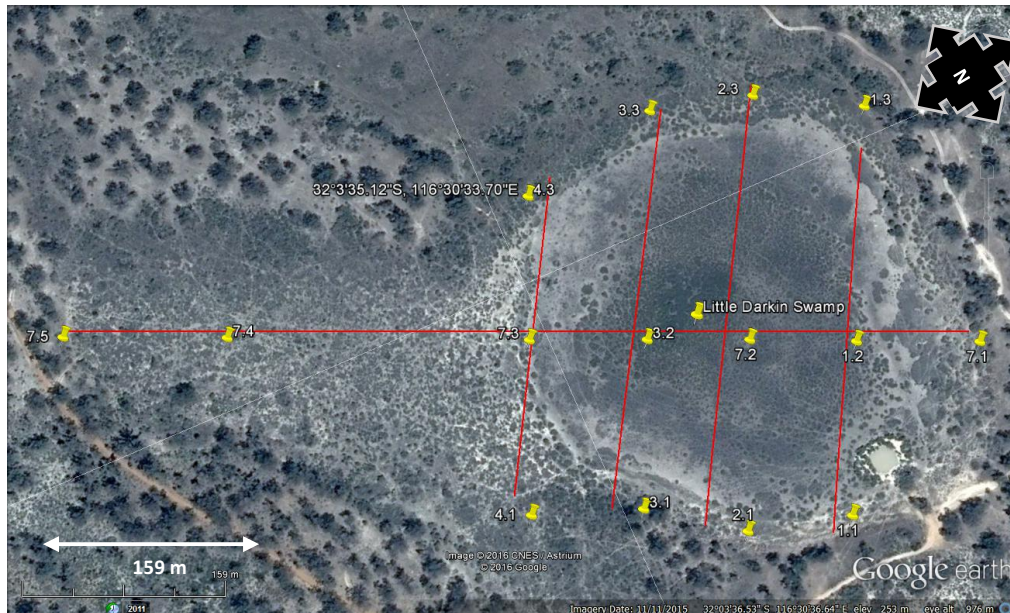
To characterise Little Darkin Swamp, the bathymetry, hydrology, vegetation, sediments, water quality, macroinvertebrates and disturbance of the wetland were examined. The bathymetry was measured through detailed elevation transects across the wetland in order to map the depth of the basin. The hydrology was determined by observing the filling and drying dynamics of the basin and measuring water levels in winter and spring. The vegetation communities were surveyed in late spring and summer to produce a vegetation map to display the variety and distribution of vegetation communities throughout the wetland. The water quality was also assessed, which included physicochemical measurements and dissolved nutrient levels during the hydroperiod. The general aquatic and terrestrial biodiversity of the wetland is briefly described, including the presence of macroinvertebrates, plants, amphibians and birds. Finally, the level of disturbance by feral animals and four-wheel drive vehicles was assessed.

All fieldwork was undertaken from September 2015 to November 2016, with different components surveyed at different times of the year according to the wetland's hydrological regime.

### 2.3.1 Elevation survey

A bathymetric survey of the dry lake bed was conducted during summer 2015-2016 to identify the change in elevation of the wetland surface to predict zones of inundation. Five line transects were laid out across the wetland (Figure 2.2) and an automatic level (Sokkisha C3E Automatic Level,

Tripod and Measuring stick) was used to measure differences in elevation. The tripod was placed at a high point in the wetland, and the elevation was read from the staff at 5m intervals along parallel transects across the wetland. The differences between readings were calculated to give the slope for each transect. A fifth transect perpendicular to the other transects was used to identify elevation levels in a southeast-northwest direction across the wetland (Figure 2.2).



**Figure 2.2.** Aerial photo of the wetland including the four transects orientated southeast-northwest and a fifth transect running southwest-northeast across the wetland used to measure the elevation levels and the therefore the relative depths across the basin (Google Earth Pro, 11/11/2015).

### 2.3.2 Water quality and hydrology

Water quality and hydrological measurements such as physicochemical variables and water depth were measured over two winter/spring seasons from September to October 2015, and from June to November 2016. Seven sites were randomly chosen in the deeper part of the wetland basin (Figure 2.3). The sites were chosen to represent the deepest area of the basin, retaining standing water for the longest period of time, and would therefore allow for a longer series of data points to cover the hydrological cycle. In order to compare densely vegetated habitats with sparsely vegetated or 'open habitats', four sites were chosen in areas with high vegetation density (sites 2, 5, 6 and 7) and three sites were chosen in areas with low vegetation density (sites 1, 3 and 4). Site 7 was added after the other six sites, to represent the deepest point of the wetland. All sites were marked with a stake and flagging tape, and GPS coordinates were taken.





**Figure 2.3.** Selected sites (S1-S7) for water quality and depth measurements within the wetland. Longitudinal cross section represents transect used to identify elevation levels.

Measurements and sampling commenced in early September 2015. Due to late winter rainfall and slow filling of the wetland, sampling started relatively late in winter and only four sets of measurements were taken. By early October surface water from most of the sites had disappeared. In general, 2015 was a relatively low (877.8 mm total) rainfall year according to historical rainfall data for the area (BOM, Annual rainfall 1994-2016, Bickley, WA<sup>1</sup>) and would not be representative of average duration of inundation and water levels at Little Darkin Swamp. Therefore, another sampling season was added in 2016, with measurements starting early May, and taken on a bi-weekly basis until late November 2016 to capture dynamics during the filling and drying of the wetland. Rainfall was much higher in 2016 (1085.6 mm total), and more representative of the average annual rainfall for the area (BOM, Annual rainfall 1994-2016, Bickley, WA).

All physicochemical measurements were taken around midday (11am-1pm) for consistency, when productivity is at its highest. A routinely calibrated Thermo Scientific Orion Star A329 pH/ISE/conductivity/RDO/DO multimeter was used, recording dissolved oxygen (DO in mg/L and % saturation), pH, electrical conductivity and temperature. The probes were submerged approximately 10-15cm below the water surface where possible. A wooden ruler was used to

<sup>1</sup> Located 40 km west of the study site.

measure water depth. All physicochemical and water depth measurements were taken as close as possible to the stakes at each site. Additionally, two HOBO U26-001 dissolved oxygen (DO) and temperature loggers with RDO sensors were deployed at two of the selected sites (site 4 and 6 in 2015, and site 6 and 7 in 2016), recording DO and temperature at 15-minute intervals. These data were used to examine continuous fluctuations in DO and crosschecked with measurements taken manually.

To measure the nutrients in the surface water of the wetland, water samples were taken at sites 2 and 4 in November 2015. Before use, all bottles were washed, rinsed and acid washed in the laboratory. In the field, one sterile 500mL bottle was submerged under water for each of the sites, and placed on ice until return to the laboratory at Edith Cowan University. All nutrient samples were analysed at ALS Global. Alkalinity was analysed by PC titrator (Department of Water, 2009). Sulphate (turbidimetric) as  $\text{SO}_4$ , chloride and ammonium were analysed by discrete analyser. Samples for dissolved nitrogen and phosphorus were also analysed by discrete analyser using the automated phenate method. Water samples were filtered through a  $0.45\mu\text{m}$  filter, placed in pre-prepared 10mL bottles and frozen until sent for analysis. Untreated water samples for other chemical analyses were kept in 200mL bottles and refrigerated until sent for analysis. Calcium, magnesium, sodium, and potassium were also analysed at ALS Global.

Chlorophyll-*a*, turbidity and gilvin were analysed for all seven sites, at Edith Cowan University facilities. Chlorophyll-*a* was used to estimate phytoplankton biomass and was measured as follows. A 2L water sample was collected in a clean, un-used plastic bottle in the field. The collected water samples were filtered using a filter tower and glass (Whatman GF/C  $1.2\mu\text{m}$ ) filter paper. All parts of the filtration tower were rinsed with de-ionised water prior to use and between samples. The volume of water filtered was recorded and filter papers used for each sample were combined and folded, wrapped in aluminium foil and labelled appropriately (Froend & Judd, 2014). The samples were analysed using the acetone method (Department of Water, 2009) for chlorophyll-*a* using a mass spectrophotometer. Gilvin was measured as a descriptor for colour of the water. A 500mL water sample was filtered through a  $0.2\mu\text{m}$  filter paper and refrigerated at  $1-4^\circ\text{C}$  and analysed within 7 days (Department of Water, 2009). The filtered samples were used to fill cuvettes and analysed using a Shimadzu Biotech BioSpec-mini spectrophotometer at 440nm. The following equation was used to calculate gilvin:  $\text{Absorbance at } 440\text{nm} \times \text{cell path length} \times 2.3030g = g_{440} \text{ absorbance/m.}$

### 2.3.3 Soil texture analysis

Soil field texture grade was determined following methods adapted from McDonald and Isbell (1987). A small sample of the soil was placed in the palm of the hand, moistened with water and kneaded until a ball was formed without sticking to the fingers. Once the sticky point was attained, the soil bolus was sheared to produce a ribbon. The behaviour of the soil bolus and the ribbon, as well as the ribbon length, was used to determine field texture grade of the soil (McDonald & Isbell, 1987).

### 2.3.4 Vegetation survey

A vegetation survey was conducted to describe and characterise the vegetation communities and their distribution at Little Darkin Swamp. The wetland vegetation was divided into interim vegetation zones based on recent Google Earth aerial images and personal observation. Then the interim zones were examined *in situ* for their vegetation density, patchiness of the vegetation, and floristics (species composition, see below). In each interim zone, a randomly selected GPS coordinate was chosen to establish one 5mx5m quadrat for ground truthing to provide a more detailed analysis of the vegetation communities. The vegetation was described based on composition of the dominant species in terms of relative projected foliage cover, average heights and patchiness (Table 2.1). Patchiness was defined as an area where the vegetation occurs in small clumps interspersed with bare sediments, rather than being continuous and evenly spread. The relative projected foliage cover and average heights were based on visual estimations. The interim zone boundaries were adjusted according to the in-field surveys, and the updated map was groundtruthed iteratively until distinct vegetation zones were established. A detailed map of the vegetation zones and boundaries was produced using the information gathered from the field-surveys.

Water depth was also measured in each established vegetation zone at the same fixed point when the wetland was full, using a wooden ruler to give a relative measure of the range of water depths between the zones. This would be used to further characterise the vegetation zones, and to examine the correlation between relative water levels and the vegetation type occurring in each zone.

Grazing disturbance was assessed in each vegetation zone during groundtruthing. A score was given based on visual measures of vegetation patchiness and disturbance of the sediments. A scale from 0-4 was used, where 4 is highly disturbed and 0 is undisturbed.



Organic matter content of surface sediments was calculated as loss on ignition (LOI) of sediment samples from each elevation level identified through the elevation survey. Four 5cm x 5cm sediment samples were taken from the surface (0-5cm) for each of the elevation levels and dried in the oven at 105 °C for approximately 24 hours until dry to achieve consistent weight. The dried samples were mixed and a small amount (16-53g) was weighed out in a crucible and burnt in the furnace at 550 °C for approximately 4 hours, and then re-weighed (Heiri, Lotter, & Lemcke, 2000). The average percentage loss of weight was given as loss on ignition and provided an estimate of organic matter content for soil samples for each elevation level and applied to the established vegetation zones corresponding to them.

### 2.3.5 Biota

#### 2.3.5.1 Macroinvertebrates

Macroinvertebrates were sampled twice during winter 2015, once in September (16/09) and once in October (8/10). A D-framed net with 250µm mesh size was swept in the water for approximately 30 seconds at each of the seven sites. The net was rinsed between sites and samples were emptied into plastic zip-lock bags and placed in an esky until arrival at the Edith Cowan University facilities. In the laboratory, samples were rinsed through sieves to remove silt and clay; coarser fractions were placed in sorting trays and finer fractions examined under a dissecting microscope. Each sample was searched for 30 minutes. Voucher species were collected for taxa found at each site, and presence/absence were recorded. Macroinvertebrates were identified to lowest taxonomic level and used to provide presence/absence data for each site and sampling time.

The samples were also used to collect specimens for stable isotope analysis in order to determine the aquatic food web and the basal carbon sources providing energy. The species were grouped into functional feeding groups and the abundant species were used for stable isotope analysis (see Chapter 3).

#### 2.3.5.2 Plant list

A plant list was collated over the duration of the field seasons in 2015 and 2016. Cuttings and photographs were taken and specimens were identified using field guides and plant lists for the region (Barrett & Tay, 2016; Western Australian Herbarium, 2016; Atlas of Living Australia, 2016). Some of the identifications were verified by flora specialist Tanya Llorens (Department of Parks and Wildlife). Specimens were collected from both within the wetland, the immediate

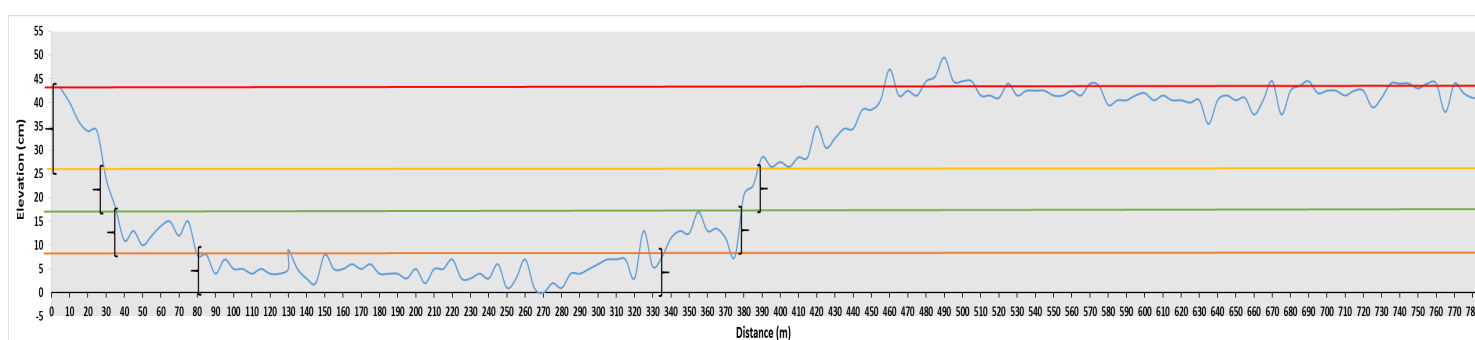
surroundings and within the close proximity (within a 0.5- 1km buffer). Species were divided into macrophytes, perennials and annuals according to lifecycle and habitat. Plant taxa within each vegetation zone were identified in 5x5m plots and also included in the overall plant list for the wetland.

## 2.4 Results

### 2.4.1 Basin depth and size

Little Darkin Swamp is a relatively shallow basin with approximately 0.5 m elevation from the deepest point in the centre of the wetland to the highest point at the boundaries. The depth of the basin was divided into four elevation levels, based on elevation along the longitudinal transect across the wetland (Figure 2.4). Assuming the bottom of the depression was 0m, Level 1 was set at 0-0.1m elevation, Level 2 was 0.1-0.2m elevation, Level 3 was 0.2-0.3m elevation, and Level 4 was 0.3-0.5m elevation where the transect plateaus.

Little Darkin Swamp has a perimeter of approximately 1845m and surface area of 165 976m<sup>2</sup> (determined from Google Earth Pro). Assuming the average depth of the basin is 0.165m based on all elevation measurements from the five line transects, the total volume of the wetland when full is approximately 26 556m<sup>3</sup> (calculation adapted from Masser & Jensen, 1991). Vernal pools typically range from 50 to 5000 m<sup>2</sup> in area and from 0.1 to 1m in depth (Rains et al., 2006).



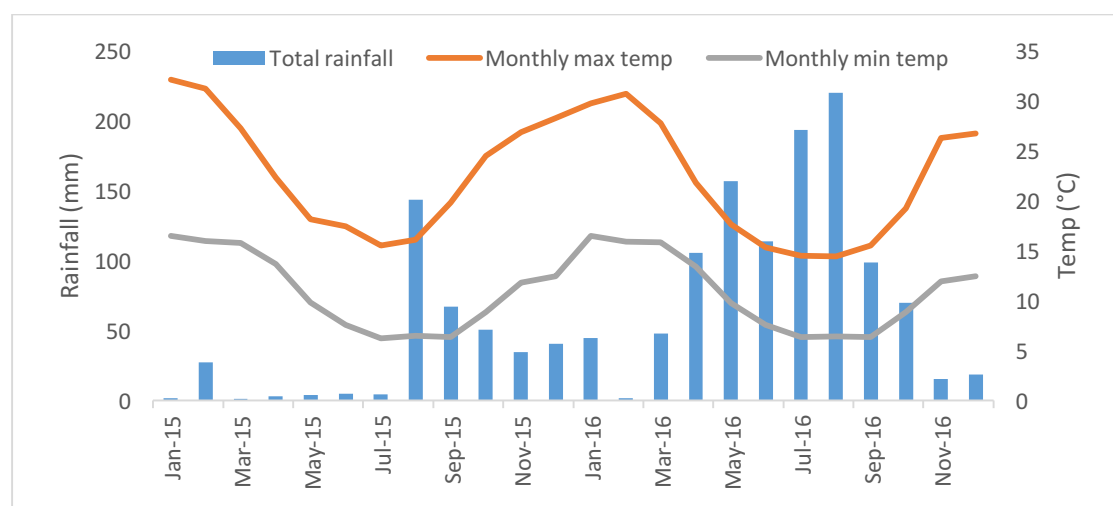
**Figure 2.4.** Basin shape and range of the four elevation levels based on an 800m longitudinal transect through the length of the wetland. Vertical axis is accentuated to better distinguish between the elevation levels.

### 2.4.2 Climate and Hydrology

The hydrological cycle of Little Darkin Swamp is highly seasonal, filling during late spring to winter and drying in summer for an extended period of the year.

The total rainfall in 2015 was 65% lower than the total rainfall in 2016 in Bickley, Western Australia (BOM, 2016), and especially early in the winter from March to July (Figure 2.5). Specifically, during the sampling season in 2015 (August-October), rainfall was 33% lower than the sampling season in 2016 (Figure 2.5).

In addition, maximum and minimum temperatures were higher during winter and early spring 2015, and likely to contribute to higher evaporation rates at the wetland (BOM, 2016). Temperatures started rising already by August in 2015, whereas in 2016 temperatures were unusual in that they remained relatively low in September and October, likely reducing evaporation rates. The overall rainfall levels were significantly lower in 2015 compared to 2016 (Figure 2.5), resulting in shorter duration of inundation and lower water levels at Little Darkin Swamp.

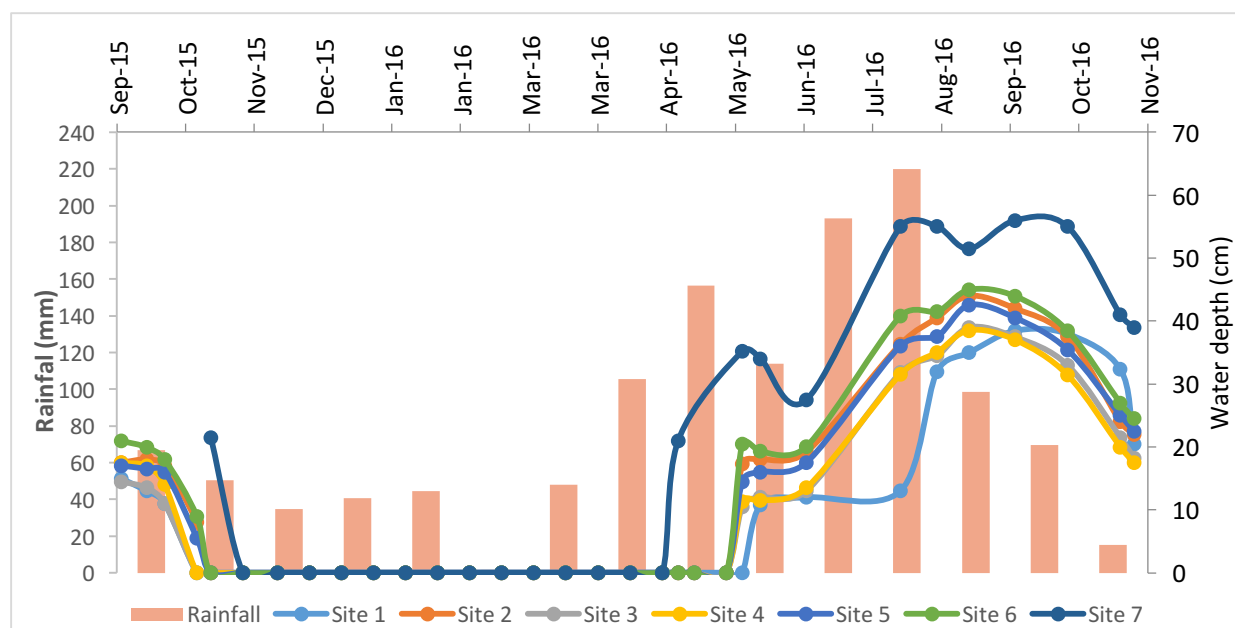


**Figure 2.5.** Monthly total rainfall and average maximum and minimum temperature during the sampling period (August 2015- November 2016) and for 2015-16 (BOM, 2016).

As a result of the low rainfall and water levels during winter 2015, the basin dried out as early as mid-October, and there was no standing water between October 2015 and April 2016 for at least 6 months. The wetland started to re-wet after the first rainfall in March 2016, and by the beginning of May there was standing water in the centre of the wetland. There was generally a lag between rainfall and increase in water depth, caused by delayed infiltration of water and runoff into the basin (Figure 2.6). Surface water accumulated in the centre of the wetland, gradually expanding outwards, as well as accumulating in other small depressions or puddles such as tracks (made by kangaroos or pigs). Site 7, located in the deepest point of the basin, filled the earliest, had standing water for the longest period, and had higher water levels compared to the other sites (Figure 2.6).

During winter 2016, water levels in the wetland increased steadily from the beginning of July to the end of September when it peaked, and began to decline in early October (Figure 2.6). At the peak in September maximum water levels were 56cm at Site 7. The highest average water depth across the seven sites during the study period for the centre of the wetland was 42.7cm.

Overall, water levels in the wetland were over twice as high from September to October 2016 compared to the same time in 2015, and the total hydroperiod for 2016 was approximately 6 months, compared to approximately 3 months in 2015 (Figure 2.6). These data suggest large inter-annual variability in water depth, extent and duration of inundation. They are highly dependent on rainfall and evapotranspiration rates which are controlled by ambient temperature, wind and exposure to light.



**Figure 2.6.** Surface water hydrograph plotted with total monthly rainfall (BOM, 2016) for Little Darkin Swamp at 7 sites within V1 for the period August 2015 to November 2016.

### 2.4.3 Soil and substrate

The field soil texture of the top soil layer across the length of a longitudinal transect showed that the majority of the basin soil at Little Darkin Swamp is classified as 'heavy clay' ( $\geq 50\%$  approximate clay content) (McDonald, Isbell, Speight, Walker, & Hopkins, 1998). The clay content of the soil increased toward the centre of the wetland, while close to the edges of the basin the soil appeared to be sandier with larger grains and less coherence. This pattern was also consistent with depth,

the areas of deeper water having higher clay content of surface sediments. On the outer rim of the wetland, the soil had a sandier composition but was more similar to that of the basin.

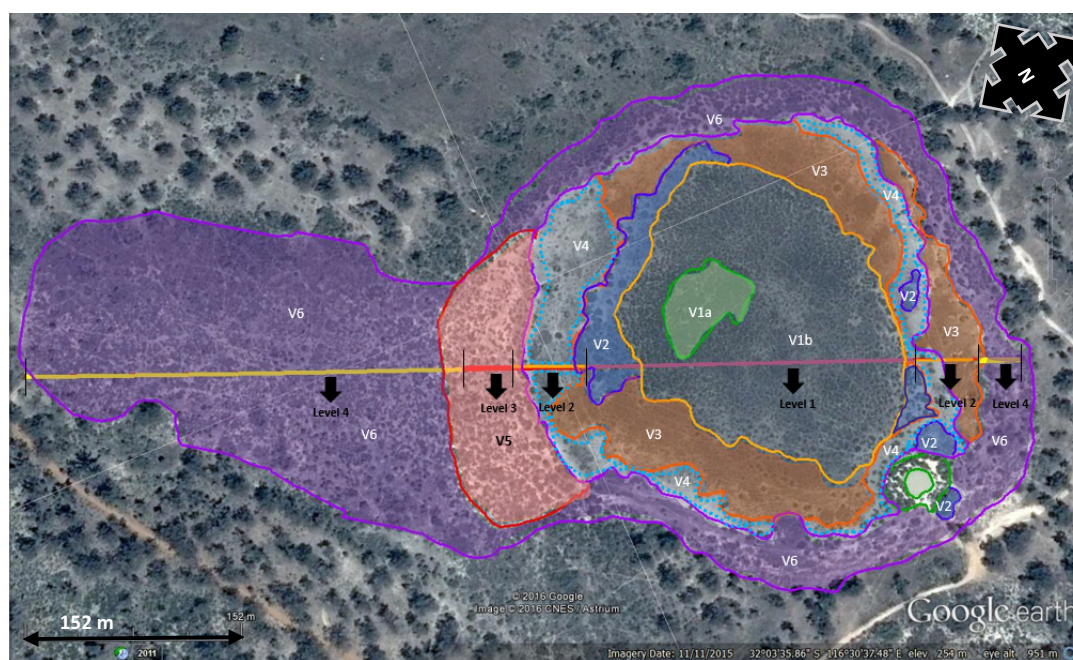
The amount of organic matter increases with depth of the basin and thus duration of inundation. The vegetation zones (Figure 2.7) in the centre of the wetland had the highest organic matter content in the soil profile, which progressively decreased towards the edges of the wetland. This was due to higher amounts of leaf litter from shrubs, sedges and macrophytes in the low elevation areas where depth was greater. Vegetation zones V1 (a+b) and V2 both had an organic matter content of 22%, followed by 13% in V3 and V4. The outer-most vegetation zones, V5 and V6, had the lowest amounts of organic matter with 2.5% and 3.3% respectively.

#### 2.4.4 Vegetation zones

The vegetation at Little Darkin Swamp can be divided into 6 zones based on differences in community structure and composition (Figure 2.7). These vegetation zones can be associated with areas of differing elevation (Figure 2.4); elevation level 1= vegetation zones V1a, V1b, V2 and V3; elevation level 2= vegetation zone V4; elevation level 3= vegetation zone V5 and elevation level 4= vegetation zone V6. Vegetation zones V1 (a+b) and V2 lie in the central portion of the wetland with the lowest elevation, and will generally hold water for the longest time during winter and spring. Vegetation zones V3 and V4 lie in the areas that mark the start of the slope outwards from the centre of the basin, while zone V5 lies in the steepest area of the basin moving into the semi-terrestrial area. Parts of zone V5 and all of zone V6 lie in the extensive shallow tail area (elsewhere in this chapter referred to as the 'plateau') of the wetland and will dry out quickest.

The vegetation within the basin consists of a few dominating perennial species including the tall shrub *Melaleuca lateritia* (Robin red-breast bush), the sedges *Chorizandra enodis* (Black bristle rush) and *Meeboldina coangustata*. During the wet season, there is also an abundance of submerged and emergent macrophytes growing in the wetland, with dominant species being emergent *Cycnogeton huegelii* (water ribbon), submerged *Ornduffia submersa* and a grass-like (*Callitriche stagnalis*) submerged plant. In the areas at the borders of the wetland and just outside the basin itself, semi-terrestrial plants also occur in addition to the perennials already mentioned. These include 3 species of *Verticordia* (*V. plumosa*, *V. densiflora* and *V. acerosa*) and two species of *Hakea* (*H. marginata* and *H. varia*). There is also a high diversity and abundance of annuals during the spring/summer months.

*Melaleuca lateritia*, *Chorizandra enodis* and *Meeboldina coangustata* have the widest distribution in the wetland, although their relative contribution (of density/percentage cover) to the community structure differs across zones (Table 2.1). The riparian zones V4-V6 have higher species diversity with the additional semi-terrestrial species (Table 2.1). As water depth increases, so does the abundance of emergent and submergent macrophytes. The deeper central zones V1a, V1b and V2 have transitional boundaries to their neighbouring zones. The rushes are found in all zones with differing abundances, while the rush *M. coangustata* only occurs in the zones further out from the centre of the wetland, indicating its lower tolerance to inundation. *Melaleuca lateritia* is the dominating species throughout the wetland, and is at its tallest and with highest density in the centre where water is deepest. This species is highly tolerant of the extreme seasonal changes to the hydrology and persists throughout the year. *Melaleuca viminea*, *Verticordia densiflora* and *Hakea marginata* are semi-terrestrial and less tolerant of high water levels (Marchant, 1987), and therefore only occur in the shallower parts of the wetland.



**Figure 2.7.** Boundaries of vegetation zones (V1-V6) and boundaries of elevation levels at Little Darkin Swamp. Black lines and arrows indicate boundaries of elevation zones.

Zones V3, V4 and V5 are most affected by feral pigs, evident by the patchiness of the vegetation and disturbed sediments, resulting in clearer boundaries of the zones. In addition, zones V3, V4 and V5 have the lowest percentage of *M. lateritia*, indicating that the species might be sensitive to the disturbances or the drier conditions of these zones. The disturbances could also explain the

low percentage of *M. coangustata* in zones V3 and V4. There are annual species growing in both the deeper and shallow zones of the wetland during spring, though they are generally found with higher abundance in the shallow areas where the soils dry out the quickest. The vegetation survey was conducted in late summer, resulting in the low occurrence of annuals at this time due to low soil moisture (Table 2.1).

**Table 2.1.** Relative species abundances and structural characteristics of the vegetation zones at Little Darkin Swamp during summer 2015-16. Disturbance categories; 1= Undisturbed, 2= Little disturbance, 3= Some disturbance, 4= Very disturbed. Water depth range refers to water levels in each zone when the basin is at maximum water depth.

Variables	V1a	V1b	V2	V3	V4	V5	V6
Water depth range (cm)	40-50	30-40	30-40	20-30	20-30	2-5	0-1
Sediment LOI (%)	22	22	22	13	13	2.5	3.3
Level of disturbance (1-4)	1	2	2-3	3-4	4	0-1	0
Nature of zone boundaries	Clear	Less clear	Less clear	Clear	Clear	Not clear	Clear
<b><i>Melaleuca lateritia</i></b>							
Cover range (%)	80-90%	50-60%	30-35%	5-10%	5-10%	50-60%	40-50%
Height range (m)	1.5-2m	0.5-1m	1-2m	0.5-1m	0.5-1.5m	0.5-1m	0.5-0.7m
<b><i>Melaleuca viminea</i></b>							
Cover range (%)	0%	0%	0%	0%	1-2%	5-10%	5-10%
Height range (m)	-	-	-	-	2-3m	1.5-3m	1.5-3.5m
<b>Cover ranges (%)</b>							
<i>Meeboldina coangustata</i>	0%	0%	1-2%	0-1%	0%	5-10%	2-5%
<i>Chorizandra enodis</i>	15-20%	40-50%	60-70%	30-40%	15-30%	5-10%	30-40%
<i>Verticordia densiflora</i> (<0.5m)	0%	0%	0%	0%	0%	20-30%	10-15%
<i>Hakea marginata</i> (<2m)	0%	0%	0%	0%	0%	0%	2-5%
<b>Macrophytes</b>							
<i>Cycnogeton huegelii</i>	15-20%	5-10%	5-15%	5-10%	5-10%	0%	0%
<i>Ornduffia submersa</i>	2-5%	2-5%	0%	1%	1%	0%	0%
<i>Callitriche stagnalis</i>	20-30%	50-60%	20-30%	70-80%	50-60%	0%	0%
<b>Annuals (spring)</b>	0%	0%	1%	0%	0%	2-5%	5-10%

## 2.4.5 Community structure of vegetation zones

Based on the information from the surveys of the vegetation zones, brief summarised descriptions of each zone have been given in Table 2.2. The descriptions include key characteristics of each zones vegetation community including details of floristics and structure.

**Table 2.2.** Community structure and characteristics of the six vegetation zones.

Vegetation zone	Description
<b>V1a</b>	Tall, dense <i>Melaleuca lateritia</i> (1.5-2m), interspersed with sedges and an abundance of macrophytes and abundant filamentous algae present when water levels are high. Boundaries quite clear, with shorter <i>M. lateritia</i> occurring at the boundaries. High amounts of organic matter in sediments due to high leaf litter fall from <i>M. lateritia</i> .
<b>V1b</b>	Relatively dense areas of shorter <i>M. lateritia</i> (0.5-1m) dominating, interspersed with sedges, yet sparser than V1a. <i>M. lateritia</i> sometimes in clusters, but generally homogenous. <i>Cycnogeton huegelii</i> and <i>Callitriche stagnalis</i> abundant and growing amongst the <i>Melaleuca</i> during inundated phase. Other macrophytes found sparsely scattered between areas of <i>M. lateritia</i> .
<b>V2</b>	Areas with dense clusters of taller <i>M. lateritia</i> (1-2m) and otherwise mainly rushes/sedges dominant. Combination of V1b and V3 and perhaps a transition zone between the two. Large amounts of new <i>Chorizandra enodis</i> growth covering previously patchy and sparse areas. Higher levels of disturbance due to exposed sediments and grazing by feral pig during the dry phase. Some annuals present during spring.
<b>V3</b>	Shorter rushland with rushes/sedges and macrophytes dominating. <i>M. lateritia</i> (0.5-1.5m) present less abundantly but more patchy and sparse, and with larger height range. Higher elevation causing a slope and lower water levels, resulting in phasing out from denser areas of <i>M. lateritia</i> . Macrophytes <i>Cycnogeton huegelii</i> and <i>Callitriche stagnalis</i> proliferate and cover large areas when wet. Not a clear vegetation pattern in these areas, high variability in abundances and heights making it difficult to define clearly. Signs of grazing and animal tracks indicating higher disturbance. Area less frequently inundated than V1 and V2 due to higher elevation, making it more prone to grazing and drying in low rainfall years.
<b>V4</b>	Areas of grazed and mainly dead sedges/rushes. Very patchy with large areas of exposed sediment. Some small clusters of <i>M. lateritia</i> present, as well as some <i>M. viminea</i> . Macrophytes present and abundant during wet season. High diversity and relatively high abundance of annuals during end of spring/summer. Occurs in areas where elevation is high, close to the riparian zone (V6) where water levels are lowest.
<b>V5</b>	Gradational zone moving towards the drier riparian tail of wetland and out of the basin. Dominated by <i>M. lateritia</i> and <i>Verticordia</i> , interspersed with sedges/rushes, <i>M. viminea</i> and macrophytes during wet season. <i>M. lateritia</i> sparse and patchy, but more abundant than in zones V3 and V4. Area lies on an up-slope out from basin, yet low enough to hold some water in high rainfall years allowing macrophytes to grow. Some patchiness in vegetation and exposed sediments. Sediment generally lighter in colour and more clayey with less organic matter from leaf litter than in the centre of the wetland.
<b>V6</b>	Riparian zone with higher species diversity/richness, marking outside borders of wetland. Semi-terrestrial species such as <i>Verticordia</i> sp. and <i>Hakea</i> sp. present, along with <i>M. lateritia</i> , <i>M. viminea</i> . Sedges/rushes also found. Very shallow (1cm) if any standing water, only present in high rainfall years (such as 2016). Higher abundance and diversity of annuals during spring/summer.



### 2.4.5 Water quality

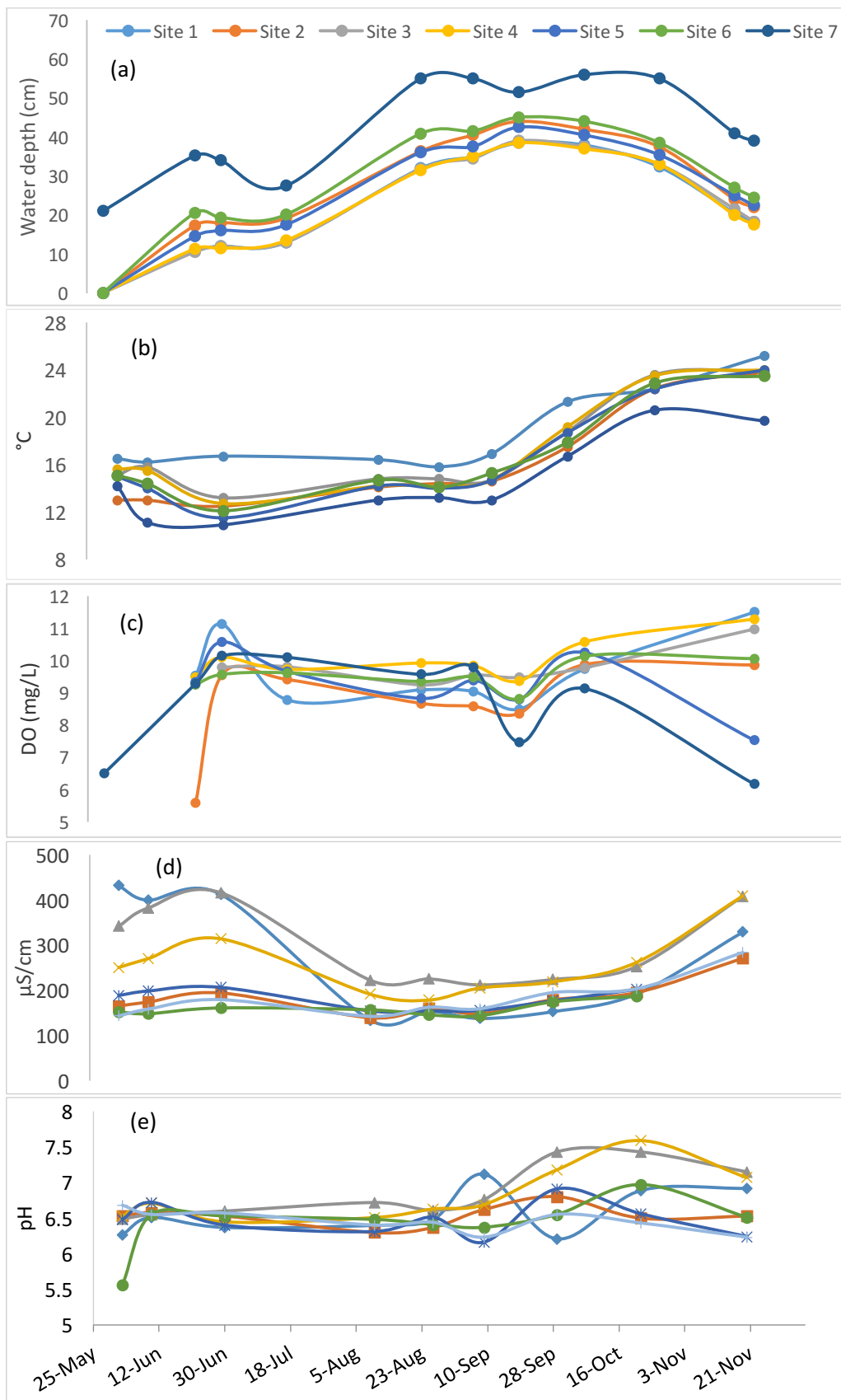
During winter 2016, dissolved oxygen (DO) levels were temporally highly variable in the wetland and ranged between 5.6-13 mg/L. On average DO stayed between 8.7-10.2 mg/L when water levels were at their highest. There was also high variability between sites (Figure 2.8c); DO was most constant at sites 3, 4 and 6, whereas sites 2, 5, and 7 had high fluctuations. This was particularly pronounced at the start and end of the winter season in 2016 when water levels were low. In comparison, dissolved oxygen levels were lower during spring 2015 (September-October) compared to 2016 as a result of higher temperature levels. In addition, variability between the sites was low (8.7- 10 mg/L) for this period of time.

During the wet-season in 2016, DO levels increased early June following the initial increase in water levels, then stabilised between 8-9 mg/L until October when water levels started declining (Figure 2.8c). During the decline of water levels and rise in temperature in spring, DO became spatially variable, increasing at some sites and decreasing at others.

The water temperature in the wetland was relatively low during the start of winter 2016 (Figure 2.8b), remaining between 11-17°C from April to August. In general, water temperature did not vary greatly between sites but increased in spring, reflecting ambient air temperatures (see Figure 2.5). Sites 1, 3 and 4 were located in low vegetation density areas, resulting in higher water temperatures compared to those in high vegetation density areas (sites 2, 5 and 6). These sites (1, 3, 4) also had the lowest water levels, which would also have contributed to higher daily temperature fluctuations. In comparison, water temperature increased sooner in 2015, as a result of higher ambient temperatures. This resulted in higher evapotranspiration rates and rapid declines in water levels in spring.

The pH stayed stable at around 6.5 for all sites and variability between the sites was low during the first months following rainfall in 2016 (Figure 2.8e). Early in spring (from September), pH became much more variable between and within sites as water levels started to recede. Specifically, pH steadily increased until the end of October at sites 3 and 4, while it fluctuated more widely at the other sites. In 2015, pH was less variable, ranging only between pH 6-7.

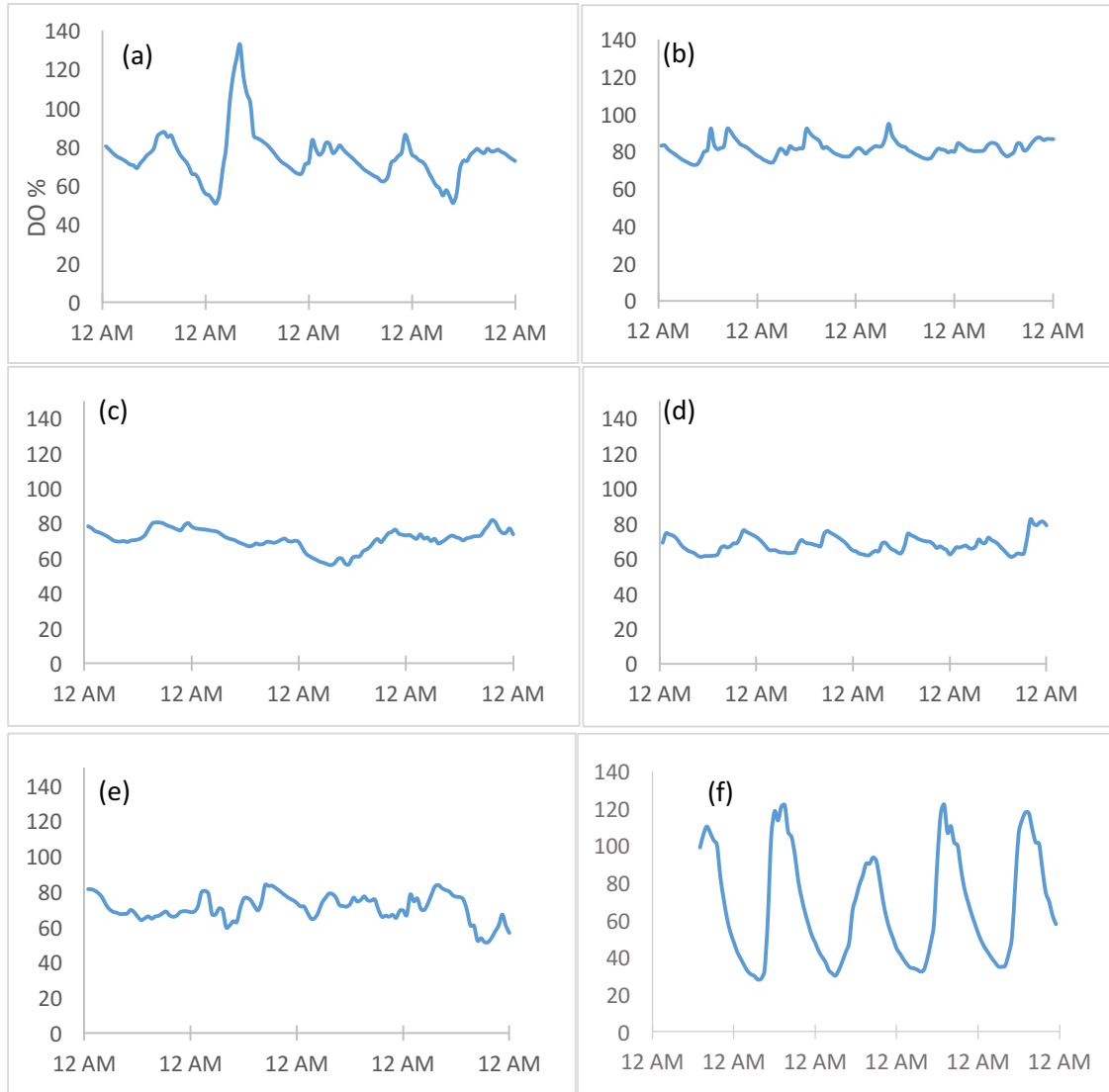
Conductivity was generally low during the wet-season 2016, ranging between 142-432  $\mu\text{S}/\text{cm}$ . Early in the wet season conductivity was spatially variable as the wetland filled, with higher conductivities at the shallower sites (sites 1, 3 and 4) (Figure 2.8d). As water levels increased and stabilised, the conductivity also stabilised between 150-250  $\mu\text{S}/\text{cm}$  for all sites. Once the water levels started to recede in spring, an increase in conductivity was observed at all sites. In comparison, conductivity was more variable in spring 2015, ranging between 160-810  $\mu\text{S}/\text{cm}$ .



**Figure 2.8.** Water depth (a), water temperature (b), dissolved oxygen concentration (c), conductivity (d) and pH (e) dynamics for 7 sites at the centre of Little Darkin Swamp, winter and spring 2016.

During the hydrological cycle in 2016, DO saturation (%) had larger diurnal fluctuations early in winter when the wetland was filling as well as later in spring when water levels were decreasing through evapotranspiration. Figure 2.9 shows diurnal fluctuations in DO over 5 consecutive days at site 7 in the months June to November 2016. The data from the loggers show that diurnal DO fluctuations were highest in June and November, and that DO was most stable in August and September when water levels were at the highest and temperatures were relatively low. Average daily temperature was more variable 3-7<sup>th</sup> June (11.7-21°C) compared to July-September. In October, temperatures had started increasing and fluctuated more diurnally, contributing to larger diurnal fluctuations in DO. As temperatures increased further in November (18.4-34.5°C), causing higher rates of photosynthesis, diurnal DO fluctuations became highly pronounced.

With the higher temperatures, and consequently higher evaporation and photosynthesis rates, DO had the largest range and diurnal fluctuations in November (28- 122 % saturation) when water levels started rapidly decreasing. Peaks in DO occurred at around 3pm. Dissolved oxygen levels were slightly higher in July (range 72- 95 % saturation), compared to August and September (range 56-80 % saturation). In October the fluctuations started becoming more pronounced, but stayed between a relatively narrow range (50- 85% saturation).



**Figure 2.9.** Diurnal changes in dissolved oxygen (%) from logger located at Site 7 at different times during the hydrological cycle in 2016: (a) June 3<sup>rd</sup> - 7<sup>th</sup> (b) July 3<sup>rd</sup> - 7<sup>th</sup> (c) August 3<sup>rd</sup> - 7<sup>th</sup> (d) September 3<sup>rd</sup> - 7<sup>th</sup> (e) October 3<sup>rd</sup> - 7<sup>th</sup> and (f) November 14<sup>th</sup> - 18<sup>th</sup>.

#### 2.4.6 Nutrients, colour and chlorophyll-*a*

Reactive phosphorus and inorganic nitrogen (dissolved) levels at Little Darkin Swamp (LD) were very low and below detection at sites 2 and 4 (P and N <0.01 mg/L) in winter 2015 (Table 2.4).

The ionic composition of Little Darkin Swamp is dominated by sodium and chloride, much like most Australian waters (Boulton et al. 2014). Dominant cations (sodium, calcium, potassium and magnesium) were all relatively low at both sites 2 and 4. Site 4 had the highest levels of sodium (68 mg/L) and chloride (89 mg/L). For both sites at Little Darkin, the ionic dominance pattern was  $\text{Cl} > \text{Na} > \text{K} > \text{Ca} = \text{Mg}$ , whereas for surface water of CA vernal pools the dominance is  $\text{Ca} - \text{Mg} - \text{Na} - \text{HCO}_3$  (Rains et al., 2006). At Goonaping Swamp, also located in the Darkin Swamp catchment in the

Wandoo National Park, the pattern of cation concentrations was  $\text{Na} > \text{K} = \text{Ca} = \text{Mg}$ , and  $\text{Cl}^-$  was the dominant anion (Cale, Halse, & Walker, 2004). Total alkalinity ( $\text{CaCO}_3$ ) at both sites 2 and 4 at Little Darkin were 15 mg/L, suggesting it was poorly buffered, and falls under the US EPA category 'sensitive' to disturbance (Sim, 2012a).

**Table 2.4.** Nutrients and ionic composition for Site 2 and Site 4 at Little Darkin Swamp, November 2015.

<b>Analyte</b>	<b>Units</b>	<b>Site 2</b>	<b>Site 4</b>
Total Alkalinity as $\text{CaCO}_3$	mg/L	<b>15</b>	<b>15</b>
Sulfate as $\text{SO}_4$ - Turbidimetric	mg/L	<20	<20
Chloride ( $\text{Cl}^-$ )	mg/L	<b>49</b>	<b>89</b>
Calcium ( $\text{Ca}^{2+}$ )	mg/L	<b>2</b>	<b>2</b>
Magnesium ( $\text{Mg}^{2+}$ )	mg/L	<b>2</b>	<b>2</b>
Sodium ( $\text{Na}^+$ )	mg/L	<b>39</b>	<b>68</b>
Potassium ( $\text{K}^+$ )	mg/L	<b>4</b>	<b>6</b>
Ammonium as N	mg/L	<0.01	<0.01
Nitrite + Nitrate as N	mg/L	<0.01	<0.01
Reactive Phosphorus as P	mg/L	<0.01	<0.01

Sites 5, 6 and 7 had the highest amount of chlorophyll-*a* (3-12  $\mu\text{g/L}$ ) in the water column, while at sites 1-4 the levels were low or below detection (Table 2.5). At sites 5-7, the higher average water depth corresponded to the higher amount of chlorophyll-*a* (3 mg/L, 2 mg/L and 12 mg/L respectively). Overall, chlorophyll-*a* concentrations were low at the wetland, although they were somewhat higher at site 7.

The shallow sites were more elevated for each of conductivity, temperature and dissolved oxygen compared to the deeper sites (Table 2.5). The conductivity was generally relatively low and varied spatially throughout the winter season, but was on average similar between the sites (range 172.7-298  $\mu\text{S/cm}$ ). Turbidity varied between sites, ranging from 2.76-14.3 NTU and were lower than or at the lower end of the broad range for southwestern Australian wetlands (10-100 NTU) (Sim, 2012b). The sites in the centre of the wetland were more turbid than the ones further out from the centre (Table 2.5). These sites have higher amounts of organic matter, as well as higher water levels (Figure 2.1). The surface water of Little Darkin was coloured, but was not particularly variable between sites (gilvin range 47- 53  $\text{g}_{440}\text{m}^{-1}$ ). It has been proposed that wetlands on the Swan Coastal Plain are considered 'coloured' beyond 52  $\text{g}_{440}/\text{m}$  (gilvin) (Sim, 2012b).

**Table 2.5.** Chlorophyll-*a* (mg/m<sup>3</sup>), turbidity (NTU) and gilvin (g<sub>440</sub>m<sup>-1</sup>) from single samples winter 2015, and water depth (cm), pH, conductivity (µS/cm), DO (mg/L) and temperature (°C) averages for winter 2016 for Sites 1-7.

Site	Depth	Chl- <i>a</i>	Turbidity	Gilvin	pH	Conductivity	DO	Temp
1	25.04	<1	5.35	52.05	6.6	260.3	9.6	18.1
2	30.08	<b>1</b>	4.16	52.74	6.6	180.4	8.7	16.1
3	25.1	<1	3.06	49.05	6.8	298.2	10.2	17.2
4	24.72	<1	2.76	49.28	6.8	255.3	10	17.1
5	28.74	<b>3</b>	12.7	47.44	6.5	192.4	9.3	16.5
6	32.12	<b>2</b>	14.3	52.90	6.4	172.7	9.5	16.7
7	44.92	<b>12</b>	-	-	6.5	180.8	8.9	14.7

#### 2.4.7 Biodiversity

Within Little Darkin Swamp there is a relatively large diversity of plants compared to what might be expected for permanent wetlands of southwestern Australia. There are at least 6 species of aquatic plants, including submerged and emergent macrophytes (Table 2.7). As expected in seasonal shallow wetlands, there are also rushes, sedges and shrubs growing in the wetland, in addition to numerous annual species (Table 2.3) appearing in spring. The highest plant diversity appears to be in the outer vegetation zones which are inundated for much shorter periods, with large numbers of annuals and geophytes such as orchids, appearing as water levels recede. The seasonal filling of the wetland attracts many animals during the winter season, such as kangaroos and emus, as well as a number of bird species such as Carnaby's cockatoos (*Calyptorhynchus latirostris*), unidentified duck species and white-necked herons (*Ardea pacifica*) that come to drink and feed in the wetland (personal observation). When the wetland is dry, the fire dam adjacent to the wetland attracts kangaroos, emus and probably feral boars, apparent by the numerous tracks throughout the wetland leading to the dam. Several species of frogs have also been observed and heard in the wetland, including the Moaning Frog (*Heleioporus eyrei*), the Whooping Frog (*Heleioporus inornatus*) and the Western Spotted Frog (*Heleioporus albopunctatus*). Among the macroinvertebrates present during sampling in October 2015 were common wetland taxa such as microcrustaceans, amphipods, nematodes, gastropods, beetles and arachnids (Table 2.6). An introduced species of crayfish, *Cherax destructor*, was collected from a deep (about 0.5m) burrow within the wetland, and is likely to have been introduced to the fire dam located at the wetland, and 'escaped' from it. The presence of a fire dam with permanent water directly adjacent to the wetland may have negative impacts on the wetland by attracting feral animals.

#### 2.4.7.1 Macroinvertebrate richness

The macroinvertebrate richness and composition of Little Darkin Swamp is based on three sampling events in 2015 (16/09, 08/10 and 14/10). A total of 16 taxa were found across 6 sites after the first sampling in September 2015, which were not identified to species level, and this is therefore an underestimation of the species richness in the wetland.

Across the three sampling occasions, the most consistently present macroinvertebrates in the wetland were Amphipods, Ostracods and Coleopterans (diving beetles) (Table 2.6). At the first sampling occasion (16/09/2015), Nematodes, Molluscs and weevils were also found at most sites, whereas at the second sampling occasion (8/10/15), when water levels had dropped significantly, their occurrences were reduced or they were not present at all. However, the occurrence of damselfly nymphs had increased. In addition, the Big Red Mite was found at a number of sites across the two sampling occasions. At least two species of tadpoles were also found at most sites at all three sampling occasions, and great numbers were found in remaining puddles of water as the wetland dried towards spring 2015.

The groups of invertebrates found at Little Darkin Swamp are similar to groups found at Goonaping Swamp, also located in the Wandoo National Park (see Figure 2.1 above and Table 6.1, Appendix). These include Nematoda, Gastropoda, Acarina, Cladocera, Ostracoda, Trichoptera and Coleoptera. These groups were also amongst those found at the Drummond Nature reserve claypans, and are common for small ephemeral wetlands (Pinder, Quinlan, Cale, & Shiel, 2013).

**Table 2.6** Macroinvertebrate groups identified at 7 sites within the basin of Little Darkin Swamp on three sampling occasions during winter 2015. Sampling at site 7 was conducted separately.

Taxon list		16.09.15						08.10.15					14.10.15		
Class/Phylum	Common name	1	2	3	4	5	6	1	2	3	4	5	6	7	
NEMATODA	Worm 1	X	X	X	X	X	X	X							
	Worm 2					X	X								
PLATYHELMINTHES	Flatworms (Turbellaria)										X				
MOLLUSCA	Freshwater snail (Gastropoda)	X	X	X	X	X				X		X	X		
CRUSTACEA	Ostracoda	X	X	X	X	X	X	X	X	X	X		X	X	
MICROCRUSTEACEA	Copepoda			X											
AMPHIPODA	Sp. 1	X	X	X	X	X	X	X	X	X		X	X	X	
	Sp. 2								X		X				
ISOPODA	Waterslater														
<i>Cherax destructor</i>	1x Yabby							X							
ARACHNIDA															
ACARINA	Big red mite		X	X	X		X		X			X		X	
ARANEAE	Wolf spider			X			X						X		
INSECTA															
ODONATA	Damselfly nymph	X		X					X	X	X	X	X	X	
EPHEMEROPTERA	Mayflies									?					
COLEOPTERA	Unidentified SP. 1								X			?	?		
	?Aquatic weevils	X	X	X	X	X	X								
	Diving beetles	X	X	X	X	X	X		X		X	X	X	X	
	Unidentified sp. 2			X	X										
TRICHOPTERA	Unidentified 1				X	X									
	Unidentified 2			X											
	Unidentified 3			X											
DIPTERA	Larvae									X					
AMPHIBIA															
	Tadpoles	X	X	X	X	X		X	X		X	X	X	X	

#### 2.4.7.2 Plant diversity and richness

At Little Darkin Swamp, there is a high cover of annual herbs in spring and early summer, which disappear by mid to late summer as the claypan dries. Of the 36 claypan specialist taxa (endemic to or with distributions centred on claypans) described for the southwest (Gibson et al., 2005), at least 8 have been identified at Little Darkin Swamp; *Eryngium pinnatifidum* subsp. *palustre*, *Rhodanthe pyrethrum*, *Schoenus natans*, *Tribonanthes* sp., *Isoetes drummondii*, *Ornduffia submersa* (previously *Villarsia submersa*), *Verticordia plumosa* and *Stylidium* sp.

The flora of Little Darkin is most similar to the claypans of the Pinjarra plain described by Sim (2012). These include shrublands dominated by *Melaleuca* species (including *M. lateritia* and *M. viminea*), and perennial sedgelands associated with these two species. Deeper claypans are usually dominated by *M. lateritia* shrubland and sustain annual grasslands, herblands and



sedgelands at different times of winter and spring. The sedgelands at the Pinjarra claypans are dominated by *Meeboldina* species (including *M. coangustata*) and *Chorizandra enodis*, which fits very well with the vegetation composition at Little Darkin Swamp. The herbland is dominated by *Tribonanthes*, *Stylidium* and *Asteracea* species, similarly to Little Darkin, which occur in spring as the clay dries. When the basin is inundated, *Ornduffia submersa*, *Cycnogeton huegelii*, and other aquatic plants form a herbland during winter (Sim, 2012c). The flora of Little Darkin Swamp is a combination of the flora described for the five clay pan community types (Brown et al., 2015).

Amongst the other annual species growing in and surrounding the basin were 3 species of orchids (*Thelymitra mucida*, *Microtis atrata*, and *Prasophyllum macrostachyum*) and six species of *Stylidium* (Table 2.7). Some of the species observed at Little Darkin Swamp are listed as threatened and priority flora by the Department of Parks and Wildlife (Tanya Llorens, personal communication) including *Stylidium asymmetricum* (Priority 2), *Phyllangium palustre* (Priority 2), *Stylidium rubricalyx* (Priority 3), *Schoenus natans* (Priority 4), *Ornduffia submersa* (Priority 4), and *Acacia cuneifolia* (Priority 4). Priority 2 species are species that are known from one or few locations, located in areas of nature conservation and are in urgent need of further research. Priority 3 species are known from several locations and do not appear to be under immediate threat, but could be affected by known threatening processes. Priority 4 species are rare, near threatened and other species in need of monitoring (Department of Parks and Wildlife, 2017).

Some common species surrounding the wetland were *Drosera* sp., *Hyaloperma cotula*, *Stylidium* sp., *Ptilotus* sp., and *Utricularia multifida* (Table 2.7). The non-aquatic annual species most common in the wetland were *Tribonanthes longipetala* and *Utricularia multifida*.

**Table 2.7.** Plant species identified within and in the proximate area of the wetland. Species with asterisk were found within the wetland basin.

Type	Species name	Type	Species name
<b>Annuals</b>	<i>Hyalosperma cotula</i> *	<b>Aquatic</b>	<i>Marsilea drummondii</i> *
	<i>Podolepis gracilis</i>		<i>Cynnogeton huegelii</i> *
	<i>Drosera glanduligera</i>		<i>Ornduffia submersa</i> *(P4)
	<i>Rhodanthe pyrethrum</i> *		<i>Crassula natans</i> *
	<i>Utricularia multifida</i> *		<i>Myriophyllum drummondii</i> *
	<i>Stylidium calcaratum</i> *		<i>Isoetes drummondii</i> *
	<i>Stylidium roseoalatum</i> *		<i>Callitriche stagnalis</i> *
	<i>Stylidium longitubum</i> *		<i>Utricularia inaequalis</i> *
	<i>Stylidium asymmentricum</i> (P2)		
	<i>Stylidium rubricalyx</i> (P3)		
	<i>Schoenus natans</i> (P4)		
	<i>Phyllangium palustre</i> (P2)		
<b>Geophytes</b>	<i>Lysimachia arvensis</i> L.	<b>Perennials</b>	<i>Acacia cuneifolia</i> (P4)
	<i>Drosera zoneria</i>		<i>Hakea marginata</i>
	<i>Drosera aberrans</i>		<i>Hakea varia</i>
	<i>Thelymitra antennifera</i>		<i>Melaleuca lateritia</i> *
	<i>Prasophyllum macrostachyum</i> *		<i>Melaleuca viminea</i>
	<i>Thelymitra mucida</i> *		<i>Verticordia densiflora</i> *
	<i>Thysanotus thyrsoides</i>		? <i>Verticordia plumosa</i>
	<i>Drosera menziesii</i>		<i>Verticordia acerosa</i>
			<i>Chorizandra enodis</i> *
			? <i>Juncus</i> sp.
			<i>Meeboldina coangustata</i> *
			<i>Orthrosanthus laxus</i>
			<i>Conostylis</i> sp.
			<i>Sowerbaea laxiflora</i>
			<i>Stylidium repens</i>
			<i>Ptilotus manglesii</i>
			<i>Eryngium pinnatifidum</i>
			subsp. <i>Palustre</i>

## 2.5 Discussion

There are many similarities between Little Darkin Swamp and the vernal pools of California. Keeley and Zedler (1998) drew similar parallels between vernal pools of California and seasonally inundated clay-based wetlands in southwestern Australia, which have many similar features. Among these are formation on clay or carbonate mud, an impermeable duripan, and precipitation as their main water source (Keeley & Zedler, 1998). The most fitting description of Little Darkin Swamp in terms of descriptors of geomorphology and hydrology is: a seasonal wetland formed in a shallow basin, with alternating cycles of surface water and extended dry periods, which fits with the definition of vernal pools. Furthermore, Little Darkin Swamp's hydrology consists of four annual phases, similar to those described for vernal pools with (i) a wetting phase in autumn, (ii) an aquatic or inundation phase in winter, (iii) a waterlogged-terrestrial phase in late spring, and (iv) a drought phase in summer.

The Drummond Nature Reserve (DNR) is located in the Julimar State Forest in the Western Australian Wheatbelt. Located within this reserve are two freshwater claypans, identified as 'claypans with mid dense shrublands of *Melaleuca lateritia* over herbs', and have been described as some of the last of their type to remain in their natural state (Keighery, Gibson, Webb, & Muir, 2002). Here we propose that Little Darkin Swamp has many similarities to these claypans, and that it could provide an additional example of this claypan type in its natural state. These similarities include location, vegetation and hydrology. The comparison will help put the wetland into a regional context and determine whether it belongs to the same claypan type.

### **Shape and size**

Little Darkin Swamp is mesoscale, which makes the wetland significantly larger in size than the average range in area for vernal pools of California. The shallow depth of Little Darkin (0.5m) falls within the range of depth for vernal pools (0.1 to 1m). The depth of the basin is also similar to the two claypans located at Drummond Nature Reserve (DNR), which have been described to be shallow with <0.5 metres maximum depth (Department of Parks and Wildlife, 2013).

### **Topography, substrate and soils**

In terms of topography, Little Darkin Swamp is a clay-based depression in the upper part of the landscape where water pools in the rainy season due to run-off from surrounding areas and poor infiltration. It is located on an area elevated above the surrounding catchment, but is surrounded by level terrain. It has no connection to any surrounding waterbodies and therefore forms a closed depression. Similarly, vernal pools can only form in closed and shallow depression on relatively level terrain (Keeley & Zedler, 1998). The DNR claypans represent the lowest point in the surrounding landscape on level terrain (Forbes & Vogwill, 2012).

The substrate of the claypan of Little Darkin Swamp consists of mainly hard clay in the soil depression which forms an imperious layer that prevents downward filtration of water and makes a perched water table. Similarly, vernal pools occur on many geological surfaces and are always underlain by low-permeability layers such as claypans or hardpans, clay-rich soils, mudflows or bedrock (Rains et al., 2006).

The soils of Little Darkin Swamp consisted of heavily weathered clay-rich topsoil typical of vernal pools and other clay pans of southwestern Australia (Featherstone & Brown, 1990; Forbes &

Vogwill, 2012; Keeley & Zedler, 1998). For example, the substrate and soils of Little Darkin Swamp conform to those of the claypans of the Drummond Reserve, with organic-rich topsoil and underlying heavy clay rich soil.

The clay dominance (>90%) in freshwater claypan sediments indicates significant nutrient retention capabilities, since they have an ability to lock N and P into the sediments (Forbes & Vogwill, 2012). However, my results indicate very low dissolved N and P in the water column. The ancient nature of Darling Range lateritic soils means that they are generally highly nutrient deficient, resulting in biota adapted to low nutrient conditions (Bunn & Davies, 1990). High clay content of the basin sediments will also readily adsorb P making it unavailable for plants. The low oxygen and high moisture, combined with high organic carbon (C) content in the sediments, may also lead to high denitrification rates that would also reduce N availability.

#### **Water permanence, depth, source and rate of water movement (hydrology)**

The Little Darkin Swamp hydroperiod depends on precipitation, local surface runoff and ambient temperatures. Like vernal pools, there is a lack of water input by long distance drainage (see Keeley & Zedler, 1998). Water loss occurs mainly by evapotranspiration, although there is also likely to be some infiltration. The common feature of the hydrology among the wetland types considered here (Little Darkin, Californian vernal pools, and claypans of the Drummond Reserve) is their shallow basins and relatively low water levels.

Standing water only occurs in the basin of Little Darkin Swamp when the rate of water input has been higher than the rate of evapotranspiration, which normally occurs during winter and early spring when temperatures are lower. This filling process also conforms to the hydrology of vernal pools (Keeley & Zedler, 1998). The dry season during summer is characterised by desiccating soils that prevent aquatic and wetland plant species from growing, and only terrestrial or tolerant perennial species can persist, such as *Melaleuca lateritia*, *Verticordia spp.* and other woody perennial wetland shrub species (Loomes, 2000). The transition phase between wet and dry occurs in mid- to late spring, when many water-dependent species disappear and there is a proliferation of short-lived annuals and geophytes in the outer areas of the wetland.

The depth and duration of inundation at Little Darkin Swamp varies on an inter-annual basis, depending on winter rainfall and temperatures, as proven by the contrast in water depth and duration in 2015 and 2016. This means that the ecology and biota of the wetland will also differ

from year to year, and that the wetland biota need to be relatively resilient to drought and lower rainfall years, a feature that is also common to vernal pools of California (Rains et al., 2006).

The pattern of filling for Little Darkin Swamp is very similar to the hydrology of the Drummond Reserve claypans (Pinder et al., 2013) and is typical of this type of ephemeral wetland (Jolly, Mcewan, & Holland, 2008; Prober & Smith, 2009). The 6 month duration of the hydroperiod at Little Darkin Swamp in 2016 was also seen at the DNR claypans in 2011 (Pinder et al., 2013). However, peak water depths at Little Darkin Swamp in 2016 were higher than at the two similar claypans in Drummond Nature Reserve in the winter of 2008. The surface water peaks measured at these wetlands were 0.2 and 0.3m, compared to a maximum depth of 0.56m at Little Darkin in 2016 (Forbes & Vogwill, 2012). However, water levels at Little Darkin in 2015 were lower and more similar to the Drummond Nature Reserve claypans in 2008, suggesting hydrology is highly dependent on rainfall and varies inter-annually in both locations.

### **Water quality**

The relatively clear, low turbidity water of Little Darkin Swamp resembled the claypans at DNR, although turbidity was somewhat lower ( $\leq 2$  NTU). However, the surface water of Little Darkin was coloured (gilvin 52 g440/m), which compares to the moderately coloured ( $\leq 140$  TCU) claypans of the DNR (Department of Parks and Wildlife, 2013).

The low conductivity at Little Darkin was similar to recorded conductivity at the Drummond Reserve claypans, where winter surface water also were fresh ( $104\text{--}477\mu\text{S/cm}$ ) (Pinder et al., 2013). In comparison, the conductivity recorded at the Californian vernal pools were much lower ( $25\text{--}66\mu\text{S/cm}$ ).

Levels of dissolved oxygen recorded were relatively high at all sites ( $8.7\text{--}10.2\text{ mg/L}$ ) at Little Darkin when the wetland was full, and similar to DO levels recorded at DNR (Department of Parks and Wildlife, 2013). The levels of DO are considered within the 'normal' range for freshwater environments ( $6\text{--}10\text{ mg/L}$ ) (Sim, 2012b). The diurnal changes of DO was more pronounced at Little Darkin Swamp when water levels are low, and especially once water evaporated. The shallow basin results in high surface water to volume ratios and causes high diurnal changes in temperatures which in turn affects levels of dissolved oxygen.

The surface water pH of Little Darkin during winter, ranging between pH 6.4–6.8, is also similar to the range at the Drummond Reserve clay pans (pH 6–7) (Forbes & Vogwill, 2012). The water

generally has poor buffering ability so that once water evaporates, the pH becomes variable both temporally and spatially, and generally becomes more basic as opposed to acidic.

The low chlorophyll-*a* concentrations at Little Darkin indicated low phytoplankton abundance, and were not of concern. It also indicated that the low nutrient levels in the wetland, together with lower lights and low temperatures during winter suppressed algal growth. The low concentrations could thus have been a result of winter sampling, rather than spring. However, the levels were similar to values recorded at the clay pans of Drummond Nature Reserve (Department of Parks and Wildlife, 2013), suggesting water quality is generally good at these claypans.

### **Nutrients and hydrochemistry**

Nutrients were measured during the winter of 2015 when water levels were low, from two locations in the wetland. As temperature and irradiance are lower during winter, slower decomposition will cause less nutrients to be released into the water column. As spring approaches, higher decomposition of detritus will cause higher levels of nutrients in the water. Therefore, the nutrient concentrations measured are only representative of winter 2015 at Little Darkin Swamp, and would likely have been higher during spring.

Nonetheless, the nutrient status of Little Darkin in winter 2015 was oligotrophic, with low nutrients such as phosphates and nitrates. There was a relatively high amount of dissolved oxygen in the water, due to relatively low water temperatures and an abundance of aquatic plants (Sim, 2012b). Vernal pools also tend to have low levels of nutrients that are similar to oligotrophic lacustrine habitats (Keeley & Zedler, 1998). In addition, this is also similar to the low nutrient concentrations at the claypans at the Drummond Nature Reserve (TN 0.1-5mg/L and TP  $\leq$ 0.5mg/L) (Forbes & Vogwill, 2012).

Compared to vernal pools of California (CA), there were some similarities in specific nutrient concentrations. Ammonium-nitrogen levels were below detection for both Little Darkin Swamp and vernal pools of CA, although nitrate-nitrogen levels were slightly higher for CA vernal pools (0.17mg/L) (Rains et al., 2006). Much like at Little Darkin Swamp, phosphate-phosphorus concentrations were also recorded to be below detection limits at CA vernal pools.

The proposed total nitrogen and phosphorus threshold required for protection of native vegetation in the Western Australian Wheatbelt is 4mg/L and 5mg/L respectively (Prober & Smith, 2009), well above those recorded at Little Darkin. The low N and P values reflect low nutrient inputs (since there is no agricultural and urban run-off in the small catchment), and as mentioned

above could also be a result of high clay contents (>90%) of the sediments causing high nutrient retention (Forbes & Vogwill, 2012). Seasonal drying of the wetland can result in high annual variation in total-nitrogen concentration, due to variable amounts of accumulated material being de-nitrified or volatilised (Department of Parks and Wildlife, 2013).

In terms of ionic composition of the surface water, there were similarities between Little Darkin and the DNR claypans (Table 2.8), but also with other areas of the Perth region (Table 2.8). Specifically, the sodium and chloride concentrations at Little Darkin Swamp were lower compared to the wetlands of the Gnangara mound (Lake Gnangara) and the Swan Coastal Plain (SCP) (Table 2.8) indicating that it is less saline (Boulton et al., 2014). However, the relative levels of the most common ions at Little Darkin Swamp were similar to those of the SCP, Gnangara mound and DNR claypans. Sodium and chloride were the dominating ions at Little Darkin, followed by potassium, similar to the other regions. Magnesium levels were more similar to the SCP than the Gnangara mound, and were the same as at the DNR claypans. In addition, the ionic dominance of Little Darkin and the claypans of Drummond Reserve were similar in that they were both dominated by  $\text{Na}^+$  and  $\text{Cl}^-$  (Forbes & Vogwill, 2012) due to the relative influence of atmospheric salt in the surface water, rather than salts of geological or marine origin. This contrasts with Californian vernal pools.

**Table 2.8.** Comparison of average ionic composition (mg/L) of Little Darkin Swamp, Gnangara Mound (Lake Gnangara) (Williams & Buckney, 1976), the Swan Coastal Plain (Bekele, 2006) and Drummond Nature Reserve Claypans (Forbes & Vogwill, 2012).

Location	$\text{Na}^+$	$\text{Cl}^-$	$\text{K}^+$	$\text{Mg}^{2+}$	$\text{Ca}^{2+}$
Little Darkin Swamp	53	69	5	2	2
Gnangara mound	76	90	2	20	2
Swan Coastal Plain	97	96	<1	<1	<1
Drummond Nature Reserve Claypans	30	45	6.5	2	1.25

### Vegetation scale, areal extent and distribution pattern

Following the wetland vegetation classification proposed by Semeniuk et al. (1990), the vegetation cover of Little Darkin is a micro-scale wetland complex with complete (>90%) cover (sensu Semeniuk et al., 1990). It has concentric vegetation cover, meaning that the vegetation organisation is zoned (Semeniuk et al., 1990). This structure, forming concentric zones from the outsides to the centre of the basin, is also typical of vernal pools in California. California vernal pools typically have dense coverage with mainly native annual grasses, forbs, and pool-bed algae (Rains et al., 2006), which is similar to the vegetation pattern observed at Little Darkin Swamp and the clay pan community type 'claypans with shrubs over herbs' in southwestern Australia (Brown

et al., 2015). However, the dominant plants at Little Darkin Swamp consist of tall shrubs rather than grasses and herbs, which make up the understory.

We divided Little Darkin Swamp into 6 zones based on differences in community structure and composition, which are associated with areas of differing elevation. The identified zones were based on elevation which influences their frequency and duration of inundation, and leads to variation in the vegetation type. Similarly, vernal pools in the State of Washington, USA, have also been described to have six vegetation zones with differing widths and presence (Crowe, Busacca, Reganold, & Zamora, 1994). However, the sequence of the zones are consistently sequentially ordered in these vernal pools, whereas at Little Darkin Swamp and the community type 'claypans with shrubs over herbs' in southwestern Australia they are not. This may be due to disturbances such as grazing or weed invasion in the basin of these claypans described by Brown et al. (2015), or due to a more undulating substrate surface, causing patchiness of the vegetation zones.

Little Darkin Swamp is dominated by a terrestrial and semi-aquatic flora adapted to periods of alternating annual inundation and desiccation as well as species that have a specialised aquatic lifeform during the inundation phase. Within the basin of Little Darkin Swamp there are sedges and rushes growing in patches, but mainly occurring closer to the edges of the basin. In vernal pools, many of the species that experience little or no inundation are annual grasses and forbs, which occur outside the pool basin as the surrounding grassland flora (whereas claypans of southwestern Australia are generally surrounded by forest). In the lower areas in the centre of the basin of Little Darkin, plant taxa that are restricted to the claypan habitat occur, most likely due to intolerance of desiccating and hot soil during summer as well as varying periods of flooding in the wet season. This structure has also been described for Californian vernal pools (Crowe et al., 1994). This prevents many aquatic wetland species from growing in claypans and vernal pools, and only specialised species can survive.

In terms of structure and floristics, the vegetation at Little Darkin swamp is similar to the claypans found at Drummond Nature Reserve. The vegetation structure consisted of an aquatic and amphibious herb layer (in winter) with an overlying shrub layer (Chow, Vogwill, & Forbes, 2010). The flora of DNR claypans is characterised by aquatic (e.g. *Hydrocotyle lemnoides*) and amphibious (e.g. *Glossostigma diandrum*, *Villarsia capitata* and *Eleocharis keigheryi*) taxa, combined with the shrub layer dominated by *Melaleuca lateritia*. The DNR claypans also contain a number of the state's uncommon taxa including *Hydrocotyle lemnoides* and *Schoenus natans* (Keighery et al., 2002), with the latter species also found at Little Darkin Swamp.



## Macroinvertebrates

The macroinvertebrate list from this study is probably not a complete record of the taxa at Little Darkin Swamp. We found a low taxon diversity, however this may be due to the unusually short period of inundation at the wetland in the year of survey (2015), combined with inadequate taxonomic knowledge. Many of the taxa were only able to be identified to class or phylum. Generally, years with shorter hydroperiod and less frequent flooding result in lower species richness in these claypans, mostly through senescence and reduced replenishment of resting stages, as well as reduced likelihood of colonisation (Pinder et al., 2013). This topic will be revisited in Chapter 4.

A survey of the aquatic invertebrates of the DNR claypans (Pinder et al., 2013) identified a very diverse community compared to other wetlands of southwestern Australia, suggesting a high diversity could also be present at Little Darkin. Many of the invertebrate species cannot tolerate major changes in water chemistry, in particular salt and nutrients (Forbes & Vogwill, 2012). However, salinity at Little Darkin was low (0.124 ppt), suggesting that this would not affect richness, as low levels of salinity generally does not decrease species richness in the Wheatbelt of Western Australia (Delaney, Shiel, & Storey, 2016). In addition, nutrient levels were below detection (although rehydration of sediments showed detectable, but low concentrations, see Chapter 4), suggesting that nutrient enrichments would not be negatively affecting the richness, as has been found previously on the Swan Coastal Plain (e.g. Balla & Davis, 1995). It has also been found that wetlands with marked seasonal changes in water levels have higher macroinvertebrate richness than permanent ones (Balla & Davis, 1995). These characteristics all indicate potentially high macroinvertebrate diversity and richness at Little Darkin Swamp.

Generally, seasonal clay-based wetlands of southwestern Australia have a high invertebrate species richness, ranging from 30-95 species each sampling time and place, though this can be highly variable due to timing and duration of flood events prior to sampling (Appendix, Figure 6.1) (Department of Parks and Wildlife, 2013). Water depth is another factor that could affect species richness for these seasonal wetlands, and might have contributed to the low taxa richness at Little Darkin in 2015. At Goonaping Swamp, also located in the Darkin Swamp Catchment (see Figure 2.1), 71 taxa were collected in 1998, of which 28 were insects and 29 crustaceans (Appendix, Table 6.1) (Cale et al., 2004). This gives us an idea of the numbers to expect at Little Darkin Swamp. At the Drummond Reserve wetlands, a combined total of 160 species were collected between 2004-2012, which comprised taxa such as *Hydra*, Turbellaria, Nematoda, Gastropoda, Polychaeta, Oligochaetes, Hirudinea, Tardigrada, *Acarina*, Copepoda, Cladocera, Ostracoda, Conchostracans,

Ceratopogonidae, Culicidae, Chironomidae, Coleoptera and Trichoptera (Pinder et al., 2013). Most of these invertebrates are strongly adapted to highly variable hydrological regimes (Brock, Nielsen, Shiel, Green, & Langley, 2003).

To get a broader picture of the macroinvertebrate communities and species richness at claypans, it is recommended to sample once a year and consider monitoring results over several years (Department of Parks and Wildlife, 2013). This will even inter-annual differences caused by inconsistent timing of sampling, among other factors. Annual macroinvertebrate sampling at Little Darkin Swamp would aid detection of changes in diversity, as well as any declines in water quality. Macroinvertebrate assessment, together with other physical and chemical assessment, is a good indicator of water quality in wetlands (Davis et al., 1999).

Another approach to monitor the condition of the macroinvertebrate assemblage at Little Darkin Swamp is to set targets for triggers. A suggested trigger for management action at the DNR claypans is for no less than four indicator species to be present in each wetland on any sampling occasion (Department of Parks and Wildlife, 2013). The indicator species comprise seven species which are closely associated with seasonal clay-based wetlands in inland southwestern Australia: a clam shrimp (*Lynceus sp.*), an ostracod (*Bennelongia australis*), two beetles (*Paroster courage* and *Berosus approximans*), a copepod (*Calamoecia attenuata*), a phantom midge (*Promochlonyx australiensis*), and a cladoceran (*Latonopsis brehmi*). Similar targets could apply to other seasonal claypans of southwestern Australia, such as Little Darkin Swamp.

## Conclusion

Little Darkin Swamp conforms to the descriptions of vernal pools of California in terms of many of its wetland attributes. There were similarities particularly in terms of topography, soil and substrate, vegetation structure and communities, hydrology, nutrients and hydrochemistry. However, there were also some differences, such as size and floristics, and due to the lack of information about macroinvertebrates both at Little Darkin Swamp and vernal pools of California it is not known whether the aquatic fauna conforms. These similarities in addition to the similar Mediterranean climate and their biodiversity significance, make these wetlands uniquely comparable.

Based on the available information about the claypans at the Drummond Nature Reserve it is also reasonable to conclude that Little Darkin Swamp is indeed very similar to these systems in terms of the wetland attributes used for classification by Semeniuk and Semeniuk (2011). However, long-

term data are needed to determine whether they also respond in the same way to inter-annual variations in climate, such as temperature and rainfall, and disturbances such as grazing, weed invasions and physical alterations by humans. Furthermore, it is not known whether their wetland functions or internal processes, such as ecosystem metabolism and responses to changes in hydrological regime, will be the same.

The apparently low nutrient status at Little Darkin, and the confirmed low nutrient levels for the clay pans of DNR and the vernal pools of CA, raise a question about their trophic status: would they be regarded as autotrophic or are they generally heterotrophic and reliant on external sources of carbon? Furthermore, what does the inter-annual variation in frequency, duration and depth of inundation in these wetlands mean for the biotic response when sediments are annually re-hydrated? To permit future comparisons of these systems in southwestern Australia, as well as to gain understanding of how Little Darkin Swamp functions, these wetland processes will be examined in the following chapters.

# Chapter 3. Temporal and spatial variation in ecosystem metabolism and food web carbon transfer

## 3.1 Introduction

Gross primary production (GPP) and respiration (R) are considered the most fundamental processes in ecosystems (Hu et al., 2015). They describe biochemical pathways that make organic carbon molecules and energy available to the cells, and integrate biogeochemical and trophic processes at the ecosystem level. These processes are known as ecosystem metabolism, which is an integrated measure of overall rates of organic matter production (GPP) and consumption or respiration (R) (Odum, 1956). In recent decades, there has been increasing interest in understanding the controls on ecosystem metabolism in aquatic systems (Hu et al., 2015).

Gross primary production (GPP), ecosystem respiration (R) and their relative contribution given by net ecosystem production ( $GPP - R = NEP$ ) and the P/R ratio ( $GPP:R$ ) (Hunt et al., 2012) show large temporal and spatial variations across aquatic ecosystems (Staehr & Sand-Jensen, 2007). NEP can be used to understand the role of lakes as sources or sinks of atmospheric  $CO_2$  through net heterotrophic or autotrophic annual balances, and can be used to evaluate the trophic state of ecosystems (Hu et al., 2015). It has been shown that most freshwater systems are heterotrophic, contrary to the previous assumption that autotrophy or metabolic balance occurs in most aquatic systems (Hu et al., 2015).

In practice, a heterotrophic state is defined as area-normalised gross carbon oxidation indicated by the total production of carbon dioxide from respiration (production < respiration), while autotrophic state is the area-normalised gross carbon fixation rate (GPP) over at least a 24-hour period (production > respiration) (Dodds & Cole, 2007). An autotrophic system has a net addition of energy and is represented by  $NEP (GPP - R) > 0$  and a P/R ratio of  $>1$ , meaning that more oxygen (and therefore carbon) is produced via photosynthesis than that consumed via respiration. A heterotrophic system is represented by  $NEP < 0$  and a P/R ratio of  $< 1$  and will indicate the breakdown of a major organic carbon resource from outside of the system (Hanson et al., 2003; Hu et al., 2015; Staehr & Sand-Jensen, 2007; Staehr, Sand-Jensen, Raun, Nilsson, & Kidmose, 2010; Staehr et al., 2010). In a heterotrophic system the microbes, fungi or detritivores are the most

important part at the base of the food web, whereas in autotrophic systems photosynthesising algae and higher plants and the herbivores that feed on them are the most vital link for higher trophic levels.

By measuring concentrations of dissolved oxygen (DO) in wetlands, gross primary production (GPP) and ecosystem respiration (R) can be estimated (Grace & Imberger, 2006). GPP is the assimilation of inorganic carbon into organic plant material and release of oxygen via photosynthesis. R is the release of CO<sub>2</sub> and oxygen uptake due to the organism's aerobic degradation of organic material, and anaerobic respiration causing oxidation of reduced compounds (Staeher & Sand-Jensen, 2007). Using new, reliable and accessible technology, diurnal, seasonal and annual measurements can be obtained. This allows us to examine how changes in environmental factors, such as changing water levels, affect aquatic metabolism.

Measurements of open water diurnal dissolved oxygen (DO) concentrations, called the oxygen mass balance method, are widely used to examine ecosystem primary production and respiration (Staeher et al., 2010). Changes in DO concentrations in a lake reflect the biological balance that is a result of photosynthetic production and respiratory consumption as well as physical exchange of oxygen between air and water (Staeher et al., 2010). Thus the long term monitoring of DO dynamics in lakes allows quantification of lake metabolism (Hu et al., 2015). During daylight hours, photosynthesis produces DO, while DO consumption occurs continuously and is the only metabolic process to occur at night. Thus, by measuring changes in DO over a 24 hour period, we can quantify NEP, R and GPP (Staeher et al., 2010).

Many factors affect the rates of metabolism in aquatic systems. Metabolism is driven by different physical, chemical, and biological forces such as temperature, irradiance, wind speed, pH, nutrients and carbon (Hu et al., 2015). These factors create temporal variations in metabolism, which can be observed at different scales. Daily and weekly variability in metabolism can be caused by variations in irradiance, whereas monthly and seasonal changes in metabolism suggest changes in trophic group activity and ecosystem structure (Staeher & Sand-Jensen, 2007).

Particularly for small, shallow systems, such as Little Darkin Swamp, with large seasonal fluctuations in water levels, and large seasonal and diurnal fluctuations in temperature, oxygen and carbon dioxide, drying and filling can change nutrient concentrations and carbon sources for the food web (Christensen, Sand-Jensen, & Staeher, 2013). These fluctuations affect decomposition rates, and can alter the amounts of organic material present in the sediments, in turn impacting nutrient concentrations available. This seasonality or ephemerality of these systems can therefore support net autotrophy during inundated periods due to active plant growth, and net

heterotrophy during drying periods reducing water volume due to higher temperatures stimulating respiration (Christensen et al., 2013). Furthermore, the seasonal hydrology of Little Darkin Swamp may reduce the organic matter load present in the sediment due to high exposure to air, radiation and temperatures during summer. This may reduce the rates of respiration during the inundated period of the wetland and changes the sources of carbon available for the food web if carbon is limiting.

Food web studies provide information about fluxes of nutrients and energy in the ecosystem, as well as a broader understanding of ecosystem function (Pettit et al., 2016). Aquatic ecosystems receive organic carbon from multiple autochthonous and allochthonous sources. Primary production by plants including algae and macrophytes within the system make up the autochthonous basis of the food web, whereas terrestrial inputs of particulate and dissolved organic carbon is the allochthonous basis for the food web (Cole et al., 2000). The allochthonous organic carbon loading can be much greater than the autochthonous primary production, and thus the respiration of this material, even small portions of it, has the potential to affect the metabolic balance of the ecosystem greatly (Cole et al., 2000). This is due to higher rates of microbial respiration, which can exceed primary production in the system. In fact, input of allochthonous organic matter is often equal to or larger than internal primary production in lakes (Cole et al., 2006). This causes total R to be higher than GPP and as a result NEP is negative (i.e.  $NEP < 0$ , net heterotrophy). As such, the sources of carbon fuelling the food web can support or explain the trophic status of the ecosystem.

Identifying the carbon sources supporting aquatic consumers and their quality is central for understanding food web dynamics (Blanchette, et al., 2014). Carbon stable isotope ratios in organisms closely reflect those of their diet, whereas nitrogen stable isotopes in organisms are often enriched compared to their diet (Jones et al., 1998; Peterson & Fry, 1987). Given that different sources of carbon (i.e. food) have distinct carbon isotope ratios, their relative contributions and importance in the consumer diets of the food web can be determined. Dual stable isotope ratios of carbon and nitrogen are more informative about food web relationships than single ratios, and have been used to trace flow of organic matter in aquatic food webs (Jones et al., 1998). Stable isotope analysis (SIA) is a technique frequently used to estimate the relative importance of autochthonous and allochthonous sources of organic carbon in aquatic food webs (Gladyshev, 2009). The  $^{13}\text{C}:^{12}\text{C}$  ratio is used to determine the relative contributions of autochthonous and allochthonous carbon sources in freshwater ecosystems, whereas the nitrogen isotope ratios  $^{15}\text{N}:^{14}\text{N}$  help determine the trophic level of food web components (Lancaster & Waldron, 2001).

This chapter aims to describe and interpret the ecosystem metabolism and trophic dynamics of Little Darkin Swamp. By examining temporal and spatial nature of ecosystem metabolism it will be possible to determine whether the system is autotrophic or heterotrophic, and how this changes in the context of the hydrological regime. In addition, the sources of carbon fuelling the food web and the trophic status of the biota within the wetland will be examined.

## 3.2 Methods

### 3.2.1 Sampling design

Ecosystem metabolism was measured during the inundated phase at two sites at Little Darkin Swamp chosen to reflect quite different vegetation cover: one site was categorised as vegetated (dense patches of *Melaleuca lateritia*) (Site 2, logger 1, see Chapter 2) and one site categorised as open water (little or no *M. lateritia* but with some occurrence of submerged and emergent aquatic plants) (Site 4, logger 2, see Chapter 2). Samples for food web analysis were collected at 7 sites within vegetation zones V1a and V1b (see Chapter 2) twice during winter in 2015, at the end of that hydroperiod.

### 3.2.2 Physical and chemical characteristics

Measurements of physical and chemical characteristics were collected as outlined in Chapter 2. Average daily water temperature is important in explaining changes in metabolic rates at different times between days and over the season and was used to convert dissolved oxygen from mg O<sub>2</sub>/L to %DO saturation (see Grace & Imberger, 2006). Several spot measurements were also taken using a water quality meter at the two DO data logger sites, including dissolved oxygen, pH, conductivity and temperature (see Chapter 2).

Site characteristics were also recorded, as they are important when evaluating and explaining patterns in the metabolic parameters (see Grace & Imberger, 2006). Site characteristics included: presence and coverage of vegetation in water body (such as sedges and submerged macrophytes), substrate of the sediment, organic matter, sign of animal access to the wetland (significance for addition of nutrient and suspended solids), geomorphology and site topography of surrounding area (Grace & Imberger, 2006) (see Chapter 2). In addition, wind speed data and sunrise/sunset times were retrieved from the Bureau of Meteorology for the nearest weather station (Bickley, WA, 40 km west of the study site) for calculation and interpretation of metabolism results.

### 3.2.2 Ecosystem metabolism

To determine the trophic status of Little Darkin Swamp, the GPP and ecosystem respiration (R) was attained directly from the amount of production and consumption of dissolved oxygen in the water body ( $\Delta DO = GPP - R \pm E$ , where E represents gaseous O<sub>2</sub> exchange across the air-water boundary). The balance between GPP and R, given as NEP or the GPP:R ratio, is used to define lake trophic classification (Hanson et al., 2003).

An open water diurnal dissolved oxygen method, which uses sondes to log continuous measurements by deployment in free water, was used to measure the system-level metabolism (Grace & Imberger, 2006). One advantage of the method is that it does not change the integrity of algae, soft sediment or macrophyte beds. The flow, light and temperature of the wetland are not changed, and there is no depletion of nutrients or oxygen caused by the fluorescence DO probes. Further, it requires shorter intervals to resolve productivity changes and allows for significantly higher estimates of mean metabolism in comparison to chamber methods (Grace & Imberger, 2006).

Open water metabolism measured the diurnal variation in DO concentrations in the water, which result from photosynthesis and respiration. Being a perched, lentic system additional inputs of O<sub>2</sub> from atmospheric gas exchange (or re-aeration), groundwater and hyporheic water were assumed to be negligible. The open water method allows determination of GPP, R and/or NEP, and the rate of primary production to respiration (P/R ratio) can be derived (Grace & Imberger, 2006; Hanson, Bade, Carpenter & Kratz, 2003; Hu et al., 2015; Hunt et al., 2012; Staehr et al. 2010; Staehr & Sand-Jensen, 2007).

Although the idea behind the open water diurnal technique is that whole lake metabolism can be estimated from a single sonde, the placement of the sonde can highly affect the outcome of the measurements. Therefore, it is suggested that lakes with large horizontal and vertical heterogeneity in primary production require deployment of multiple sondes in various habitats (Van de Bogert, Carpenter, Cole, & Pace, 2007). For this reason, we deployed two sondes at the two major habitat types present at Little Darkin Swamp; open water habitat and vegetated habitat (see Chapter 2). This allows for examination of at least some spatial variability of metabolism within the wetland.



### 3.2.2.1 Field measurements

For the purpose of this study, fluorescence DO probes (HOBO U26-001 Dissolved Oxygen Data Logger) were used. In contrast to electrochemical DO probes, no oxygen is consumed during recordings by the fluorescence probes and they are therefore more accurate in very low-flow conditions (Grace & Imberger, 2006) such as those found at Little Darkin Swamp. The HOBO logger uses RDO® Basic (Rugged Dissolved Oxygen) optical DO sensor technology with 0.2 mg/L accuracy. This provided a set of measurements for DO and temperature at two sites and allowed for calculation of metabolism and comparison between the locations, and a representative measure for the whole system. The NEP was calculated ( $NEP = GPP - R$ ) and expressed in terms of carbon. The results were expressed in % DO saturation and the suitable value in  $mg\ O_2\ L^{-1}$  was determined for calibration of the probe (using expected DO values for water-saturated air).

The DO probes were located with the membrane submerged in the water column without touching the bottom. As water levels decreased some parts of the probe were exposed to air, but never the membrane. Diurnal logging during the majority of the study period was used to assess the temporal dynamics of system-level metabolism for the whole hydroperiod. The probes were checked on at least monthly if not fortnightly, following deployment. Rates of DO concentration and temperature were continuously logged at 15 minute intervals for a total of 5 months from June-November during autumn and winter 2016. Before any initial DO measurements were recorded, an on-site probe calibration was performed on the afternoon of day 1 and until morning of day 3. As recommended by Grace and Imberger (2006), the calibration was then checked.

### 3.2.2.2 Data analysis

The Optic USB Base Station and couplers were used to offload the data from the HOBO DO loggers, and data were viewed using the HOBOWare® Pro. This software provided logger management, data graphing and data export for further analysis. Observed DO changes were related to the identified re-aeration change, respiration and GPP. Average night-time respiration rate was determined and assumed to be the same as daylight respiration (Staehr et al., 2010). Calculation of ecosystem metabolism from the logger data followed the steps and equations outlined in Staehr et al. (2010). Parameters calculated were: the depth of the mixed layer ( $Z_{mix}$ ), oxygen concentration in water in equilibrium with the atmosphere at ambient temperature ( $O_{2sat}$ ), actual DO concentration ( $mg\ L^{-1}$ ), Schmidt number, piston velocity ( $k$ ), oxygen exchange with the atmosphere ( $F$ ) ( $gO_2\ m^{-2}h^{-1}$ ), dayfraction, daytime NEP ( $NEP_{daytime}$ ), respiration ( $R$ ) and gross primary production (GPP) (Staehr et al., 2010).

The statistical program R (version 3.0.1, 5/6-2013) was used to manipulate the metabolism dataset and to render plots of GPP, R, GPP/R ratio and NEP, as well as DO concentration and temperature. More specifically, the ‘reshape’ package was used to handle the dataset and the ‘ggplot’ package was used to produce plots (R Development Core Team, 2013; Wickham, 2007, 2009).

### 3.2.3 Food web sampling and analysis

Stable isotope analysis (SIA) is a well-established approach for characterising food web structure and investigating flow of carbon in aquatic food webs (Bunn & Boon, 1993; Gladyshev, 2009; Jones & Waldron, 2003; Karlsson et al., 2012; Pingram, Collier, Hamilton, David, & Hicks, 2012).

In order to cover potential variation within the wetland, habitat types were identified to stratify sampling of biota for stable isotope analysis. Habitats were categorised into two categories: open water vs. with plant cover, following the categorisation given in Chapter 2. A structured random sampling approach was used, with three random sites per habitat (total of 6 sites) (Table 3.1). All consumers present, including macroinvertebrates and juvenile frogs, were sampled at each site and three potential sources per site (Table 3.1). Samples were collected twice during the hydroperiod in spring 2015 on the 16<sup>th</sup> September and 8<sup>th</sup> October and were combined across sites and sampling events to produce enough material for SIA. In total 60 samples of primary producers and consumers were collected from the 6 sites per sampling event, giving a total of 120 samples for stable isotope analysis.

**Table 3.1.** Sampling strategy adopted for each habitat type. Three sites of each of two habitat types sampled for each primary producer source (s) and consumer (c).

Open water	With vegetation cover
<b>3 sites:</b> Leaf litter (s) Biofilm (s) Particulate Organic Matter (seston) (s) Macrophytes (s) Macroinvertebrates (c) Amphibians (c)	<b>3 sites:</b> Leaf litter (s) Biofilm (s) Macrophytes (s) Particulate Organic Matter (seston) (s) Macroinvertebrates (c) Amphibians (c)

Four categories of primary carbon sources were sampled and included 1) leaf litter from dominant woody plant species (*Melaleuca lateritia*) (conditioned leaves); 2) biofilm attached to surfaces; 3)

macrophytes (submerged and emergent); and 4) particulate organic matter (POM) (seston) (Table 2) (see Bunn, Davies, & Winning, 2003; Leigh, Burford, Sheldon, & Bunn, 2010).

Riparian vegetation and emergent macrophytes were hand collected. Leaves from the dominant shrub were freshly cut and collected. Biofilm (attached algae) was scraped from plants using a brush (see Hadwen & Bunn, 2004) and dissolved in distilled (DI) water then filtered through a glass fibre filterpaper (Whatman). Matter trapped by the filter paper was then used for stable isotope analysis. Seston samples were collected from the water column by filtration of a pre-determined volume of surface water through pre-combusted glass-fibre filter papers (see Leigh et al., 2010), and weights were recorded in  $\mu\text{g L}^{-1}$ . All collected samples were placed on ice immediately, transported to the university facilities and frozen as quickly as possible for stable isotope analysis (as per Bunn et al., 2003).

Aquatic consumers, including macroinvertebrates and tadpoles (Hadwen & Bunn, 2004), were collected in a 500- $\mu\text{m}$  mesh pore size dip-net swept evenly for at least 20 seconds through the varying habitats present at each location. The contents were emptied into zip-lock bags and kept on ice and stored securely until return to Edith Cowan University, Joondalup where macroinvertebrates were picked and sorted into major taxonomic groups. Voucher specimens and photos were collected from each consumer taxon for reference use.

Amphibians were also sampled using sweep nets, and consisted of tadpoles of the common frog species in the area: Glauert's Froglet (*Crinia glauerti*), Motorbike Frog (*Litoria moorei*), Slender Tree Frog (*Litoria adelaidensis*) and Western Banjo Frog (*Limnodynastes dorsalis*). Tadpoles retained for tissue samples were euthanised by adding 0.65ml of clove oil per litre of water in the buckets they were kept in. The remaining tadpoles caught were returned to their point of capture unharmed. Very low numbers of tadpoles were collected, and conformed with requirements of the Ethics Approval granted for the project.

### 3.2.3.1 Laboratory sample preparations

All samples were thoroughly rinsed with distilled water. Any foreign material was removed (Leigh et al., 2010). Macroinvertebrates were categorised according to functional feeding groups (Uwadiae, 2010) with representative taxa and prepared samples consisted of between 1–20 individuals due to varying sizes and quantity (mass) needed for analysis (as outlined by UC Davis Stable Isotope Facilities (SIF) website). Samples were dried at 50–60°C for up to 48 hours (see Bunn et al., 2003; Hadwen & Bunn, 2004; Leigh et al., 2010). Small samples that met the target weight

for SIA were encapsulated whole into tin (Sn) capsules, while larger samples such as leaves and coarse material were ground, homogenised and encapsulated into tin (Sn) capsules. An electric ball-mill grinder (Retsch MM200, Haan, Germany) was used to grind the samples, or mortar and pestle, depending on roughness (Leigh et al., 2010). Each sample was large enough to hold between 20-150µg of nitrogen and 200-2000µg of carbon. The appropriate sample weight was calculated using the Sample Weight Calculator from the SIF website. Samples were then organised into clean 96-well trays and similar sample materials was grouped together (animal tissue and plant material). Each tray had a sample list and was named appropriately. All samples were sent to the UC Davis Stable Isotope Facility in Davis, California U.S.A for stable isotope analysis.

### 3.2.3.2 Stable isotope analysis

At the Stable Isotope Facility all dried and ground samples were analysed for  $^{13}\text{C}$  and  $^{15}\text{N}$  isotopes by use of a PDZ Europa ANCA-GSL elemental analyser, which was joint to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The samples were combusted at 1000°C in a chromium oxide and silvered copper oxide-packed reactor (Stable Isotope Facility UC Davis, 2015). After the combustion, samples were placed in a reduction-reactor to remove oxides (copper reduced at 650°C). Before entering the IRMS,  $\text{N}_2$  and  $\text{CO}_2$  were separated using a Carbosieve GC column (65°C, 65 mL/min). During analysis, the samples were spread with several replicates of at least two different laboratory standards previously calibrated against NIST Standard Reference Materials. The long-term standard deviation was 0.2 per ‰ for  $^{13}\text{C}$  and 0.3 per ‰ for  $^{15}\text{N}$  (Stable Isotope Facility UC Davis, 2015). The absolute delta values were conveyed relative to the international standards V-PDB (Vienna PeeDee Belemite) and Air for carbon and nitrogen, respectively (Stable Isotope Facility UC Davis, 2015).

Isotope ratios were expressed as either  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  (relative parts per million difference between the sample and conventional standards) and related to the ratio of  $^{13}\text{C}:^{12}\text{C}$  and  $^{15}\text{N}:^{14}\text{N}$  respectively. Values were reported using following equation:  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$  where  $R_{\text{sample}}$  is the isotopic ratio for the sample and  $R_{\text{standard}}$  is the isotopic ratio of the standard (Lambert et al., 2011).

To identify the origin of the primary carbon sources (detrital vs. algal vs. higher plants) that feed the aquatic consumers, the percentages of organic carbon and nitrogen values were utilised to calculate the molar basal source C:N ratios. This helps differentiate between the detritus/living tissue from  $\text{C}_3$  plants and algal carbon (Leigh et al., 2010). The C:N ratios of aquatic sources (phytoplankton in particular) are around 14:1 molar ratio; 12:1 mass ratio, and generally much

lower compared to the terrestrial ones, which have a molar ratio of approximately 50-60:1 and mass ratio of approximately 45-50:1. For organic matter this ratio tends to increase during decomposition (Leigh et al., 2010). The C:N ratios further help estimate the nutritional value of the sources for first order consumers as greater disparities between source and consumer ratios commonly suggest a larger metabolic requirement for the consumer. More similar ratios imply smaller metabolic demand for the consumer (Leigh et al., 2010).

### 3.2.3.3 Data analysis

The IsoSource mixing model program (Phillips & Gregg, 2003) was used to calculate the percentage/proportion contributions of each source to consumer's  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  value. Mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from 1<sup>st</sup> order consumers and each primary producer were used in the mixing model, based on increments of 5% that maintained mass balance within a tolerance of 1‰. Source importance (the potential contribution of each basal source to the biomass of consumers) was assessed using the 1<sup>st</sup> and 99<sup>th</sup> percentiles of IsoSource solution ranges. Assuming all potential sources are sampled, a low 'maximum' (99<sup>th</sup> percentile) indicates a source is unlikely to be important, a high 'minimum' (1<sup>st</sup> percentile) indicates a source is likely to be important, and a small range between the 1<sup>st</sup> and 99<sup>th</sup> percentiles ('1–99 range') indicates the estimate of a source's contribution is well constrained (Benstead et al. 2006; Leigh et al., 2010).

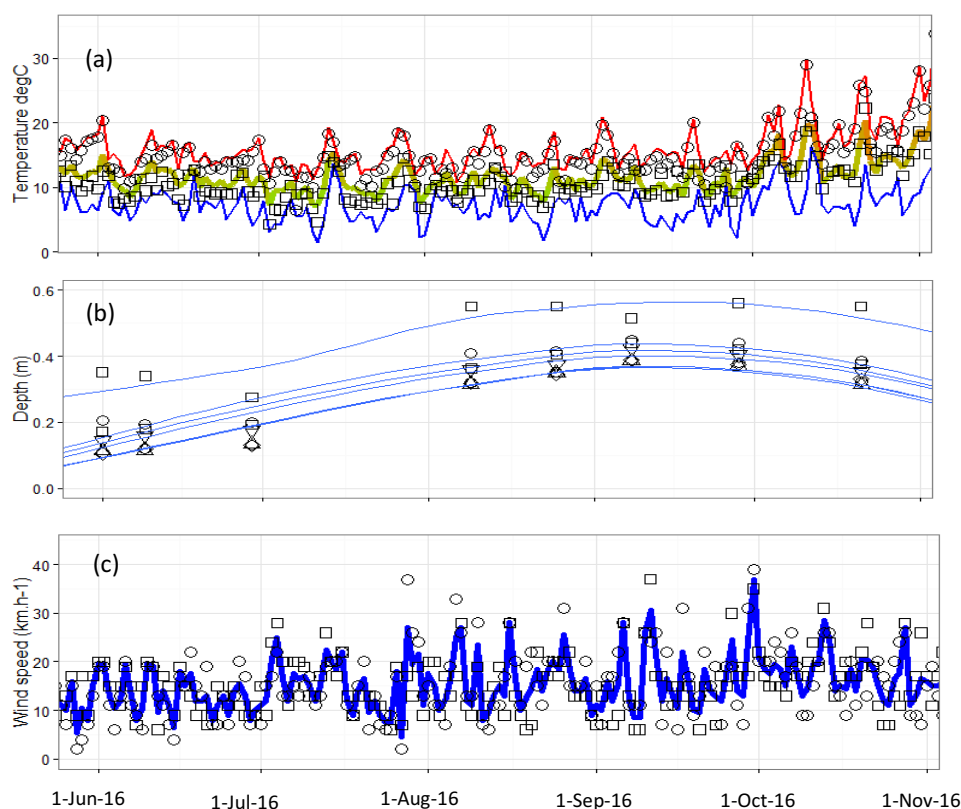
## 3.3 Results

### 3.3.1 Physical and chemical characteristics

The wetland began filling in April and continued to fill until August 2016 when rainfall was at its highest and temperatures were low. As rainfall decreased significantly in September and temperatures started increasing, water levels started decreasing (Chapter 2, Figure 2.5 and Figure 2.6).

During the sampling period, ambient temperatures stayed relatively stable between June and September, with some expected fluctuations. Average daily temperatures did not exceed 15°C until October, progressively increasing to above 20°C (Figure 3.1a). Toward the end of September, temperatures started rising together with increased wind speed at the nearest weather station (Bickley WA, BOM), causing water levels to decrease due to evapotranspiration (Figure 3.1b). Wind speed, corrected to a height of 10 m, fluctuated on a daily basis throughout the sampling period, with several wind events reaching above 20 km h<sup>-1</sup> and one event over 30 km h<sup>-1</sup> at the

start of October (Figure 3.1c). These wind events lead to increased gas exchange with the atmosphere in the shallow basin, affecting the rates of DO and ultimately ecosystem metabolism.

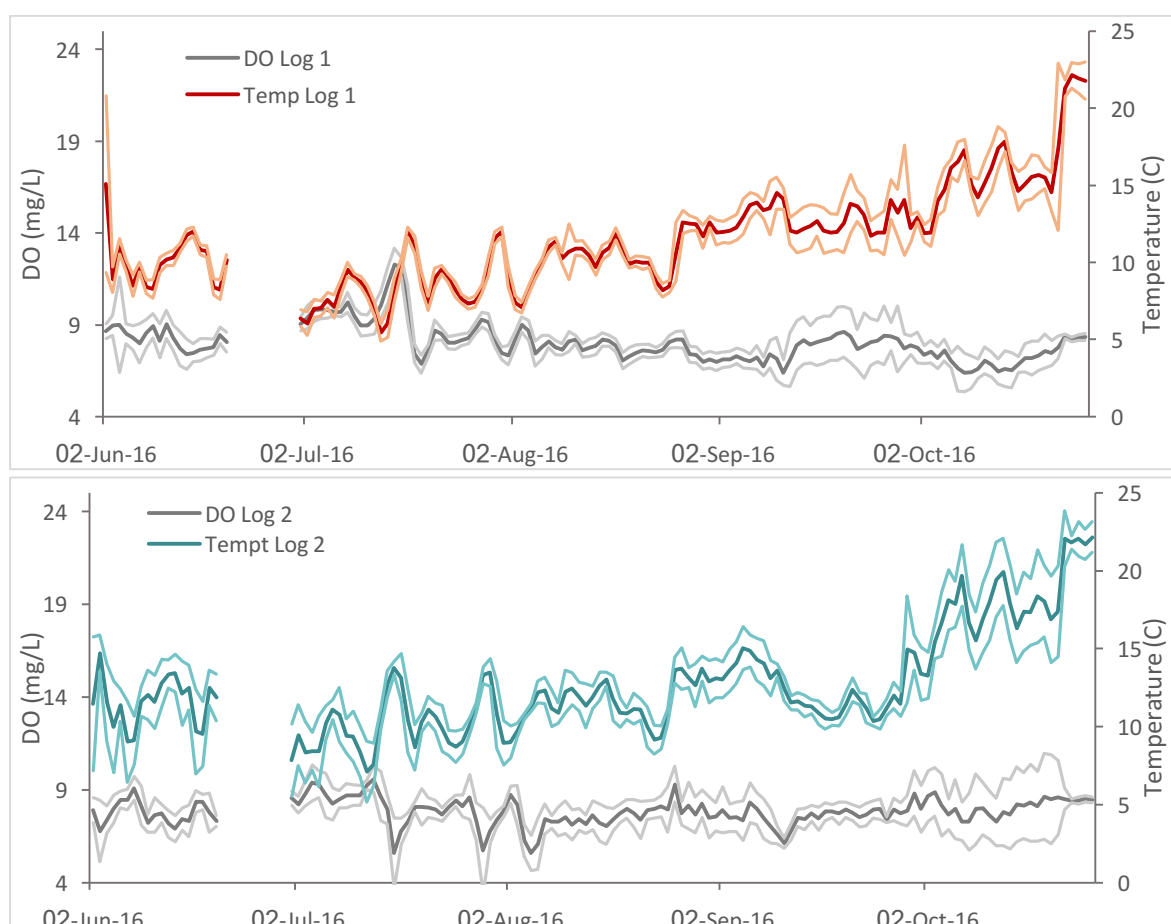


**Figure 3.1.** Daily maximum, minimum and average ambient temperature (BOM, 2016) (a), water depth (at sites 1-7, see Chapter 2) (b), daily wind speed in  $\text{km h}^{-1}$  (BOM, 2016) (c) for the period June-Nov 2016.

### 3.3.2 Ecosystem metabolism

#### 3.3.2.1 Temporal dynamics of DO concentration

Figure 3.2 shows the dissolved oxygen and water temperature dynamics during the sampling period. As temperature increased toward the end of winter and spring, so did the range in DO at both sampling locations. Daily fluctuations in DO were highest in September in the open water location, and in October in the vegetated location. Changes in DO followed changes in temperature during the sampling period, and were higher in the open area location as a result of higher water temperatures due to more light exposure (and shallower water, see Chapter 2). Maximum and minimum DO concentration were approximately 15 mg/L and 5 mg/L at the open water site, and approximately 12 mg/L and approximately 2 mg/L at the vegetated site, respectively. Water temperature fluctuated more on a daily basis in the vegetated location compared to the open water location throughout the sampling period.



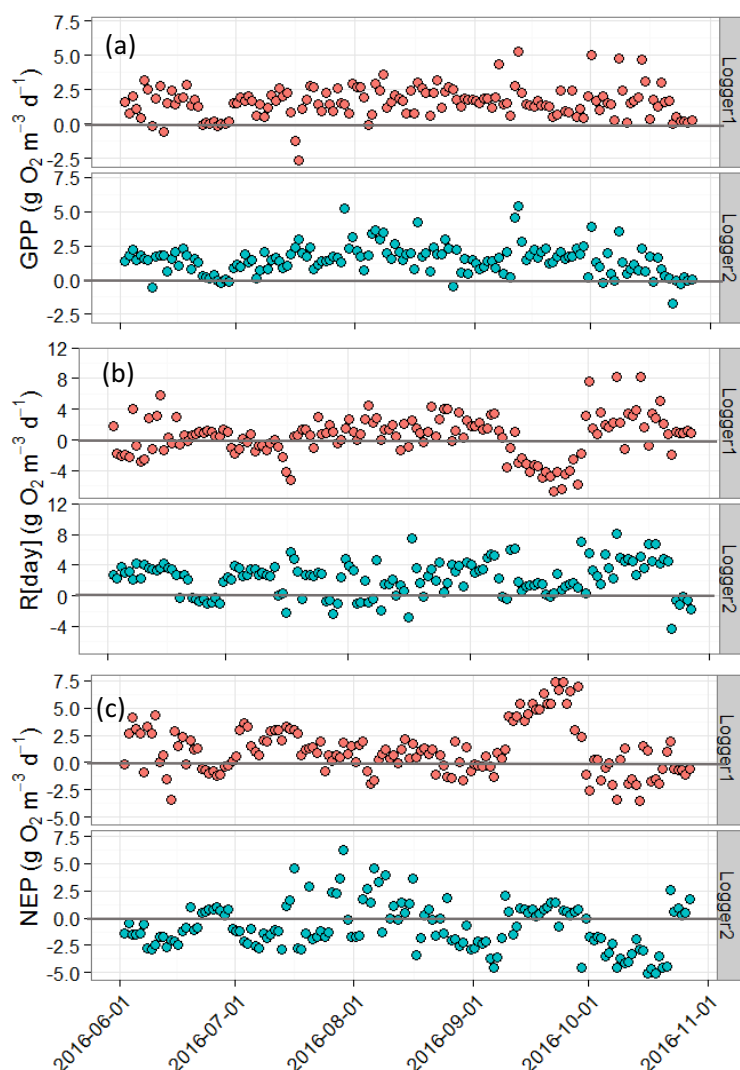
**Figure 3.2.** Average daily dissolved oxygen ( $\pm$ SD) and water temperature dynamics in 2016 (June–November) at two sites (Red Logger 1= open water, Blue Logger 2= vegetated area) at Little Darkin Swamp. Gaps represent periods where loggers were not deployed.

### 3.3.2.2 Temporal dynamics of metabolism

The open water and vegetated site had similar trends in daily GPP and R during the sampling season (Figures 3.3a and 3.3b). Highest GPP and R rates occurred in early spring (October), reaching a maximum of approximately  $5 \text{ g O}_2\text{m}^{-3}\text{d}^{-1}$  GPP and  $8 \text{ g O}_2\text{m}^{-3}\text{d}^{-1}$  R in both locations.

There was a high daily variability in NEP (GPP–R) throughout the wet-season in 2016 (Figure 3.3c). Data from both locations in the wetland suggest that Little Darkin Swamp alternated between heterotrophy and autotrophy on a monthly basis in 2016. Data from logger 1 in open water showed that NEP was particularly high between September and October ( $7.5 \text{ g O}_2\text{m}^{-3}\text{d}^{-1}$  in late September), i.e. highly autotrophic. This was similar to the same period of time in 2015 (Figure 6.4, Appendix). However, data from the vegetated area showed particularly high NEP from mid-July to mid-August, although with much higher daily variability.

Overall, NEP was both higher (more positive) and more variable in September-October 2016 than for the same period in 2015 when water levels were lower and the wetland was drying. For both sampling locations, NEP decreased toward the end of the wet-season in 2016 and was at its lowest (most negative) in October (minimum  $-5 \text{ g O}_2\text{m}^{-3}\text{d}^{-1}$  in open water, and  $-3.5\text{--}4 \text{ g O}_2\text{m}^{-3}\text{d}^{-1}$  in vegetated), resulting in a shift to net heterotrophy.

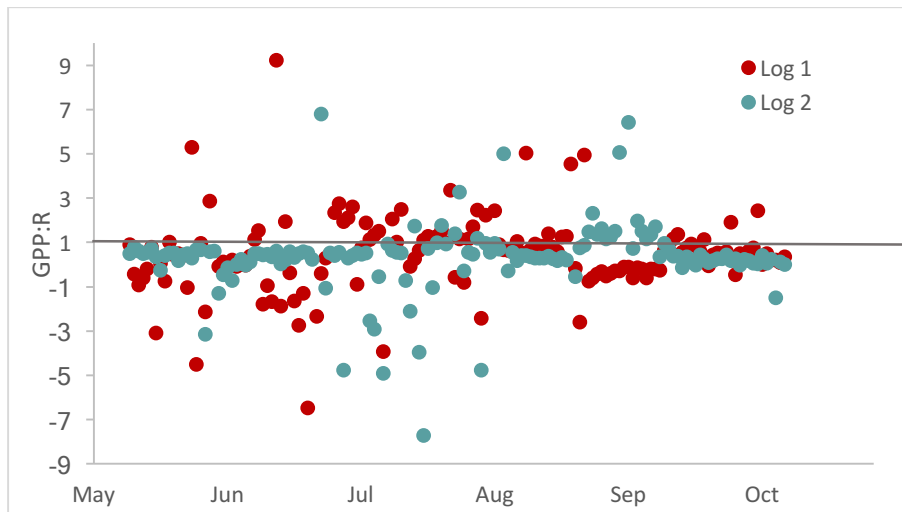


**Figure 3.3.** Average daily (a) gross primary production (GPP), (b) ecosystem respiration (R) and (c) net ecosystem production (NEP=GPP-ER) (relative contribution of GPP and ER) in 2016 (Jun-Nov) as determined from continuous  $\text{O}_2$  measurements at two locations (Red Logger 1= open water, Blue Logger 2= vegetated). NEP > 0 = net autotrophy, NEP < 0 = net heterotrophy.

There was also high variability in the GPP:R ratio on a daily basis during most of the sampling period for both locations, alternating between net heterotrophy and net autotrophy (Figure 3.4). This was most pronounced during the high rainfall months (June-July), and dropped off toward



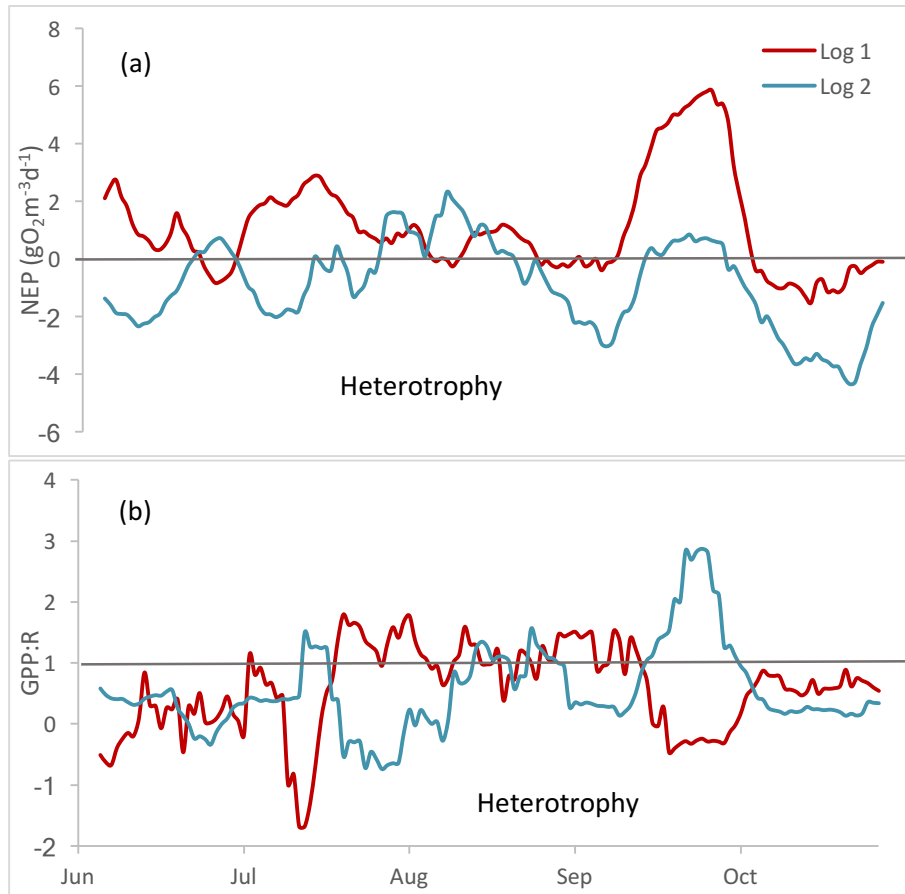
spring in September and October when the GPP:R ratio became more stabilised. However, the GPP:R ratio was below 1 most days for both locations, showing net heterotrophy.



**Figure 3.4.** Daily estimates of the GPP:R ratio for the two locations (Red Log 1= open habitat, Blue Log 2= vegetated habitat) at Little Darkin Swamp between June and October 2016.

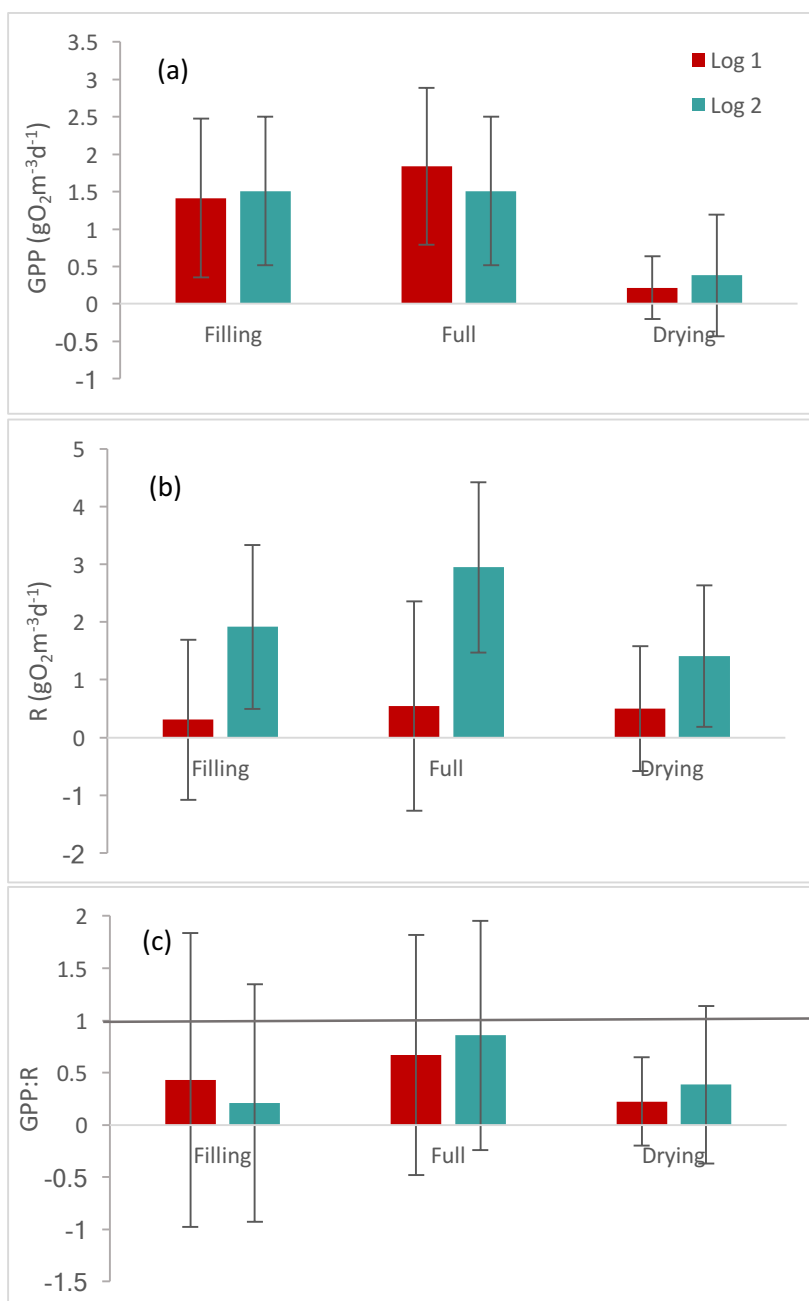
The 7-day moving averages of NEP and GPP:R smoothed the daily variations (Figure 3.5). However, the alternation between heterotrophy and autotrophy was still apparent for both locations. The net heterotrophy ( $NEP < 0$ ) of the vegetated location and the net autotrophy ( $NEP > 0$ ) of the open water location for the majority of the hydroperiod became more pronounced (Figure 3.5a). On a monthly basis, the NEP in the vegetated habitat reached a peak in August, when it became net autotrophic. In the open water location, NEP fluctuated more widely on a monthly basis and reached maximum NEP and autotrophy in September before becoming heterotrophic in October. However, the apparently less variable rates of NEP at longer timescales, were accompanied by a significant increase in the variability of the estimated mean rates.

The 7-day moving averages of the GPP:R ratio also showed different trends at the two locations; the open water location seems to have become more autotrophic in late July to September when the water levels were highest (Figure 3.5b). The vegetated site, however, fluctuated more on a monthly basis, becoming net autotrophic on three separate occasions during the hydroperiod and remaining heterotrophic otherwise.



**Figure 3.5.** 7 day moving average values of GPP:R ratio (a) and NEP (b) for the period June-November 2016. Red Log 1= open water habitat, Blue Log 2= vegetated habitat. GPP:R < 1, NEP < 0 = net heterotrophy, GPP:R > 1, NEP > 0 = net autotrophy.

On a seasonal basis, means of the production versus respiration ratio for both locations were net heterotrophic (mean GPP: mean R<1) during the period of rising water level, peak water levels and declining water levels (Figure 3.6c). However, the ratio increased when water levels were at the highest and the basin was 'full'. Rates of respiration were much lower in the open water area during all stages during the hydroperiod, likely due to the lack of dense vegetation respiring during night. In contrast, rates of respiration were high in the vegetated habitat, and peaked during highest water levels. Both locations had similar trends in rates of GPP during the three stages of the hydroperiod, with higher rates at peak water levels, and lowest rates when water levels were declining (Figure 3.6a).



**Figure 3.6.** Average GPP (a), R (b) and GPP:R ratio (c) for the filling, full and drying stages of Little Darkin Swamp for open habitat (Logger 1) and vegetated habitat (Logger 2) locations. Error bars represent standard error. Filling= 2 June-10 August, Full= 11 August-22 October, Drying= 23 October-27 October.

Overall average values of GPP and R were relatively low for both locations during the study period (Table 3.2). On average for the entire sampling period, the open water location was net autotrophic ( $\text{NEP} > 0$ ,  $\text{GPP}:\text{R} > 1$ ), whereas the vegetated location was net heterotrophic ( $\text{NEP} < 0$ ,  $\text{GPP}:\text{R} < 1$ ) (Table 3.2). The reason for the difference in metabolism between the two locations could be due to the zone of influence on the sensor and the different processes occurring in vegetated and open water sites. Previous studies have shown that there is a zone of influence on

the sensor, and sensors in different locations within the same system can yield different estimates of metabolism (Staeher et al., 2010). However, the combined average NEP for the wetland for the entire sampling period was  $0.12 \text{ g O}_2\text{m}^{-3}\text{d}^{-1}$  with a GPP:R ratio of 1.09, meaning that it was overall net autotrophic.

**Table 3.2.** Mean R, GPP, NEP and GPP:R ratio for the two locations (Log 1= open water, Log 2= vegetated) and the average of the two for the entire sampling period (June- November 2016). GPP:R >1, NEP > 0 = net autotrophy, GPP:R <1, NEP < 0 = net heterotrophy.

Location	R	GPP	NEP	GPP:R
Log 1	0.45	1.59	1.14	3.54
Log 2	2.39	1.49	-0.89	0.63
Average	1.42	1.54	0.12	1.09

### 3.3.2 Carbon sources sustaining the aquatic food web

The results from the stable isotope analysis reflect the structure and carbon sources of the aquatic food web in the wetland. The  $\delta^{14}\text{N}$  values show the trophic levels of the organisms sampled; primary producers are found at the base of the food web, followed by the 1<sup>st</sup> order consumers, 2<sup>nd</sup> order consumers and the top predator (red mite) (Figure 3.7). The mites are likely to be parasites on other organism and hence sitting high in the trophic structure. The  $\delta^{13}\text{C}$  values show the carbon sources sustaining the 1<sup>st</sup> order and 2<sup>nd</sup> order consumers, where specific primary producers are feeding on them.

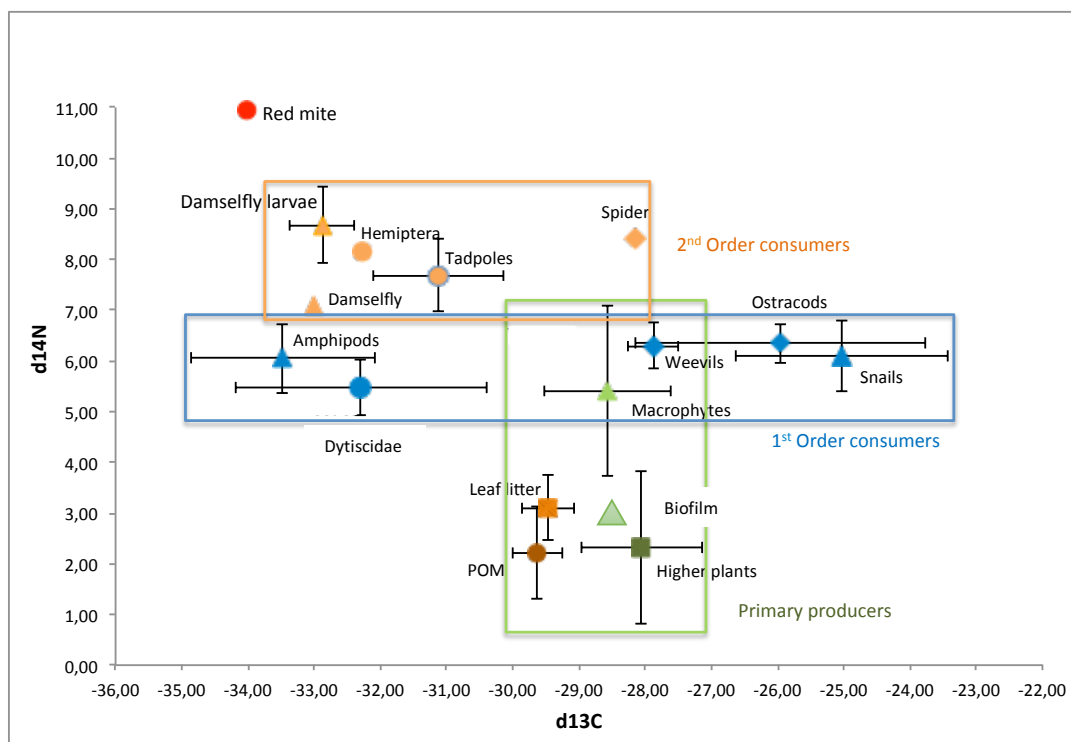
The primary producers all had a relatively narrow  $\delta^{13}\text{C}$  range (-26.5 and -31), indicating that they are attaining their carbon from the same source (e.g.  $\text{CO}_2$  from the atmosphere). In contrast, the 1<sup>st</sup> order consumers have a wider  $\delta^{13}\text{C}$  range (-23 to -31) indicating that their sources of carbon vary. The 2<sup>nd</sup> order consumers all have relatively depleted  $\delta^{13}\text{C}$  values compared to the 1<sup>st</sup> order consumers, which suggests they are feeding on animals with more depleted values such as beetles or tadpoles, or on a microbial source missing from the plot.

#### 3.3.2.1 Trophic levels

The primary producers comprised higher plants, macrophytes, leaf litter, POM and biofilm. The higher plants consisted solely of fresh *Melaleuca lateritia* foliage and the rushes/sedges *Chorizandra enodis* and *Meeboldina coangustata*. Leaf litter consisted of fallen, dead leaves from *Melaleuca lateritia* and rushes floating on the water column, with traces of macrophyte material. The  $\delta^{13}\text{C}$  values suggests that POM consists mostly of leaf litter, rather than macrophytes, as it sits

lower on the trophic scale (Figure 3.7). Macrophytes consisted mainly of *Cyanogeton huegeli* and *Ornduffia submersa*, and showed a higher  $\delta^{14}\text{N}$  value than the other primary producers. Biofilm was initially scraped off macrophyte leaves, which caused mixing of materials producing an unclear isotopic signature more similar to that of macrophytes. To re-sample biofilm, material from the re-hydration experiment was scraped off the aquaria walls (see Chapter 4). Average C:N ratio for macrophytes was 15.3, leaf litter was 33.3, higher plants was 35.3 and POM was 14.3. This further suggests that POM was mainly composed of macrophyte material, and that leaf litter consisted mostly of *Melaleuca lateritia* leaves.

The 1<sup>st</sup> order consumers comprised Dytiscidae (water beetles), Amphipoda, Curculionioidea (weevils), Ostracoda and Gastropoda (snails). The 2<sup>nd</sup> order consumers comprised Zygoptera (damselfly) larvae, adult Zygopetrans (damselflies), Amphibians (tadpoles), Arachnida (spiders) and ?Hemiptera. The Red Water mite (Hydracarina) sits at the top of the aquatic food web as the top predator (Figure 3.8). Mites in their larval stages are parasitic on aquatic insect nymphs, and it is therefore likely that they parasitized 2<sup>nd</sup> order consumers.



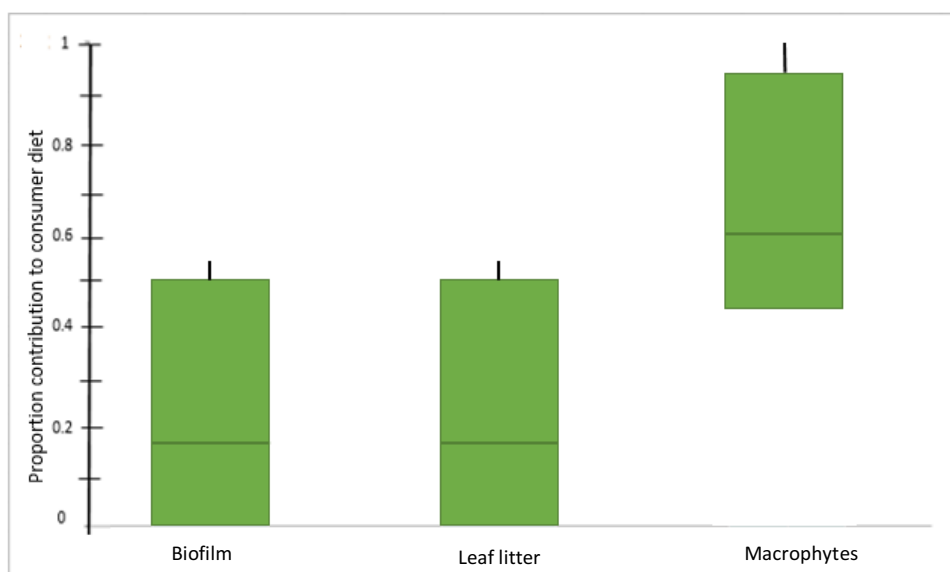
**Figure 3.7.** Dual isotope plot of macroinvertebrates and basal sources in Little Darkin Swamp in the late wet season (Sep-Oct 2015). Values represent averages with total range of lipid-corrected values.

### 3.3.2.2 Basal carbon sources for the macroinvertebrates

The biplot (Figure 3.8) shows basal sources of carbon and three distinct feeding groups, including 1<sup>st</sup> and 2<sup>nd</sup> order consumers and the mites. The depleted  $\delta^{13}\text{C}$  of the leaf litter separates it from biofilm and macrophytes, which may indicate that the litter is from terrestrial sources and colonised by microbes. The  $\delta^{13}\text{C}$  enriched consumers are possibly feeding on terrestrial sources, while the group of consumers with depleted  $\delta^{13}\text{C}$  are feeding on a basal source that was not sampled. Possibilities of alternative carbon sources which are depleted in carbon include microbial sources or through methanogenesis in anaerobic conditions in the sediments, especially in low water and high POM conditions (Siebers, 2015). There is also a more enriched  $\delta^{13}\text{C}$  source that was not sampled, which the snails and Ostracods appeared to be feeding on. Phytoplankton or benthic microalgae are possible sources with less depleted  $\delta^{13}\text{C}$  signal (Doi, et al., 2010).

Some links can be made for some of the food web components. Weevils and spiders, both likely to be 'terrestrial' invertebrates found within the wetland basin, have a  $\delta^{13}\text{C}$  signature similar to higher plants, macrophytes and biofilm. This suggests that they are either feeding on these primary producers directly or on other invertebrates that are feeding on this material. Food sources for the grazers (ostracods and snails) and shredders (beetles and amphipods) remain unknown. A source with a more depleted value and one with a more enriched value seems to be missing from the biplot. These might be sources that were not sampled from the wetland, such as filamentous algae or microbes, which tend to have a more depleted  $\delta^{13}\text{C}$  value (Siebers, 2015). The 2<sup>nd</sup> order consumers are feeding on animals at the lower end of the  $\delta^{13}\text{C}$  scale, such as beetles and amphipods (Fig 3.7).

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were used in IsoSource mixing models to determine potential contributions of three basal sources (leaf litter, macrophytes and biofilm) to the diets of primary consumers (1<sup>st</sup> order) (Figure 3.8). Macrophytes appeared to constitute the majority of the diet of the 1<sup>st</sup> order invertebrate consumers with a mean contribution of 63% (1-99<sup>th</sup> percentile: 45-95%), while leaf litter and biofilm contributed equally smaller amounts of mean 18.5% (1-99<sup>th</sup> percentile: 0-50%). This suggests that the greatest proportion of carbon for the primary consumers is autochthonous, and allochthonous sources only contribute a minor proportion. However, the large 1-99 ranges indicate the estimates of the contributions are not well constrained.



**Figure 3.8.** Mean, 1<sup>st</sup> and 99<sup>th</sup> percentile proportion contribution from three primary sources to 1<sup>st</sup> order consumer diets calculated with IsoSource mixing model on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope data. Error bars represent minimum and maximum values.

The dual stable isotope analysis was not successful in identifying all sources of carbon fuelling the food web at Little Darkin Swamp. There were unexplained food sources for parts of the food chain, and due to the narrow range in  $\delta^{13}\text{C}$  signatures of the primary producers.

## 3.4 Discussion

### Spatial variability in metabolism

The results from the open water and vegetated habitat sites at Little Darkin Swamp showed different dynamics in metabolism during the hydroperiod. This is likely to have been caused by the structural differences at the two sites; one being highly vegetated and with more shading and the other with sparse vegetation, more sun-exposure and lower water levels. These structural differences can in turn result in environmental differences such as water temperature, DO and the amount of organic matter (leaf litter) present.

Higher mean irradiance in the mixed surface layer and higher light absorption by photosynthetic pigments in the open water location compared to the vegetated habitat might have caused the positive NEP values (i.e. net autotrophy) observed here (Staehr et al., 2010), which showed overall net autotrophy during the study period. In contrast the vegetated location, with the dense macrophyte, sedge and woody vegetation cover (see Chapter 2), has higher amounts of leaf litter input causing higher R and therefore overall net heterotrophy.

Spatial variability of metabolism within lakes have been previously recognised, as lakes often have several different habitat types and are generally not homogeneous in their physical, chemical and biological characteristics (Coloso, Cole, & Pace, 2011). Morphology and spatial complexity, together with mixing by wind, can affect the spatial heterogeneity of metabolism within lakes, explaining the differences observed at the two different habitat types at Little Darkin Swamp. However, apart from these two habitats, the wetland was fairly uniform (see Chapter 2). Further, the wetland bottom is also very homogenous with the gradual slopes rather than a steep and narrow littoral zone. Thus, the two loggers would have picked up most of the variability caused by habitat differences. This spatial variability in metabolism has also been observed in other lakes. For example, other studies have found differences in metabolism in the open water pelagic zone and the benthic dominated littoral zones; in unproductive lakes the littoral zones can have higher metabolism than the pelagic zone (Coloso et al., 2011). Furthermore, vertical stratification of the water column can cause differences in temperature, light, oxygen and nutrients, which in turn result in different metabolic rates vertically.

#### **Trophic status (importance of autochthonous and allochthonous carbon to metabolism)**

Little Darkin Swamp was on average net autotrophic during the study period (average  $NEP=0.12 \text{ gO}_2\text{m}^{-3}\text{d}^{-1}$ ,  $GPP:R=1.09$ ). It has been suggested that nutrient-rich, non-coloured (low dissolved organic matter) and productive aquatic ecosystems with high irradiance are net autotrophic (Staeher et al., 2010), however this description does not fit Little Darkin based on measurements from 2015 (see Chapter 2). Being a nutrient-poor, coarse particulate organic matter (CPOM) -rich and relatively unproductive system, Little Darkin Swamp would be expected to be net heterotrophic. The lack of information about Little Darkin Swamp's nutrient dynamics in 2016 makes it difficult to examine the correlation with the alternating trophic state. However, a previous study on ecosystem metabolism in a shallow seasonal Mediterranean lake also showed annual net autotrophy ( $NEP=0.008 \text{ gO}_2\text{m}^{-3}\text{d}^{-1}$ ,  $GPP:R=1.16$ ) (Lopez-Archilla, Molla, Coletto, Guerrero, & Montes, 2004), with annual average  $GPP:R$  ratio and  $NEP$  similar to that of Little Darkin. Although, similarly to this lake, the temporal pattern of the  $GPP:R$  ratio at Little Darkin showed that the system was heterotrophic most of the time, and the  $GPP:R$  ratio of virtually 1 is likely caused by a slight decoupling of  $GPP$  and  $R$ .

Generally, high concentrations of total phosphorus (TP) and low dissolved organic carbon (DOC) result in autotrophy in lakes, while low TP and high DOC causes heterotrophy (Hanson et al., 2003). This occurs because TP is limiting and stimulates  $GPP$ , hence controlling the autotrophic



component, and DOC, mainly from allochthonous sources, increases R and thereby net heterotrophy in lakes (Coloso et al., 2011). Furthermore, accumulation of humic matter can depress primary production in small nutrient-poor lakes, due to denitrification and uptake of scarce nutrients by decomposing microorganisms, which would also promote heterotrophy (Staeher et al., 2010). Therefore, the net autotrophy of Little Darkin Swamp during peak water levels suggests higher algal abundance and low import of allochthonous organic matter during this time.

Large amounts of allochthonous organic matter entering usually dominate ecosystem metabolism of dystrophic lakes (Staeher et al., 2010). However, the net autotrophy at Little Darkin Swamp suggests that autochthonous organic matter is the most important energy source to the community respiration during peak water levels. Net autotrophy within systems can support an export of organic matter from the ecosystem, and/or accumulation of organic matter within the system (Staeher & Sand-Jensen, 2007), whereas net heterotrophy occurs when more organic matter is respired than produced (Hu et al., 2015).

However, the majority of primary productivity at Little Darkin Swamp is terrestrial with higher rates of primary production due to the high abundance of *Melaleuca* shrubs (rather than aquatic production). This results in addition of large amounts of 'terrestrial' leaf litter to the system, contributing to heterotrophy through decomposition. When water is added, decomposition of this material increases the ratio of respiration versus production, thus shifting the trophic status towards heterotrophy.

### **Temporal variability in metabolism and relationship with hydrology**

There was high daily, weekly and monthly variability in GPP, R and NEP in 2016 at both the open water and vegetated habitat at Little Darkin Swamp during the study period. Daily variability in R is usually caused by temperature stimulation, while GPP is only stimulated by temperature at high irradiance (Staeher et al., 2010). Furthermore, DO measured at a given place and time in any lake is a function of metabolism, degree of mixing, and exchange with the atmosphere. Therefore, estimates of metabolism on two days with identical metabolic activity, but with different rates of mixing, can result in different estimates of metabolism when non-metabolic processes are not accounted (Staeher et al., 2010). This could explain the high daily variability in NEP observed at Little Darkin, particularly as it is a shallow wetland where rate of mixing is highly affected by wind.

The high monthly variability in R, GPP and NEP at Little Darkin Swamp may be due to high phytoplankton biomass and large light availability in the mixed water layer (Staeher et al., 2010). For example, the collapse of phytoplankton blooms and mixing of productive surface waters with bottom waters can cause shifts to net heterotrophy (Staeher et al., 2010). In contrast, increasing irradiance, chlorophyll-*a* (i.e. phytoplankton biomass), low wind and little mixing often shift lakes from net heterotrophy to net autotrophy (Staeher et al., 2010). However, phytoplankton was relatively low at Little Darkin, likely due to the coloured water (see Chapter 2).

During the filling stage of the hydroperiod the overall metabolism showed net autotrophy. This was likely due to the growth of aquatic plants at attached algae (periphyton) at this time, as there is little phytoplankton production despite the clear water conditions. In the vegetated habitat the GPP:R ratio was higher when the wetland was full, and showed higher autotrophy. Large amounts of organic matter and thus nutrients are likely to have fuelled aquatic plant and algal growth at this time. However, in the open water habitat, autotrophy was higher (NEP >0) when temperatures were high and water levels were declining. These conditions would favour higher algal production and more concentrated nutrients as water levels were lower and contribute to the overall net autotrophy of the wetland.

The alternation between net heterotrophy and net autotrophy during the hydroperiod suggests that the wetland experiences both net degradation of organics and net accumulation within the system. This shift between net autotrophy and heterotrophy with changes in rates of primary production is not uncommon for aquatic systems, and especially lakes (Sadro, Melack, & MacIntyre, 2011). During net heterotrophic periods the wetland experienced import of organic matter from outside the system or experienced net degradation of organic pools within the system, whereas during net autotrophic periods there was export of organic matter from the system and/or accumulation within the system (Staeher & Sand-Jensen, 2007). It has been shown that a switch from net autotrophy to net heterotrophy occurs at DOC concentrations higher than 4–6 mg l<sup>-1</sup>, and that bacterial respiration (BR) tends to exceed NEP in aquatic systems with NEP below 100 mg C l<sup>-1</sup> day<sup>-1</sup> (Laas, Nöges, Koiv, & Nöges, 2012).

The continuous measurements of DO showed that GPP and R were relatively low at Little Darkin Swamp during the study period but within the range recorded for metabolic rates in shallow (<5m depth) standing water ecosystems worldwide (Lopez-Archilla et al., 2004). However, it has been suggested that primary production in shallow seasonal Mediterranean lakes experiencing large fluctuations in water levels tends to be high (Lopez-Archilla et al., 2004). This is due to the increased nutrient cycling and increased irradiance reaching the whole of the aquatic habitat in

these systems. However, at Little Darkin the GPP was low because of the low nutrient levels and the small size of the catchment. The overall R was low due to the ephemerality of the wetland, causing leaf litter to mostly burn away during summer when conditions are dry, leaving little organic matter on the sediments before winter rainfall inundates the surface once again.

The observed temporal patterns of GPP and R at Little Darkin Swamp, however, conformed with those usually expected in this type of ecosystem as a result of the seasonal variations in light and temperature. As expected, an increase in GPP and R was observed at Little Darkin Swamp with increased temperature at the end of September and October (Lopez-Archilla et al., 2004). This increase in GPP and R is likely also caused by the decrease in water levels, increasing the surface-volume ratio, as well as higher wind speed causing more mixing. The relatively low GPP and R of the system resulted in a finely balanced metabolism, bordering between heterotrophy and autotrophy, and switching between the two depending on weather conditions.

### **Metabolism and carbon sources for food webs**

While the GPP:R ratio suggested that Little Darkin is heterotrophic for most of the hydroperiod, the results from the stable isotope analysis and mixing models show that autochthonous sources of carbon, in the form of macrophytes, are a major contributor to the primary consumer diet. This is despite the large amounts of organic matter from allochthonous (*Melaleuca* litter) and autochthonous (macrophytes) sources available in the wetland. The results do however show that macrophytes are a more important food source than leaf litter. The breakdown of litter within the wetland may be a major source of nutrients and carbon contributing to macrophyte growth in this nutrient poor wetland. This suggests that there is tight recycling of nutrients and carbon driving autochthonous production within the wetland. However, wetland metabolism was measured in 2016, whereas the food web study was conducted in 2015 when water levels were lower and hydroperiod was shorter (see Chapter 2). There is a possibility that the difference in hydrology could have affected the outcomes of the two methods for assessing importance of allochthonous vs. autochthonous sources of carbon. This is also particularly true due to the fine balance of the metabolism in the wetland caused by the low levels of GPP and R.

The isotope bi-plot of the food web showed that there are sources of carbon fuelling the system that were not sampled such as filamentous algae and bacteria fuelled by methane. Methane produced in wetland sediments or anoxic water could provide a source of carbon and energy for

methanotrophic bacteria in the water column (Bastviken, Ejlertsson, Sundh, & Tranvik, 2003). In fact, in many lakes these bacteria can be a significant food source for zooplankton. Due to fractionation by methanogenesis causing highly depleted values of  $^{13}\text{C}$ , these bacteria are also  $^{13}\text{C}$  depleted (Jones, Grey, Sleep, & Arvola, 1999), similar to the 1<sup>st</sup> and 2<sup>nd</sup> order consumers at Little Darkin such as amphipods, beetles and damselflies. During the 2015 wet season sampling, there was not much filamentous algae observed, and this was therefore not included in the food web analysis and not sampled for SIA. During the 2016 wet season sampling however, filamentous algae was observed in large amounts, suggesting that this might be a food source for the primary consumers in years with average or higher rainfall. However, the mucilaginous coating of filamentous algae often makes it a poor or difficult to access food source for aquatic consumers.

In terms of implications for management, care needs to be taken to ensure the amount of input of allochthonous material is not altered due to disturbances such as grazing by feral (pigs) and native (kangaroos and emus) animals or fire. There are already signs that grazing is causing patchiness of the vegetation, potentially reducing the primary production and growth of plants in the wetland. This could have serious negative consequences for the food web of Little Darkin Swamp, as well as the potential to alter the trophic status of the wetland. Furthermore, the dynamics of the metabolism during the hydroperiod seems to depend highly on water levels and temperature. As rainfall decreases and temperatures increase, it is important to be aware that dynamics of the metabolism will also be affected. Management actions to reduce these impacts are therefore necessary to maintain the pristine integrity of the wetland.

## Conclusion

This chapter shows that the metabolism of Little Darkin Swamp is temporally and spatially highly variable, and driven by the hydrology of the system. Due to the ephemerality of the system, it is finely balanced in terms of trophic status, fluctuating between autotrophy and heterotrophy. The wetting and drying of the system alters the properties of the sediment, reducing organic matter content in some areas. With this knowledge in mind, the question arises as to how the different durations of inundation might affect the response of the sediments following a dry period. This will be explored in detail in the following chapter.

# Chapter 4. Biotic response following sediment re-hydration

## 4.1 Introduction

Temporary wetlands vary considerably in their frequency, duration and timing of flooding (Boulton et al., 2014). As the predictability and duration of flooding decreases, the variability of their water regimes increase, and the ratio of dry phase to wet phase becomes greater. Wetland water regimes are categorised into permanent or semi-permanent with highly predictable flooding patterns, intermittent with irregular flooding, seasonal or episodic with unpredictable and temporary flooding (Brock et al., 2003). For all the non-permanent wetlands, a dry phase is considered a stress but not a catastrophe for the organisms that have evolved to live in these habitats (Brock et al., 2003; Nielsen, Hillman, Smith, & Shiel, 2002). In temporary waters, the resilience of the ecosystem can be gauged by an ability to return to the community structure and function once reflooded, after a drying event (Brock et al., 2003).

Periods of drying can force aquatic biota to disperse or go into dormancy as a strategy to survive the disturbance. In fact, wetland flora and fauna have physiological, morphological and behavioural traits that are adapted to both dry and wet conditions. Dry sediments in seasonal wetlands are not biologically inactive, they are habitat for spore, seed and egg bank of phytoplankton, algae, plants, and zooplankton and other aquatic invertebrates. Large numbers of dormant aquatic plant seeds and vegetative propagules, as well as eggs of zooplankton, are found in the sediments. These propagules can persist from days to months to years to decades, and recolonise when the wetland once again becomes inundated. The communities rely on a specific cycle of dormancy, dormancy breakage, hatching, germination, establishment and reproduction to overcome dry phases. There is patchiness of the species in the wetland; not all species are present in all areas of the wetland, and different species hatch and germinate at different times. This allows for community resilience through spatial and temporal patterns of species richness (Brock et al, 2003). Communities that have species with quick reproduction and a high production of propagules establish rapidly, which in turn aids recovery following re-flooding.

Drying and re-wetting also causes changes in water quality (McComb & Qui, 1998), an effect dependent on the properties of the sediment, type of drawdown of water, degree and duration of drying, and the origin and transportation of water when reflooded (Sommer, 2006). Nutrient

transformations and the exchange of nitrogen, phosphorus and carbon between sediment and water are a critical consideration for the physical, chemical and biological responses (McComb & Qui, 1998).

As drying usually is a gradual process due to evaporation rates and drainage, it causes different levels of inundation in different areas of the wetland. Slight gradients in the wetland bathymetry will cause slow egression and recession of water and broad zones, whereas steep gradients will result in rapid water movement, narrow zones and sharp boundaries. This in turn may lead to zonation during the transition from wet to dry or dry to wet phases. These zonation patterns can be affected by the sediment characteristics; high clay content soil will retain water for longer than sandy soil (Boivin, Garnier, & Tessier, 2004). Indeed they in turn affect the sediment characteristics (Sommer, 2006); generally the centre of the wetland will hold water for the longest, while the edges will dry the quickest. Organic matter accumulates in areas where the sediments are wet for longer periods because biomass production is greater, and decomposition is slower, creating a gradient from the centre and outwards.

The hydrology of Little Darkin Swamp is highly seasonal; water is only present during winter and spring when rainfall is at its highest, and when evapotranspiration is at its lowest. The only unpredictable element in the hydrology is the timing, extent and duration of the inundation, which depends almost entirely on the rainfall pattern. Due to the position in the landscape of this wetland, surface runoff will be local only, and groundwater fluctuations have little influence in wetland filling or drying (see Chapter 2).

Based on the above considerations I can assume that the biota will be dominated by those taxa that are adapted and resistant to this filling and drying pattern, that the sediments will hold dormant propagules that will be activated once water is added to the system, and that zonation (distance from the centre, duration of wetting and drying) will affect the response to rewetting.

The objective of this chapter is, therefore, to characterise the water quality and biotic responses of dry sediments at Little Darkin Swamp, and to determine the specific influence of different inundation zones to re-hydration. To standardize for the extent and duration of the inundation, a laboratory re-wetting experiment was conducted.

The specific hypothesis being tested is that sediments taken from inundation zones with a more extended period of saturation will reveal a greater productivity, a richer community, and more functionally heterotrophic response to rehydration.

## 4.2 Methods and Materials

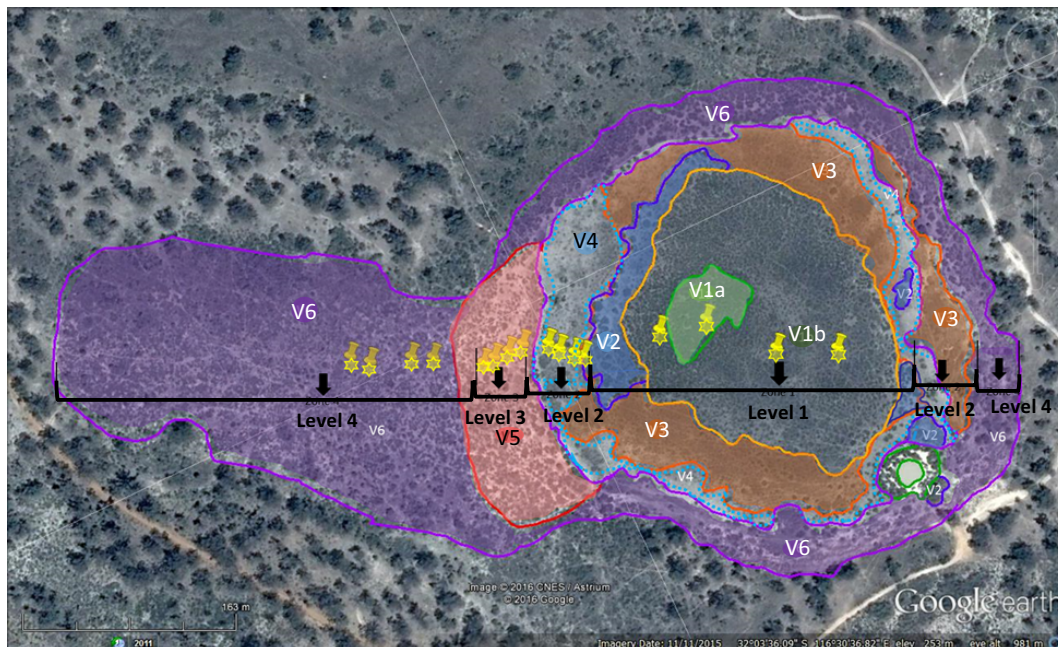
The wetland was divided into 4 elevation levels, corresponding to the 4 inundation zones described in Chapter 2 (Figure 2.4), and replicated sediment samples from each zone were compared. Water chemistry, productivity (primary production, secondary production, organic matter, production/respiration rates), emergence of macroinvertebrates and physicochemical parameters were measured in the experiment.

### 4.2.1 Study site and sampling design

Samples for the re-hydration experiment were collected at Little Darkin Swamp on April 14<sup>th</sup>, 2016, during the dry-phase of the wetland in autumn when the sediments were closest to their driest. The re-hydration experiment was conducted at the aquatic restoration laboratory facilities at the Edith Cowan University's School of Science.

Sediment sampling was designed according to elevation levels as a surrogate for inundation zones. At the wetland, a longitudinal survey was conducted for one transect and 4 elevation levels were identified, with level 1 being the lowest point of the wetland in the centre and level 4 being the highest point at the peripheries. These levels were mapped (together with vegetation zones, see Chapter 2 and Figure 4.1).

Four sediment samples were taken from each of four inundation zones (elevation levels).



**Figure 4.1.** Vegetation types V1-V6 in relation to elevation levels 1-4, and samples collected for the re-hydration experiment at Little Darkin Swamp. Yellow stars indicate samples taken and black lines and arrows indicate boundaries of elevation levels. Aerial image taken from Google Earth, 11/11/2015.

Table 4.1 shows inundation zone and number of replicate sediment samples collected, with corresponding vegetation type along the transect where samples were taken.

**Table 4.1.** Distribution of sediment samples taken across elevation levels above wetland baseline and the corresponding vegetation zones, together forming proposed inundation zones.

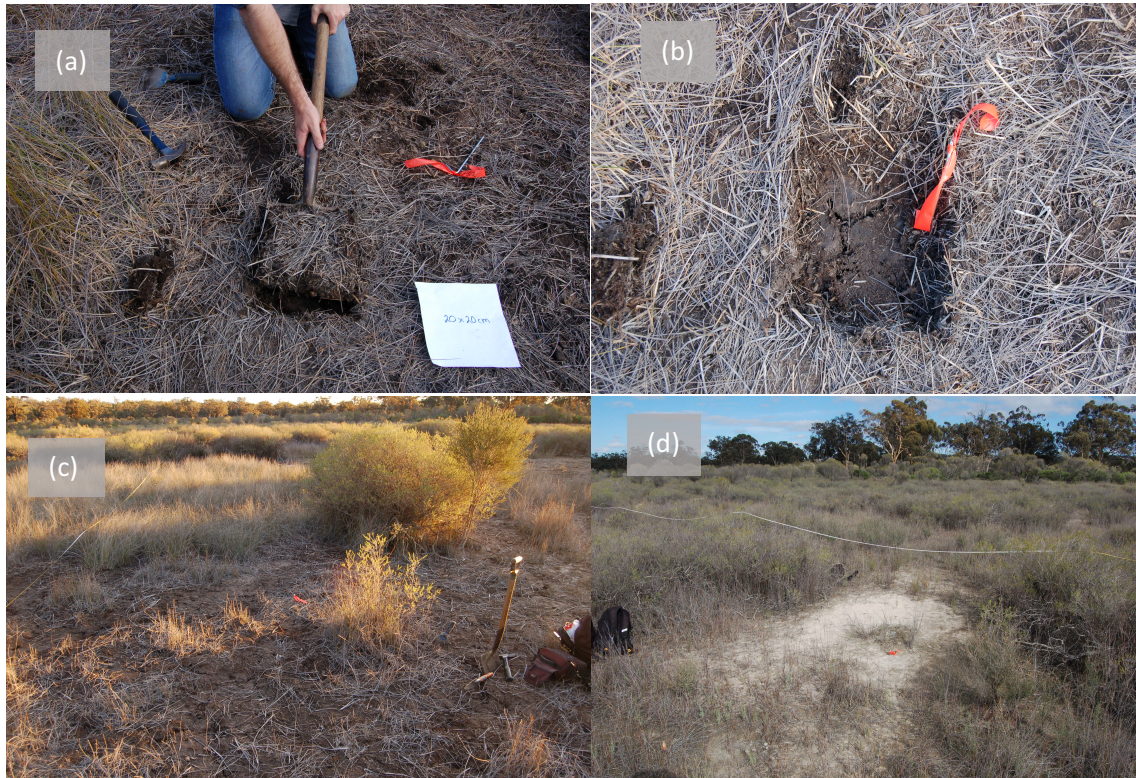
Inundation zone	Vegetation type	Elevation	Samples
1	V1a + V1b	0-10cm	4x
2	V4	10-20cm	4x
3	V5	20-30cm	4x
4	V6	30-50cm	4x
<b>Total</b>			<b>16</b>

#### 4.2.2 Sample collection and preparation

The samples were taken from randomly selected quadrats of bare sediment (20cm x 20cm) lying 10m either side of the transect line.

The area around each stainless-steel quadrat frame was exposed, carefully removing large woody vegetation, and a flat bladed spade was used to horizontally slice off the top 5-10 cm of the consolidated sediment. Site descriptions and zone characteristics were recorded for each sample and photos were taken (Figure 4.2) (as per Benier, 2004). The intact sediment sample was then sealed in airtight plastic zip-lock bags, labelled and stacked in solid boxes to prevent breakage. The samples were returned to ECU Joondalup, School of Science facilities where they were stored in cool, dark conditions until rehydration commenced the following week (as per Benier, 2004). Prior to re-hydration, the samples were weighed and measured to ensure consistent sample weight and size, and where necessary cut to the same depth (approx. 5cm) and approximate weight by removing deeper layers. The samples were then cut to fit the shape of the aquaria into which they would be placed.





**Figure 4.2** Sample collection and site characteristics for sediment re-hydration. Sediment collection (a and b) and site characteristics for zone 1 (c) and zone 4 (d).

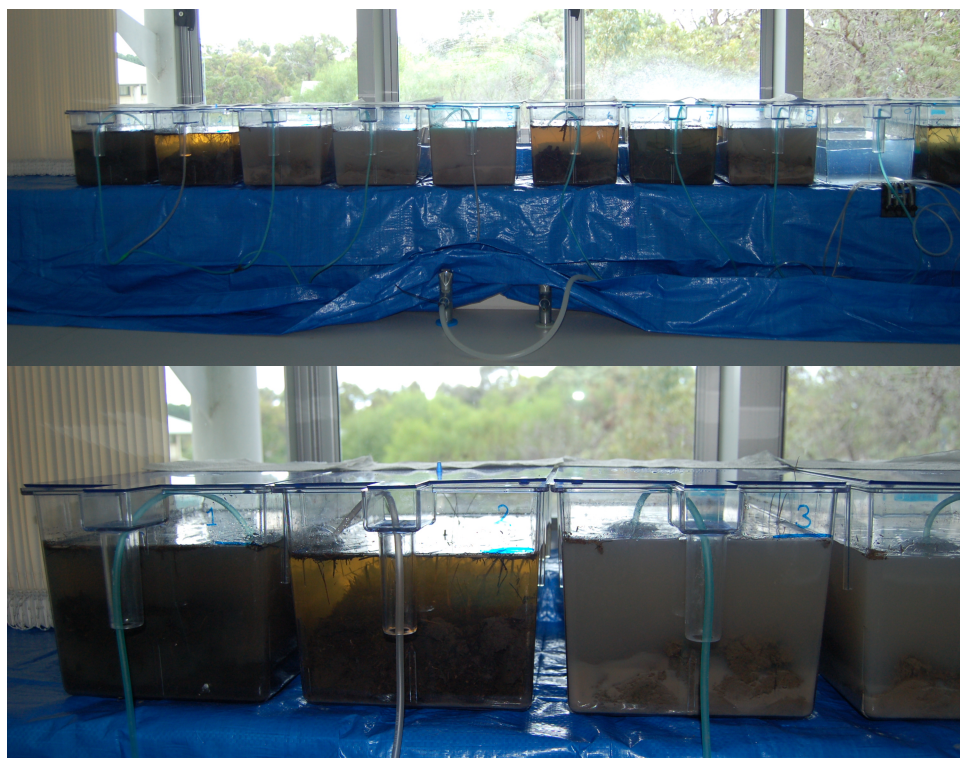
#### 4.2.3 Experimental set-up

The experiment commenced April 20<sup>th</sup> and terminated May 25<sup>th</sup>, 2016, a total of 33 days. A total of 17 aquaria with lids were set up in randomised order along a window bench in the laboratory for the re-hydration experiment. The aquaria and lids were made of Perspex glass, the lids were removable and had several small holes to allow air circulation. The aquaria were cleaned with a 5% bleach solution prior to use to prevent any contamination and then thoroughly rinsed with deionised water (Benier, 2004).

A control aquarium was established and treated in the same way as the experimental aquaria, except without sediment. Each aquarium was given a number from 1-17 and aquaria from each zone were evenly distributed across the laboratory window bench to standardise for differences in temperature and sunlight exposure, should any occur (Figure 4.3). The aquarium number, sample-zone and replicate number was recorded and kept separately (Figure 6.3, Appendix).

The samples were gently placed in the aquaria with the surface of the sediment uppermost. The aquaria received normal diurnal autumn-sunlight through the northern facing windows. There was no temperature regulation in the laboratory other than regular air-conditioning. API all natural aquarium-salt for freshwater fish (Mars Fishcare) was mixed with DI water in order to hydrate

each tank with water of similar, but with standardized, conductivity levels (300 $\mu$ S to match a starting conductivity level, see Chapter 2). Each aquarium was filled with a total of 6L of the water, poured slowly down the side of a tilted wall. Once filled, the water line was marked and aquaria were topped up with DI water throughout the experiment to maintain constant volume. A network of air-tubes connected to the laboratory air-compressor tap, was set on low pressure and airflow delivered under approximately equal pressure to each tank. The pressure was regulated and monitored throughout the experiment. A maximum and minimum thermometer was used to record ambient temperature on a daily basis.



**Figure 4.3** Experimental set-up for sediment re-hydration. Each aquarium was covered with a lid and was oxygenated through an air tube connected to the air-compressor tap.

#### 4.2.4 Physicochemical measurements

All water sampling methods and analysis followed standard procedures used in Western Australia (Department of Water, 2009).

Physicochemical measurements included dissolved oxygen (DO), temperature, pH and conductivity, and were measured using a Thermo Scientific Orion Star A329 pH/ISE/conductivity/RDO/DO meter. During the first and last 48 hours of the experiment, DO supply was turned off and these variables were measured over 4-hourly intervals in all aquaria

without aeration of the water. The depletion of DO provided a measure of the metabolism in the aquaria. In addition, two loggers were placed in aquaria containing samples from elevation levels 1 and 2 recording DO and temperature with 15-minute intervals throughout the course of the experiment (Figure 6.3, Appendix).

Following the initial 48 hours, the physicochemical parameters were measured on a daily basis until values stabilised. Once stabilised, the frequency of measurements decreased to every second or third day. All measurements were taken at approximately midday (11 am-1 pm).

Prior to each measurement event, air-tubes were closed, lids of the aquaria were removed and the multimeter probes submerged as far as possible in the water without touching the sediments. The probes were rinsed between aquaria. The aquaria were measured in the same order and a complete set of physicochemical measurements took about 40-45 minutes.

#### 4.2.5 Nutrients, colour, organic matter and biomass production

Other experimental measurements included chlorophyll-*a* and pheophytin, periphyton biomass, gilvin, plant biomass, nutrients (P and N) and organic matter (%). These were measured only once at the end of the experiment, following the last 48-hour period of the experimental set up.

Chlorophyll-*a* was used to estimate phytoplankton biomass and was measured as follows. A known volume of up to 1L of water from each aquarium was filtered onto a 1.2 µm glassfibre filterpaper (Whatman GF/C). All parts of the filtration tower were rinsed with de-ionised water prior to use and between samples. The volume of water filtered was recorded and filter papers used for each sample were combined and folded, wrapped in aluminium foil and labelled appropriately (Froend & Judd, 2014). The samples were analysed using spectrophotometric method 10200 H. (APHA/AWWA/WEF, 2012) for chlorophyll-*a* using a mass spectrophotometer.

Total phosphorus and total nitrogen were sampled for each zone. Unfiltered water samples used for nutrients were frozen at -21°C and sent to ALS Global Balcatta, Perth for analysis within 30 days (as per Government of Western Australia, 2009). Gilvin, a measure of colour, was measured in the laboratory using a filtered water sample from each aquarium and the Shimidzu Biotech BioSpec-mini spectrophotometer at 440nm wavelength and absorbance was converted to a one metre path length and multiplied by 2.303.

Periphyton biomass was sampled by scraping attached material from a known surface area of the same sidewall of each aquarium with a brush; the brush was thoroughly washed and dissolved in a known volume of DI water which was then filtered onto a pre-weighed 1.2 µm (47mm) Whatman

GF filter paper. The filters were dried in an oven at 60°C for approximately 24 hours until consistent dry weight was achieved and reweighed. Periphyton biomass was measured as ash-free dry weight; the dried filters were burnt in a furnace at 500°C for approximately 2 hours to remove all organic matter and samples were re-weighed (Heiri et al., 2000).

Plant biomass was measured by cutting all new growth in the aquaria at the base as close to the sediment as possible, drying in oven at 60°C until dry (approximately 24h) and weighing.

Organic matter contents of the sediments was measured as loss on ignition (LOI) so that a small (6 cm diameter) core sample of the sediment, once the water was drained from the aquaria, were dried in the oven until dry at 60°C for up to 48 hours and homogenised. A small sample of the homogenised sediment (35-42g) was then weighed to the nearest µg in a porcelain crucible (50mL) and burnt in the furnace at 500°C for 4 hours then re-weighed to give sediment organic matter loss-on-ignition (Heiri et al., 2000).

#### 4.2.6 Macroinvertebrate sampling

At the end of the experiment, aquatic macroinvertebrates were sampled by sieving (250µm) the entire water column (6L) from each aquarium. Each sample was rinsed into a petri dish and sorted by eye for 15 minutes and then by dissecting microscope for another 15 minutes (see Benier, 2004). To sample microorganisms in the sediment, a 9cm diameter corer was used to take one sediment core sample per aquarium. The sediment samples were rinsed to remove sediment and then wet sieved (500µm sieve in combination with a 250µm sieve). The sieved sample was placed into one or more petri dishes and sorted for 15 minutes under dissecting microscope.

All macroinvertebrates were identified to lowest taxonomic level possible. Each species or group identified was given an abundance or approximate abundance if numbers were too high to count during the 15 minutes. For analysis purposes, macroinvertebrates were categorised at a higher taxonomic level to reduce species uncertainty due to sex and juvenile stages.

#### 4.2.7 Analysis

Formulae for organic matter content (LOI), gilvin, periphyton biomass and plant biomass were adapted from 'Monitoring Wetlands' prepared by Glen Daniel (Department of Parks and Wildlife, 2012). All descriptive statistics were conducted in Microsoft Excel 2011. One-way ANOVAs in SPSS were used to test for differences between the zones. For total nitrogen, total phosphorus and



periphyton, only 3 replicate samples were taken. Therefore, for the univariate and multivariate analysis, the fourth replicate was an average of the three samples.

The statistical software PRIMER v6 with PERMANOVA+ add-on was used for univariate and multivariate analysis (Clarke & Gorley, 2006). All biological (i.e macroinvertebrate) and environmental variables (i.e physicochemistry, nutrients, chlorophyll-*a*, periphyton, organic matter and plant mass) were checked for homogeneity using PERMDISP in PRIMER, and where necessary transformations were performed. When homogeneity was not possible, the significance level was dropped to  $p < 0.01$ . The environmental dataset was normalised before further analysis. The biological dataset was square root transformed to decrease the effect of more abundant taxa. Principal Coordinates Analysis (PCO) based on Euclidean distances in PRIMER was used to produce ordination plots of the environmental variables for visualisation of the differences in biogeochemical conditions between the zones. PERMANOVA was used to test for overall and pair-wise differences between the zones for individual variables. Where appropriate, ANOSIM in PRIMER was also used to perform overall and pair-wise test between zones across variables. Twenty environmental variables were initially selected to examine the relationship between the environmental and biological variables. These included:

- 1) Average DO during last 24 hours (DO 24)
- 2) Average DO consumed over last 48 hours (DO lost 48)
- 3) Starting DO (baseline) after the first 48 hours (DO start)
- 4) Average DO during stabilised time (day 8-33)\*
- 5) Average conductivity during last 24 hours (cond 24)
- 6) Change in conductivity between day 0 to day 5 (cond 0-5)\*
- 7) Starting conductivity after the first 48 hours (cond start)
- 8) Average conductivity during stabilised time (day 8-33)
- 9) Average pH during last 24 hours (pH 24)
- 10) Starting pH (baseline) after the first 48 hours (pH start)
- 11) Average pH during stabilised time (day 8-33)\*
- 12) Starting water temperature after first 48 hours (Temp start)
- 13) Average water temperature during stabilised time (day 8-33)\*
- 14) Total nitrogen (N)\*
- 15) Total phosphorus (P)\*
- 16) Organic matter LOI (%)\*
- 17) Plant mass (g)\*
- 18) Chlorophyll a (mg/L)\*

19) Periphyton biomass (g)\*

20) Gilvin (g440)\*

The BEST procedure was used in PRIMER to identify which measure of each physicochemical variable (DO, pH, conductivity, and water temperature) most closely matched the macroinvertebrate data. BEST was further used to identify the variables that had the highest correlations with the biological data. The dataset was reduced to 11 variables (marked with an asterisk in list above). A cross correlation matrix of the remaining 11 environmental variables was produced (Table 6.5, Appendix) to identify correlations to remove those with high correlations ( $r > 0.5$ ).

Prior to multivariate analysis of the macroinvertebrate data, rare taxa which only occurred in one aquarium and in very low abundance, were removed from the dataset. A Principal Coordinates Analysis (PCO) plot of the macroinvertebrate data based on the Bray-Curtis similarities was used to visualise the distribution and variability between the zones, with the environmental vectors superimposed showing in which direction the variables were driving the variation. DistLM in PRIMER produced a dbRDA plot of the macroinvertebrate data with the environmental vectors superimposed, and also showed the percentage of total variation explained by each dbRDA axis. PERMANOVA was used to test for significant differences in the macroinvertebrate assemblages between the zones.

DIVERSE analysis of the macroinvertebrate data was used to calculate species richness, abundance, species evenness, Shannon's diversity index and Simpson's index for each aquarium. PERMANOVA for each of these variables was then used to determine differences between zones.

## 4.3 Results

### 4.3.1 Overall physicochemical response following re-hydration of sediments

The physicochemical response to re-wetting differed between the zones. This section describes the patterns for each parameter (see Figure 6.5 and Figure 6.6 in Appendix III) for graphical representations).

The DO fluctuated widely during the initial 48 hours of the experiment before the addition of oxygen supply, then remained unstable for the first week of the experiment, and stabilised across all zones until air supply was cut off during the last 48 hours. At the end of the first week, the temperatures dropped by 3 degrees across all aquaria, causing a drop in DO by 1 mg/L.

Generally, fluctuations in DO followed changes in water temperature caused by ambient temperatures. Replicate aquaria from zones 1 and 2 had overall significantly lower average levels of dissolved oxygen than zones 3 and 4 during the experiment (One-way ANOVA,  $p=0.00$  and  $p=0.002$ ), suggesting a higher rate of oxygen consumption in these more frequently inundated zones. Replicates from zone 1 had the highest range in DO (7.9-8.3 mg/L), although those from zones 3 and 4 had the highest average concentrations of DO (8.13 mg/L and 8.6 mg/L respectively). Overall, in terms of DO, zones 1 and 2 had a similar response to re-hydration, which differed to zones 3 and 4.

Immediately following re-hydration of the sediment, all aquaria had acidic water with pH ranging between 5.3- 5.5. The pH steadily increased in all aquaria, and by day 20 it had risen to pH 5.7- 6. This is assumed to be the result of photosynthetic activity by algae developing on the aquaria-walls. During the last 48 hours of the experiment, once air-supply was cut off, the pH dropped slightly. Differences in pH between the zones were small; zone 2 was most acidic (geometric mean pH 5.6), followed by zone 1 (geometric mean pH 5.7) and zone 4 (geometric mean pH 5.78), and zone 3 which was the least acidic (geometric mean pH 5.8). There were some fluctuations in pH during the experiment in all zones, such as the slight drops seen in all zones at day 7 and 13, most likely due to changes in temperature and DO. After day 20, the pH stabilised in all zones and remained relatively constant during the rest of the experiment.

In contrast, the conductivity of the water stayed relatively stable in all zones throughout the experiment). The four zones followed the same trends in average conductivity. During the initial 5 days after re-hydration of sediments there was a steady increase in conductivity in all zones, however this was much higher for zones 1 and 2. With the starting conductivity of 300 $\mu$ S/cm, after the first 5 days of the experiment, the conductivity of aquaria from zone 1 had increased by 183  $\mu$ S/cm, and zone 2 by 166.2  $\mu$ S/cm, whereas those from zones 3 and 4 only increased by 37.2  $\mu$ S/cm and 26.5  $\mu$ S/cm respectively, reflecting the highest to lowest conductivities (Table 4.2; Figure 4.3). Overall, the average conductivities over the duration of the experiment of zone 1 and 2 were significantly different to each other and to zones 3 and 4 (One-way ANOVA,  $p=0.001$ ).

Water temperature was not different across all aquaria. Due to cooler ambient temperatures, water temperatures showed a slight declining trend, which lead to an overall steady increase in DO concentrations in all zones.

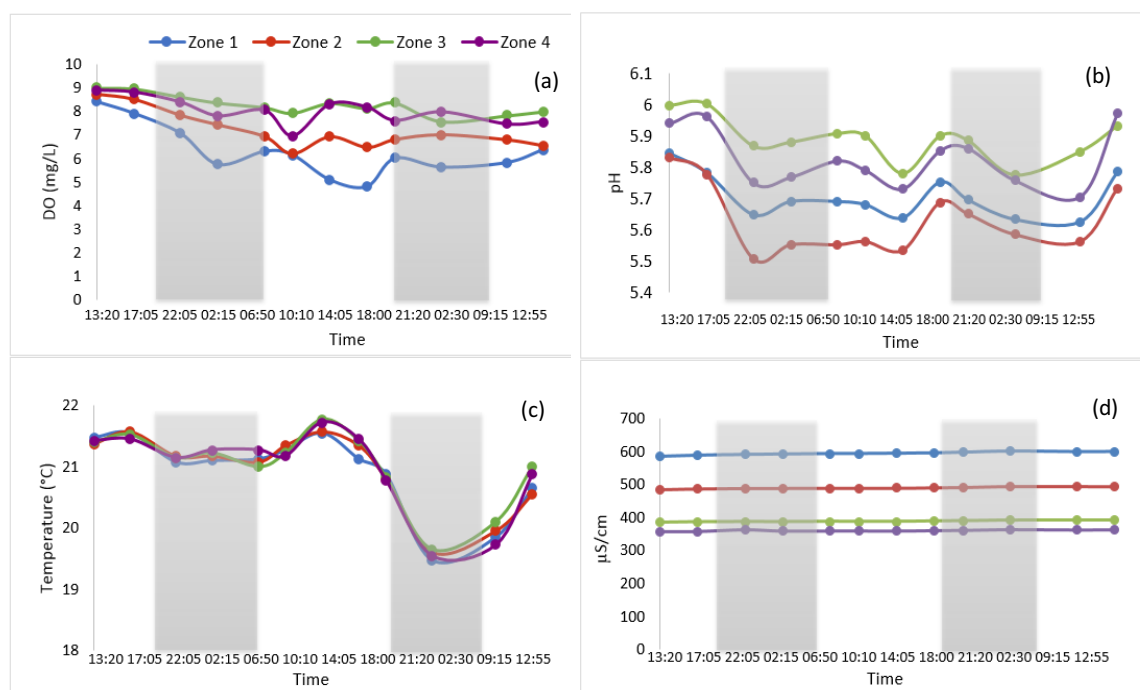
### 4.3.2 Diurnal physicochemical response without oxygen addition

The diurnal response of the aquaria during the last 48 hours of the experiment, without addition of oxygen, differed between the zones.

Following cessation of oxygen supply, DO concentrations decreased steadily, as expected, from approximately 8-9 mg/L in all aquaria to around 6.5 mg/L in those from zones 1 and 2, and approximately 7.5 mg/L for those from zones 3 and 4 (Figure 4.2a). On average, oxygen consumption was highest in aquaria from zone 1 and 2, about double that from aquaria from zones 3 and 4 (Table 4.2).

There were large diurnal fluctuations in pH during the last 48 hours, but no decrease at the end of the period (Figure 4.2b). The pH followed clear diurnal patterns; decreasing during the day due to photosynthesis and increasing at night due to respiration. The aquaria from zones differed in the same way as for the rest of the duration of the experiment.

There was no increase or decrease in conductivity in any of the aquaria over the last 48 hours, maintaining the differences between zones as above.



**Figure 4.2.** Average diurnal changes in (a) dissolved oxygen, (b) pH, (c) water temperature, and (d) conductivity during last 48 hours after cessation of oxygen supply. Shaded areas represent night-time hours.



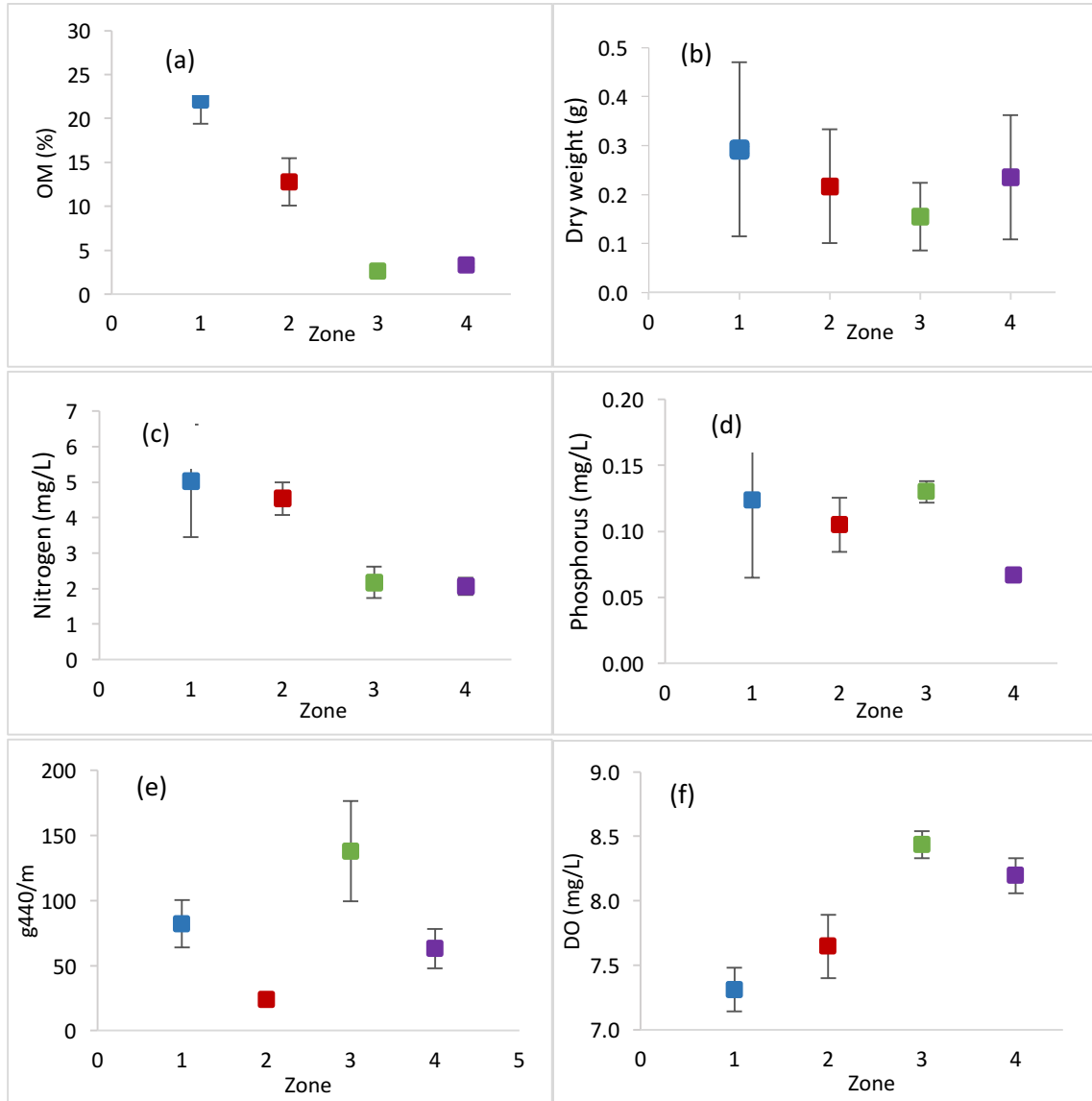
### 4.3.3 Differences in periphyton production, plant growth, nutrients, colour and organic matter

The organic matter (OM) contents of the sediments gradually decreased from the centre of the wetland to the edges, and was significantly different between the zones (PERMANOVA,  $p=0.001$ ). Aquaria from zone 1 had the highest average organic matter content (LOI 22%), followed by zone 2 (LOI 12.7%), zone 4 (LOI 3.3%) and zone 3 (LOI 2.6%) (Figure 4.3a). The higher amount of organic matter in the sediments of the centre of the wetland could be explained by the higher density of vegetation, particularly *Melaleuca lateritia* and sedges, occurring in these areas as a result of longer inundation. This pattern could also be explained by faster accumulation of OM in sediments that are saturated for longer duration. This in turn could explain the higher rate of oxygen consumption, due to decomposition, in these lower elevation and higher vegetation density zones (Figure 4.3f).

Aquaria from zones 1, 2 and 4 had similar average amounts of within-tank macrophyte production during the experiment with 0.29g, 0.24g and 0.22g respectively (Figure 4.3b), while those from zone 3 had around half the production of those from zone 1. However, these differences were not significant. Water column chlorophyll-*a* levels were either very low or not detected in most aquaria, with average chlorophyll-*a* and pheophytin values  $\leq 1 \text{ mg/m}^3$ , apart from one aquarium in zone 4 ( $3 \text{ mg/m}^3$ ). Periphyton production was also low with an average of 0.02g in each zone. This means that phytoplankton and periphyton production was negligible during the experiment.

The highest average concentrations of total nitrogen (5mg/L and 4.4mg/L) were observed in aquaria from zones 1 and 2, while those from zones 1 and 3 had the highest amount of total phosphorus (0.123mg/L and 0.130mg/L). However, only average total nitrogen was significantly different, zones 3 and 4 having only half the concentrations of zones 1 and 2 (PERMANOVA,  $p=0.007$ , pseudo- $F=6.4$ ).

Overall, the gilvin values in the aquaria were very high and variable. Average colour as gilvin was highest in aquaria from zone 3 ( $138 \text{ g}_{440}$ ), followed by those from zone 1 ( $81.16 \text{ g}_{440}$ ) and zone 4 ( $62.22 \text{ g}_{440}$ ). Zone 2 had the lowest gilvin ( $23.95 \text{ g}_{440}$ ) (Figure 4.3e). The differences in gilvin were significantly different (PERMANOVA,  $p=0.02$ ).

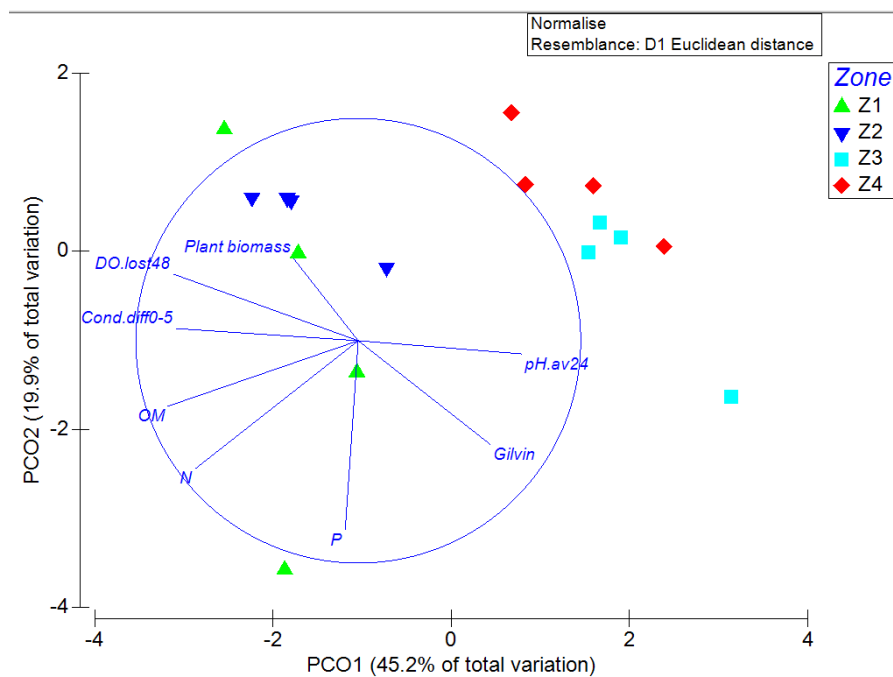


**Figure 4.3.** Average organic matter content by weight (a), macrophyte mass (b), total N (c) and P (d), gilvin (e) and mean DO concentrations (f) for the four zones. Bars represent standard errors.

#### 4.3.4 Overall differences in environmental response between the zones

The Principal Coordinates Analysis (PCO) based on all 20 environmental variables revealed that almost two-thirds of the variation was explained by the first two principal components (PCO1 45.2%; PCO2 19.9%). The PCO clearly shows the separation of zones 1 and 2 from zones 3 and 4 in terms of environmental response to re-hydration (Figure 4.4). The PERMANOVA indicated that the differences between the zones were significant ( $p=0.003$ , pseudo- $F=3.9775$ ), and pair-wise test in ANOSIM revealed that only zones 1 and 2 (sig. 22.9%), and zones 3 and 4 (sig. 82.9%) were not significantly different (Global  $R=0.492$ , sig. 0.03%). The PCO shows that aquaria from zone 1 were more variable and distributed across the PCO2 axis, possibly a consequence of the sediments being collected from a wider area and the distance between the samples.

On the horizontal axis, the vectors show that the samples are separated by, in particular, average pH during the last 24 hours (pH.av24), difference in conductivity in the first 5 days of the experiment (cond.diff 0-5), organic matter content (LOI) and consumption of dissolved oxygen during the last 48 hours of the experiment (DO.lost48) (Figure 4.4). The vectors on the PCO plot indicate that the separation of zones 1 and 2 from zones 3 and 4 is driven mainly by differences in organic matter content (LOI %), total N, pH and conductivity. This is supported by the PERMANOVA results for these variables (Table 4.2). On the vertical axis, the separation in environmental response to re-hydration is caused by differences (negative correlation) in total phosphorus concentrations and plant biomass. The sediments with higher plant biomass have lower concentrations of P, which could be due to higher uptake of P from the water column.



**Figure 4.4.** Principal Coordinates Analysis plot (PCOA) of environmental parameters showing the separation between the four zones. Vectors indicate the direction and strength of each variables' contribution to the overall distribution. Correlation was set to >0.45.

Individual tests (PERMANOVA) for each variable further revealed that DO lost during the last 48 hours (DO.lost48) only differed between zones 2 and 3, and between zones 2 and 4 (Table 4.2). However, there was further separation of zones 1 and 2 from zones 3 and 4, in terms of differences in average conductivity (cond0-5, cond24, cond start and cond8-33), total nitrogen (N), average chlorophyll-*a* and organic matter content measured as loss on ignition (OM%). Gilvin, surprisingly,

only differed significantly between zones 1 and 2 and zones 2 and 3. There were no significant differences between any other environmental variables between the zones.

**Table 4.2.** Means and PERMANOVA results of 20 environmental variables for the four zones (n=4). Letters indicate significant differences between zones as determined by pairwise test in PERMANOVA.

Variable	Zone 1	Zone 2	Zone 3	Zone 4	PERMANOVA p-perm
N (mg/L)	5.03 <sup>a</sup>	4.53 <sup>a</sup>	2.17 <sup>b</sup>	2.07 <sup>b</sup>	<b>0.007</b>
P (mg/L)	0.12	0.08	0.10	0.07	
Gilvin ( $g_{440}$ )	82.16 <sup>ac</sup>	23.95 <sup>b</sup>	138.01 <sup>ac</sup>	63.22 <sup>abc</sup>	<b>0.02</b>
Plant mass (g)	0.29	0.22	0.15	0.24	
OM (% LOI)	22.03 <sup>a</sup>	12.77 <sup>a</sup>	2.56 <sup>b</sup>	3.28 <sup>b</sup>	<b>0.001</b>
Periphyton (g)	0.02	0.02	0.02	0.02	
Chl a (mg/L)	0.63 <sup>a</sup>	0.63 <sup>a</sup>	0.50 <sup>a</sup>	1.25 <sup>b</sup>	<b>0.006</b>
DO 24 (mg/L)	9.44	6.77	8.04	8.00	
DO lost 48 (mg/L)	2.03 <sup>ab</sup>	2.20 <sup>a</sup>	1.00 <sup>b</sup>	1.34 <sup>ab</sup>	<b>0.018</b>
DO start (mg/L)	5.35	6.45	7.42	7.26	
DO 8-33 (mg/L)	8.61 <sup>a</sup>	8.69 <sup>ab</sup>	8.95 <sup>b</sup>	8.86 <sup>b</sup>	<b>0.053</b>
Cond 24 ( $\mu$ S/cm)	597.63 <sup>a</sup>	491.9 <sup>a</sup>	390.2 <sup>b</sup>	360.1 <sup>b</sup>	<b>0.007</b>
Cond start ( $\mu$ S/cm)	548 <sup>a</sup>	516 <sup>a</sup>	353.3 <sup>b</sup>	340.1 <sup>b</sup>	<b>0.002</b>
Cond 8-33 ( $\mu$ S/cm)	597 <sup>a</sup>	513.9 <sup>a</sup>	383 <sup>b</sup>	355.9 <sup>b</sup>	<b>0.007</b>
Cond 0-5 ( $\mu$ S/cm)	183 <sup>a</sup>	166.2 <sup>a</sup>	37.2 <sup>b</sup>	26.50 <sup>b</sup>	<b>0.002</b>
pH 24	5.69 <sup>a</sup>	5.63 <sup>a</sup>	5.85 <sup>b</sup>	5.83 <sup>ab</sup>	<b>0.031</b>
pH start	5.4 <sup>a</sup>	5.2 <sup>b</sup>	5.5 <sup>ab</sup>	5.5 <sup>b</sup>	<b>0.001</b>
pH 8-33	5.79	5.70	5.93	5.87	
Temp start (°C)	22.1	22.1	21.9	22.2	
Temp 8-33 (°C)	21.7	21.6	21.7	21.7	

BEST routine (PRIMER) was used to reduce the number of variables to 11, choosing only the measure of each physicochemical variable (DO, pH, temperature and conductivity) which correlated most strongly with the macroinvertebrate data.

A cross-correlation matrix showed that there were still correlations among these 11 environmental variables (Table 6.5, Appendix). Sediment organic matter content (%LOI) correlated negatively with DO during stable period of experiment (day 8-33) across the zones, meaning that higher organic matter contents yielded lower DO concentrations due to decomposition ( $r = -0.5$ ). Furthermore, sediment organic matter (%LOI) correlated positively with nitrogen ( $r = 0.7$ ), i.e. higher organic matter contents yielded higher N concentrations. Sediment organic matter (%LOI) also correlated positively with difference in conductivity during first 5 days of the experiment ( $r = 0.6$ ), and pH during the stable period of the experiment (day 8-33). Nitrogen correlated positively with difference in conductivity during first 5 days ( $r = 0.5$ ), as well as with total phosphorus concentrations ( $r = 0.5$ ). In terms of physicochemical variables, the increase in conductivity during the first 5 days of the experiment correlated negatively with DO and pH during

the stable period of the experiment ( $r=-0.5$  for both), meaning that aquaria with a larger increase in conductivity had lower concentrations of DO and pH.

#### 4.3.5 Macroinvertebrate emergence following re-hydration

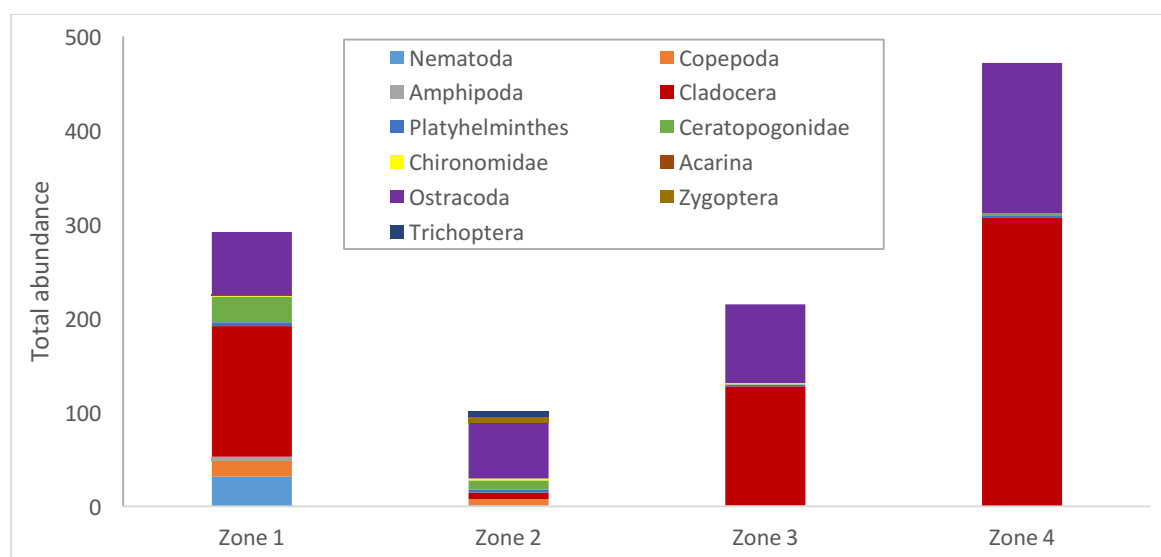
A total of 20 macroinvertebrates morphospecies emerged from the sediment during the re-hydration experiment (Appendix, Figure 6.4). There were no macroinvertebrates in the control aquarium. The macroinvertebrate richness generally decreased with distance from the centre of the wetland. Aquaria from Zones 1 and 2 had the overall highest taxa richness with 9 taxa each (Table 4.3). Only about half the number of taxa present in aquaria from zones 1 and 2 were present in those from zone 3 (5 taxa), while zone 4 had the lowest richness with 4 taxa (Figure 4.5). Cladocera, Ostracoda and Platyhelminthes were the most common taxa in the wetland and cladocerans and ostracods were the most abundant taxa from all zones, although their abundance was highest from zone 4. Copepoda and Chironomidae were only found in zones 1, 2 and 3, while Ceratopogonidae (biting midges) were only found in zones 1, 2 and 4. There was a high abundance of nematodes in zone 1, but they did not occur in any other zone.

A number of taxa such as Acarina, Amphipoda, Zygoptera and Trichoptera were only found in aquaria from one of the four zones (Table 4.3).

**Table 4.3.** Average macroinvertebrate abundance (counts) and total taxa richness from aquaria for each zone (n=4).

Taxa	Zone 1	Zone 2	Zone 3	Zone 4
<b>ARTHROPODA</b>				
Acarina	1	0	0	0
<b>Crustacea</b>				
Amphipoda	4	0	0	0
Ostracoda	67	60	84	160
Copepoda	17	7	1	0
Cladocera	139	7	127	307
<b>Insecta</b>				
Zygoptera	0	5	0	0
Chironomidae	1	2	1	0
Ceratopogonidae	27	10	0	3
Trichoptera	0	6	0	0
<b>NEMATODA</b>	32	1	0	0
<b>PLATYHELMINTHES</b>	4	3	2	2
<b>Total taxa</b>	9	9	5	4

Despite the differences between the assemblages found from zone 1 and zone 2 aquaria, DIVERSE showed no significant separation between zones in terms of richness, abundance, evenness or diversity.

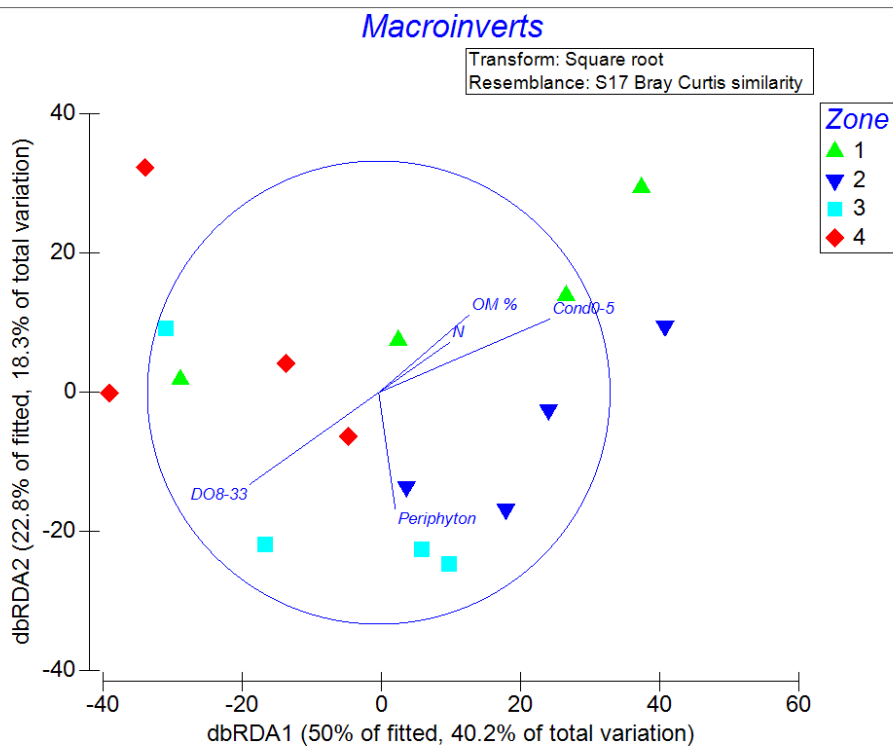


**Figure 4.5.** Macroinvertebrate abundance and taxa richness from re-hydrated sediments.

#### 4.3.6 Influence of environmental parameters on the overall distribution of macroinvertebrate assemblages

The distance-based redundancy analysis (dbRDA) plot showed some separation in macroinvertebrate communities between the zones (Figure 4.6). Aquaria from Zone 1 showed the highest variability in their macroinvertebrate data. Aquaria from zones 1 and 2 were closely related to the right of the plot, and mostly separated from those from zones 3 and 4. The environmental parameters with the largest effect on this separation and highest correlation to the macroinvertebrate data were organic matter content (%LOI), total nitrogen, change in conductivity during first 5 days of the experiment (Cond0-5), dissolved oxygen during stabilised part of the experiment (DO8-33) and periphyton biomass (dbRDA plot vectors, correlation set to >0.3). Higher organic matter content, conductivity and N seemed to be separating out aquaria from zones 1 and 2 from the others, whereas higher periphyton biomass separated aquaria from zone 3, and higher DO separated aquaria from zone 4.

However, PERMANOVA and ANOSIM of the macroinvertebrate assemblages with environmental variables (dbRDA plot) both showed that the separation between the zones was not significant (PERMANOVA  $p=0.08$ , ANOSIM  $R=0.189$ ,  $p=3.6\%$ ).



**Figure 4.6** Distance based redundancy analysis (dbRDA) plot of the DistLM based on the environmental variables fitted to the macroinvertebrate assemblages. Correlation was set to >0.3. Vectors indicate the direction of the parameter effect in the ordination plot. OM%= organic matter content (LOI) of the sediments by weight, N=nitrogen, DO8-33= mean DO during stabilised time (day 8-33), Cond0-5= change in conductivity from day 0-5, Periphyton= amount periphyton by weight.

The best distance-based linear model explained 58.5% of the total variation in macroinvertebrate distribution; axis one explaining 40.2% and axis 2 explaining 18.3% of the variation. Overall, 85.35% of the total variation in the macroinvertebrate data was explained by the 7 first axes of the dbRDA plot in the DistLM. The marginal test in DistLM showed that only change in conductivity during first 5 days (Cond0-5) and mean DO during the stable time (DO8-33) were significantly driving the separation of samples ( $p=0.004$ , pseudo- $F=4.51$  and  $p=0.025$ , pseudo- $F=3.03$  respectively). The BEST of the 11 environmental variables showed change in conductivity during the first 5 days, periphyton and mean dissolved oxygen during stable time in combination had the highest correlation with the macroinvertebrate data (Global Rho =0.416). This means that these variables best explain the patterns in the macroinvertebrate communities. However, the correlation coefficient was not significant ( $p=23\%$ ).

## 4.4 Discussion

The results of the sediment re-hydration experiment indicate that there were distinct differences in the environmental and biotic responses to re-wetting of sediments, particularly between the frequently inundated and less frequently inundated zones of the wetland. In general, the zones in low elevation areas with higher frequency and duration of inundation had more detrital material. The higher amounts of organic matter can be attributed to higher densities of emergent and submerged vegetation (particularly *M. lateritia*, *Triglochin* sp., *Meeboldina coangustata*), coupled with slower decomposition rates. High levels of detritus create an environment richer in nitrogen which may promote plant growth (Baldwin & Mitchell, 2000; Bornette & Puijalon, 2011). Thus, the higher total nitrogen concentrations in zones 1 and 2 can be explained by the higher plant mass production, with nitrogen being released from the organic matter. Specifically, mineralisation of organic matter produces ammonia, which is converted to nitrite by bacterial action in oxic sediments.

Particularly for emergent macrophytes, increased plant productivity and uptake of nutrients are usually observed following rehydration of dried sediments (Baldwin & Mitchell, 2000). In fact, it has previously been established that duration, frequency and magnitude of inundation affects the biotic communities and ecosystem processes in wetlands (Langhans & Tockner, 2006). For example, depth, duration and frequency of inundation influences plant community composition in temporary wetlands (Warwick & Brock, 2003), which was evident by the high density vegetation zone observed at the centre of Little Darkin (see Chapter 2). Frequent inundation promotes high plant species richness and biomass, particularly of amphibious fluctuation tolerator and responder species in Australian shallow freshwater wetlands (Casanova & Brock, 2000).

The rate of plant litter decomposition indirectly affects ecosystem metabolism (contributing to heterotrophy, see Chapter 4) and nutrient cycling, which in turn contributes to productivity. However, the rate of algal/phytoplankton productivity was generally low following re-hydration of sediments, confirming the low levels found in field at Little Darkin Swamp (Chlorophyll-*a* range 0-12 mg/m<sup>3</sup>, see Chapter 2). As the basin is shallow with little shading, this could be attributed to high levels of turbidity and gilvin (due to being deeply coloured or turbid with clay) reducing light, in combination with low levels of nutrients (Liboriussen & Jeppesen, 2003). However, in general, plant productivity is higher in wet zones due to higher moisture content of the soils for longer periods (Xiong, Johansson, Hughes, & Nilsson, 2003).



As the DO in the more inundated areas were lower, together with slower decomposition rates, it can be inferred that rates of accumulation of organic matter will be higher in the centre of the wetland. Due to the higher amounts of organic matter, microbial activity and lower elevation in these areas, moisture is retained for longer and therefore plant productivity is greater (Brinson, Lugo, & Brown, 1981). The longer periods of moisture retention allow for a longer growing period and the high amounts of organic matter leads to availability of nutrients in the sediments. In contrast, decreased bacterial activity in the drier inundation zones causes higher DO (and lower consumption), possibly due to lower availability of organic matter in these areas. This pattern has also been found in sediment rehydration studies of North Lake, Western Australia (Qiu & McComb, 1994).

Desiccation of sediments generally alters the nutrient dynamics following re-hydration of sediments (Boulton et al. 2014). It has previously been found that rehydration of dried river and lake sediments results in a pulse of nitrogen concentration, which promotes macrophyte growth (James, Barko, & Eakin, 2004). Higher amounts of leaf litter in the higher inundation frequency and duration zones in the centre of the wetland result in quick leaching of nutrients, such as nitrogen, following inundation. This was evident by the positive correlation between organic matter and nitrogen following re-hydration of the sediments in the experiment. The release of nutrients promoted microbial activity and nutrient cycling processes, which in turn made these parts of the wetland fertile and productive in relative terms (Baldwin & Mitchell, 2000). However, the experiment indicated very low nutrients in the water column following rehydration of sediments, suggesting that this high productivity is a result of tight recycling of nutrients, i.e. rapid take up by plants. Further, the effect of organic matter on light penetration and benthic photosynthesis could outweigh possible positive effects of nutrients on pelagic production, such that productivity is controlled by light availability rather than nutrient input (Karlsson et al., 2009).

Furthermore, the zones with longer duration of inundation also had higher electrical conductivity, indicating that there are higher amounts of anions and cations or nitrogenous ions. As there is no outflow in the wetland (see Chapter 2), any ions (mainly  $\text{Na}^{2+}$  and  $\text{Cl}^-$ ) in the wetland will accumulate due to evapoconcentration. Furthermore, nitrogen correlated positively with conductivity, indicating that conductivity might be influenced by  $\text{NO}_x$  and  $\text{NH}_4$ , as well as other ions. As gilliv increased, plant biomass and DO decreased, suggesting that higher conductivity is ionic and not related to organic acids. Thus, it is the dominant anions and cations (see Chapter 2) or nitrogenous ions that caused the observed higher conductivity, and not organic acids. In addition, the wetter zones (zones 1 and 2) had lower pH as a result of rehydration of the sediments

(Sommer, 2006). This could also be a result of higher bacterial activity, consuming O<sub>2</sub> and producing CO<sub>2</sub>, methane and H<sub>2</sub>S. Oxidation of H<sub>2</sub>S in particular increases the acidity of the water. In addition, pH correlated negatively with sediment organic matter, such that increased organic matter content yielded lower pH. Together, higher nutrients (N), conductivity, organic matter, and lower DO levels in the longer inundation zones suggest a more heterotrophic response to rehydration of dried sediments, as was hypothesised.

As the higher organic matter content of the frequently inundated sediments can improve water retention, it can affect drying and rewetting rates (Strachan, 2016). In terms of the biota and specifically the macroinvertebrates, moisture content of the sediments determines the viability of some components of the seedbank, as sediments with higher organic matter content are more suitable for aquatic organisms during summer drought (Stubbington & Datry, 2013). It has also been found that assemblage abundance and richness declines as the duration of the dry-phase increases (Stubbington & Datry, 2013; Stubbington, Gunn, Little, Worrall, & Wood, 2016). Therefore, it was expected that the invertebrate assemblages in areas near the centre of Little Darkin Swamp would have higher abundance and richness, as stated in our hypothesis. The qualitative results did indeed show that these wetter areas had higher richness of emergent taxa from the re-hydrated sediments, but not abundance for all species.

These results suggest that areas retaining moisture for longer, due to lower elevation and higher sediment organic matter, can be inhabited by species that are less tolerant of desiccation. It also shows that this habitat (moist organic rich consolidated sediment) enables some invertebrate taxa to persist during the summer/autumn drought in temporary wetlands of southwestern Australia (Strachan et al., 2015). Such taxa (Trichoptera, Zygoptera, Amphipoda etc.) do not have drought resistant life histories, but rather responses to desiccation (Strachan et al., 2015). However, the fact that the community compositions were not statistically different between the frequently inundated and less frequently inundated zones is likely affected by the small sample size and large variation in the data. The ordination (dbRDA) plot (Figure 4.6) showed that the separation between the zones is not as clear when environmental parameters are combined with the macroinvertebrate data. This indicates that this is a complicated system, with many factors affecting the community compositions at Little Darkin Swamp.

A high abundance of microcrustaceans, such as Ostracods, Cladocerans and Copepods emerging from the dried sediments of Little Darkin Swamp were expected. These organisms have multiple drought survival strategies, and can survive drying by going into dormancy, closing their carapace and lowering metabolic activity (Delorme, 2001; Strachan et al., 2015), and would have persisted

during the long dry summers of southwestern Australia. The emergence of these microcrustaceans have often been observed from dried wetland sediments after reflooding (Strachan, Chester, & Robson, 2014).

Despite the outcomes supporting our hypothesis, that longer duration of site saturation results in higher productivity, community richness and heterotrophy, this study was unable to categorically distinguish between all four inundation zones at Little Darkin Swamp. This suggests that, in terms of the aquatic ecology, there are only two functionally different inundation zones rather than four, although in terms of vegetation zonation there is definitely more (see Chapter 2). Little Darkin Swamp has a relatively shallow basin with diffuse gradients that could make it difficult to distinguish between the zones. Due to annual variability in the amount and timing of rainfall, as well as degree of grazing (see Chapter 2), there is also likely to be annual variability in the extent and boundaries of the zones.

One explanation for the lack of separation observed might be the small sample size and large variability between the samples. This was due to the patchiness of the system, larger areas sampled yielded larger variation. Because some zones had larger surface areas than others (such as zones 1 and 4), the sampling design might not have sufficiently captured the differences between them. A consequence of this might have been un-even sampling of the eggs, spores and seeds in the sediments, as they are not distributed evenly across the surface of the sediment. This was evident from the wave cast lines observed at Little Darkin Swamp. One solution to this problem would be to sample a larger area in the larger zones by increasing the number of samples and decreasing the size of each. This would ensure that the same amount of sediment is collected in each zone, but also cover a larger spatial area in larger zones.

The high variability within the zones could also be explained by the high degree of vegetation and sediment patchiness, due to disturbances such as prolonged drying and pig grazing. This can alter the properties of the sediment and their response to rehydration. These disturbances caused some zones to be more homogenous than others, producing differences in variability. In terms of differences in macroinvertebrate emergence, the cause could also have been inconsistency in sampling and identification due to lack of experience. This improved with every sample examined, and could have affected the variability between the samples. However, it is likely that these differences would even out over the four replicates. A larger sample number would have evened out these differences further.

#### 4.4.1 Conclusion

The outcomes of this study confirm that the different frequency and duration of inundation at Little Darkin Swamp does create zones, which respond differently to rehydration of sediments. There are clear differences in productivity and physicochemical response between the high and low elevation areas; deeper areas in the centre of the basin have higher sediment organic matter content, plant growth, nutrients, ions and richness of macroinvertebrates. However, while qualitatively apparent, this separation is not as clear in terms of the diversity of the aquatic community. A larger sample size and improved spatial coverage of the zones is needed to confirm whether there are two or four functionally different zones.

# Chapter 5. Synthesis

In contrast to the majority of seasonal wetlands in southwestern Australia, which are connected to regional groundwater, claypan wetlands rely solely on rainfall to fill (Brown et al., 2005). These ephemeral clay-based wetlands of southwestern Australia have significant biodiversity values, and are considered to be one of the biodiversity jewels in one of the world's biodiversity hotspots (Gibson, 2010). Maintenance of the specific and complicated hydrological regimes of these systems will be imperative for the survival of these ephemeral claypans in the future, particularly in light of the ongoing changing climate of southwestern Australia (Gibson, 2010; Hanna, Coletti, Hipsey, & Vogwill, 2011). As the wetlands dry over spring, temporally overlapping suites of annual herbs flower and set seed, making this dynamic change a unique feature of these claypans. During summer, the wetlands completely dry to impervious pans (Brown et al., 2005). Similar to Californian vernal pools, these ephemeral claypan wetlands act as repositories of biodiversity, where threatened, priority fauna and flora species and restricted communities concentrate (Gibson, 2010). Due to a number of threats, such as urban development, the plant communities of seasonal clay-based wetlands are some of the most threatened in Western Australia (Brown et al., 2005).

In addition to a wetland classification through a general description of their wetland attributes, effective management of these ephemeral wetlands will require an insight into how these systems function, and what processes drive them. Therefore, the overall aim of this thesis was to understand what drives Little Darkin Swamp, one such claypan system, and how its ephemeral nature affects wetland processes and functions. By describing the wetland attributes and systematically classifying Little Darkin Swamp, an understanding of the inflows and outflows of water and material was gained, as well as a baseline for subsequent chapters (Chapter 2). This revealed that its hydrology is highly dependent on rainfall, that it is an endorheic system, with a basin that is structurally spatially heterogeneous with distinct vegetation zones, and that surface waters have nutrient levels that are similar to oligotrophic systems. These features make it similar to other claypan wetlands of southwestern Australia and vernal pools of California, USA.

An understanding of the temporal and spatial dynamics of the wetland's ecosystem metabolism and trophic status provided information on the sources of carbon that drives the system (Chapter 3). This established that there are large temporal and spatial variations in ecosystem metabolism, that the trophic status of the wetland is finely balanced and was overall slightly autotrophic over the study period. Dual isotope analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) revealed that aquatic macrophytes make a

higher contribution to the food web compared to other sampled sources, although it is probably also supported by other sources, such filamentous algae and methanotrophic bacteria.

The functional heterogeneity of the wetland was shown by the differences in biotic response to rehydration of dried sediments in areas of varying duration and depth of inundation (Chapter 4). Experimental re-hydration emphasized that the seasonality of the water regime, and the shallow bathymetry of the basin, influence organic matter content, nutrient levels, oxygen consumption, plant growth and macroinvertebrate richness, together showing differences between the centre of the wetland and the shallower areas. This confirmed that there are at least two distinct zones in the wetland in terms of biotic response following rewetting.

In this chapter I show how the multiple wetland processes and functions of Little Darkin Swamp described in the previous chapters indicates that the ephemeral nature and ecology of this wetland separate it from other seasonal wetlands in southwestern Australia.

## 5.1 Characterisation and classification of Little Darkin Swamp

The ecological characterisation of Little Darkin Swamp indicates the many similarities with the vernal pools of California and claypans of southwestern Australia in terms of wetland attributes (Chapter 2). These systems differ from other seasonal wetlands due to their impervious clay base, which is generally perched and not connected to groundwater. However, relatively little is still known about their connectivity to groundwater, apart from some knowledge on the hydrodynamics and surface water inflow to the claypans in Drummond Nature Reserve (DNR), as well as support from perched aquifers in some vernal pools of California (Forbes & Vogwill, 2012; Rains et al., 2006).

Although this study suggests there is little or no connection to groundwater at Little Darkin Swamp, research into groundwater connectivity was out of the scope of this project. Further research will be beneficial for management, as hydrology is a key aspect for conservation in aquatic ecosystems (Davis & Froend, 1999; Fennessy, Jacobs, & Kentula, 2007; Pettit, Froend, & Davies, 2001). The wetland receives water from rainfall and surface water runoff from the surrounding area, however, the lack of outflow from Little Darkin to other water bodies makes it an endorheic system. Although this may not be the case in very high rainfall years. This means that surface water is lost almost exclusively through evapotranspiration. In turn, this results in the accumulation of salts and nutrients as the wetland dries, changing the conditions for the biota (Jolly, Mcewan, & Holland, 2008; Sim, 2012; Sim, Davis, Chambers, & Strehlow, 2006). The lack of recharge, in combination with evapotranspiration, also creates zones with differing duration and

frequency of inundation at Little Darkin. The variation in inundation and, thus, moisture content of sediments results in zonation of the vegetation as described in Chapter 2, and differences in the biotic response of sediments to re-hydration (Chapter 4).

The many similarities of Little Darkin to the claypans of the DNR (Chapter 2) suggests it is indeed another representative of the rare ephemeral clay-based wetlands of southwestern Australia, and the Threatened Ecological Community (TEC) 'claypans with mid dense shrublands of *Melaleuca lateritia* over herbs' (Brown et al., 2005; Chow, Vogwill & Forbes, 2010; Gibson, Keighery, Lyons, & Keighery, 2005; Gibson, 2010). Furthermore, Little Darkin can also be categorised as the southwestern Australian equivalent to Californian vernal pools, based on their numerous similarities in wetland attributes. In these systems, similarly to all freshwater systems, the hydrology drives and controls the wetland attributes and functions.

Due to the seasonal drying of these systems, coupled with a Mediterranean climate experiencing high temperatures in summer, organic matter is lost from the system and does not build up on the sediments. This, together with the ancient nature of the lateritic soils of the Darling Escarpment (Bunn & Davies, 1990) and a lack of surrounding agricultural runoff, results in low nutrient levels at Little Darkin, making it an oligotrophic system. This, in turn, affects other internal processes such as primary production and respiration, which impacts its ecosystem metabolism and trophic status (Russ, Ostrom, Gandhi, Ostrom, & Urban, 2004). The seasonal wetting and drying cycle also creates specific conditions that only specialised biota are adapted to, allowing a unique biotic community to inhabit the clay pans (Pinder et al., 2013).

Being a shallow, perched, endorheic and rainfall-fed basin, the hydrology of Little Darkin also varies on an interannual basis. This was evident by the contrast in surface water duration and depth over the two winter-seasons observed in 2015 and 2016 (Chapter 2). Although the biota might be adapted to this variation in hydrology (Horwitz et al., 2008), it makes the system fragile and susceptible to disturbances such as grazing and weed invasion. In addition, with the predicted trends in climate, the interannual variations in hydrology are expected to increase, which will have negative consequences for the biota (Bornette & Puijalon, 2011; Larned, Datry, & Robinson, 2007; Nielsen et al., 2002; Roshier, Whetton, Allan, & Robertson, 2001). Thus, appropriate management of these disturbances will be increasingly important.

## 5.2 Trophic status and temporal dynamics and spatial variability in metabolism

Being an oligotrophic system, the overall rates of production and respiration were low at Little Darkin Swamp. The low GPP and R are most likely related to low nutrients and the brevity of the flooded phase, resulting in low productivity (Bunn & Davies, 1990; Russ et al., 2004). The export (via burning into the atmosphere) of organic matter during summer may also results in low respiration, and needs to be further researched. However, there is high productivity in the emergent terrestrial vegetation (*M. lateritia*), which drops a large amount of leaf litter in the aquatic system. This low overall GPP and R causes the ecosystem metabolism to be finely balanced, and the trophic status of the wetland to border between heterotrophy and autotrophy (Chapter 3). However, overall the system was autotrophic, suggesting that autochthonous sources of carbon are important in driving the ecosystem processes.

The results from the dual isotope approach suggested that, also in terms of the aquatic food web, autochthonous macrophytes are a major source of carbon, supporting the notion of autotrophy in the wetland. However, it has been recognised in recent years that allochthonous input of organic matter represents an important source of carbon for the food webs of lake ecosystems (Jones & Grey, 2011), and that unproductive lakes are dominated by heterotrophic processes based on this allochthonous organic carbon (Jansson, Persson, De Roos, Jones, & Tranvik, 2007). In contrast, a study from the Macquarie River in eastern Tasmania, with high seasonal variability and intermittent flow, showed prevalence of aquatic macrophytes as food source for the invertebrates (Watson & Barmuta, 2011), suggesting that macrophytes may play an important role in ephemeral systems.

However, there were several other potentially important sources of carbon that were not sampled (Chapter 3), raising the need for further research into the carbon sources fuelling the food web of Little Darkin Swamp. Potential un-sampled sources of carbon which are depleted in carbon are methanogenesis by microbes and bacteria consumed by zooplankton (Bastviken et al., 2003; Jones & Grey, 2011), whereas phytoplankton or benthic microalgae are possible sources with less depleted carbon isotopes (Doi et al., 2010). Knowledge of the sources of carbon driving the aquatic food web will improve understanding of the aquatic processes supporting the system (Pettit et al., 2011; Thorp & Delong, 2002).

With the inter-annual variations in weather conditions and water regime, the metabolism, and trophic status is also expected to vary. The wetland was overall autotrophic during the study



period, although the metabolism was temporally variable, alternating between heterotrophy and autotrophy. Whether this dynamic is expected or not in these systems is difficult to determine, as there is a lack of metabolism studies in these clay pans for comparison. However, examples from other shallow seasonal systems suggest that they are highly variable in metabolism, both temporally and spatially (Hu et al., 2015; Russ et al., 2004; Sadro et al., 2011).

The structural heterogeneity of Little Darkin resulted in metabolic differences in contrasting habitats within the same system. This spatial variability in metabolism suggests that for this type of wetland with variable habitats, ecosystem metabolism cannot be derived by using a one station diurnal oxygen method (Chapter 3). It also suggests that metabolism is highly dependent on environmental factors such as temperature, light and wind, in addition to density of vegetation. Furthermore, the ephemeral nature of Little Darkin creates zonation based on frequency and duration of inundation, causing heterogeneity in terms of functional response to re-hydration of dried sediments (Chapter 4). These differences in inundation might in turn affect the metabolism in these areas, which are currently not known. To gain an understanding of the natural variability in metabolism of these types of wetlands there needs to be greater emphasis on accounting for spatial and temporal variability. This would require multi-year studies and subsampling of habitats.

### 5.3 Functional zonation of wetland: Spatial variability in response to sediment re-hydration

We found two distinctly different functional zones following experimental re-hydration of dried sediments at Little Darkin Swamp. As the wetland fills and dries on an annual basis, the clay pan experiences a highly dynamic water regime with several transitional stages. These transitions between wet and dry result in some areas of the wetland retaining water for longer, whereas other areas are more exposed to air by alternating between wet and dry more frequently. This in turn changes the characteristics of these areas, such as their sediments and the vegetation cover, changing the way they respond to addition of water, and consequently the biotic assemblages that reside there (Larned, Datry, Arscott, & Tockner, 2010).

Areas in the centre of the wetland, which experience frequent and longer duration of inundation showed a more productive biotic response to rehydration of sediments than the areas at the edges of the wetland (Chapter 4). These areas, with lower elevation, had higher vegetation density (i.e higher plant growth) (Chapter 2), producing more organic matter and in turn increasing the nutrient and ion levels. These conditions in turn resulted in higher decomposition rates, causing

lower amounts of dissolved oxygen, but also higher macroinvertebrate species richness. In contrast, the more elevated areas with lower frequency and duration of inundation are more sparsely vegetated (Chapter 2), and thus have lower organic matter, resulting in lower nutrient levels and higher DO, reducing plant growth. These conditions are suitable only for macroinvertebrate species that are highly adapted to the conditions (Stubbington et al., 2016), and therefore species richness was also lower.

Changes in the hydrology of the wetland is likely to alter the functionality of the inner zone. Lower rates of rainfall will decrease the extent of the lower inundation zone. Less moisture, for shorter durations, will presumably decrease the density of vegetation, decreasing litter fall, and lower rates of accumulation of organic matter due to higher exposure of sediments to air. This will make the wetland more oligotrophic by reducing nutrient levels (Karlsson et al., 2009).

Being an oligotrophic wetland, algal production was also generally low throughout the wetland following re-hydration (Chapter 4) and generally in the wetland which corresponds with the generally low GPP and R in the wetland, as well as the results of the food web transfer, indicating that algae was not a major source of carbon for the food web (Chapter 3).

This sediment rehydration experiment was carried out in the laboratory, and although conditions were similar to average wetland conditions, experimental conditions will always differ from those occurring *in situ* in wetlands. These limitations are common for all laboratory experiments and are widely recognised (e.g. Stubbington & Datry, 2013). However, such experiments facilitate controlled comparisons under different conditions.

## 5.4 Possible future projections for Little Darkin Swamp

The large reductions in rainfall over the last 30 years in the Perth region has likely already impacted these shallow, ephemeral clay-based wetlands, although the degree and nature of the changes are currently not known. Future changes to the hydrology of Little Darkin Swamp will continue to alter its current status and wetland functions. A decrease in rates of rainfall will result in lower frequency and shorter duration of inundation, increasing the annual duration of sediment exposure. As discussed in Chapter 3, being inundated on a seasonal basis prevents accumulation of organic matter in the basin, therefore it is reasonable to predict that longer exposure of the sediments will further reduce the organic matter content of the wetland's substrate. This leads to overall higher rates of export of material, pushing the metabolism to become more autotrophic as respiration declines. In addition, summer fires may also have this effect on the wetland, by reducing accumulation of organic material in the basin, however this need further research. In

contrast, longer and more frequent inundation will have the opposite effect. Respiration will increase due to the higher amounts of organic matter in the system, due to higher rates of decomposition, in turn increasing heterotrophy of the system.

Changes to the hydrology will also affect the biota of the wetland. Drier conditions will enhance the oligotrophy by reducing nutrients, decrease rate of vegetation growth and cover, and lower macroinvertebrate richness. The community composition will switch towards one dominated by drought tolerant species adapted to harsh exposed conditions, such as Copepods, Cladocerans, Turbellarians and Chironomids, which have drought resistant eggs and cysts (Strachan et al., 2015). Species that only have short-term resistance to drying, such as oligochaetes and caddisflies, will likely be lost during longer droughts (Strachan et al., 2015).

In addition, drier conditions will alter the sources of carbon available for the aquatic food web. For example, shorter duration of inundation and less water in the basin may result in reduced macrophyte growth, potentially increasing the importance of detritus as a basal source for the food web. For example, the growth of submerged macrophytes *Ornduffia sumbersa* (a Priority species, see Chapter 2) and *Cycnogeton huegelii*, which are currently abundant in the basin during the winter months with standing surface water, will be reduced if not lost altogether.

## 5.5 Management implications

Currently, Little Darkin Swamp is in a reasonably good condition in terms of native vegetation cover, and there is little presence of weeds and relatively few disturbances to the sediments by grazing animals. However, there are threats that have the potential to negatively alter the current condition of the wetland. There are signs of disturbances to the sediment and vegetation by feral animals, such as wild pigs, the occurrence of some weeds, and the presence of destructive recreational human activity. The wetland and surrounding area are relatively untouched by humans, however, 4-wheel drive tracks within the wetland, shotgun cartridges from hunting activities and inappropriate disposal of rubbish have been observed around the wetland. In addition, the invasive *Cherax destructor* (most likely introduced for recreational purposes) has been observed in the nearby dam, and was also observed in burrows within the wetland. These observations reinforce the need for protection and conservation of Little Darkin Swamp from future harmful disturbances.

The low water levels and prolonged dry phases of these seasonal claypans make them particularly exposed to grazing animals. Therefore, grazing animals pose a threat to claypans, by altering species composition and introducing or facilitating weed invasion (Yates, Norton, & Hobbs, 2000),

disturbing benthic surfaces, altering water quality and affecting productivity. Feral animals, such as rabbits (*Oryctolagus cuniculus*) and pigs (*Sus scrofa*) are especially of concern due to grazing and burrowing, though some native animals such as kangaroos can also have adverse impacts (Brown et al., 2015). At Little Darkin Swamp, feral pigs and kangaroos are the likely the main grazing animals. Management actions are needed to prevent further disturbances caused by these animals, such as fencing or removal of invasive species.

Changes such as increased nutrient levels due to leaching lead to weed invasions, as native flora are not adapted to these conditions. In fact, weed invasion is a major threat to the natural diversity and balance of clay pan communities, and can replace native plants (Brown, Paczkowska, Huston, & Withnell, 2005). While other activities such as grazing, trampling and 4-wheel driving will contribute to the spread of weeds, this will also be facilitated by the effects of a drying climate on the water regime. Weeds compete with native flora for light, nutrients and water, and can prevent recruitment and cause changes in their abundances, particularly following disturbances such as frequent fire, grazing or clearing. In addition, exotic species can lead to alterations to the properties of the sediment maintained by the native species, changes in carbon and nitrogen cycling, and alterations of the amounts of organic matter deposited onto the sediments. These changes can in turn have negative consequences for the aquatic ecosystem by altering the amount and type of organic material entering the system, altering internal processes such as nutrient cycling, ecosystem metabolism, primary production and carbon cycling. In addition to causing a shift in the trophic status of the system, this can have fatal consequences for the macroinvertebrate community by removing microrefuges in summer (Strachan, 2016). Furthermore, weeds may carry pests and diseases and can lead to an increase in risk of fire. According to Gibson et al. (2005), approximately 16% of the claypan flora in southwestern Australia are weeds, suggesting that monitoring and prevention of invasion is needed at wetlands such as Little Darkin Swamp.

Another major threat to these claypan communities is disturbance caused by inappropriate recreational activities such as four-wheel driving, dirt bikes and dumping of rubbish (Kate Brown et al., 2015; Threatened Species Scientific Committee, 2012). Not only can these activities lead to adverse damage to benthic surfaces where biota find refuge during the dry periods, they will also kill plants, reduce recruitment and cause weed invasion or spread of dieback. Dieback (*Phytophthora cinnamomi*) is known to be a serious threat to the viability and genetic diversity of the southwestern Australian native flora (Shearer, Crane, & Cochrane, 2004). The plant pathogen also poses a threat to the flora of claypans such as Little Darkin Swamp. The occurrence of dieback disease has already been recorded at one nature reserve where claypans occur (Bullsbrook Nature

Reserve), though claypan communities are less susceptible due to their heavy soils (Brown et al., 2015). Species in the families Proteaceae and Myrtaceae, and other woody perennials often found in claypan communities are especially affected, putting the dominating shrub (*Melaleuca lateritia*) at Little Darkin at risk.

Furthermore, inappropriate fire regimes are another significant threat to claypans such as Little Darkin Swamp, as they are naturally only burnt on an occasional basis. Fire poses a direct threat to the biota occurring on these claypans, particularly as they are dominated by wetland species which are not adapted to the effects of fire (Brown et al., 2015). Furthermore, inappropriate fire regimes are suggested to have negative impacts on water quality of wetlands that dry out during summer by altering nutrient and carbon dynamics (Horwitz & Sommer, 2005). In addition, it has been suggested that the impacts of a drying climate may be intensified by fire. The DPaW policy is that the clay pans should not be subject to fire (Threatened Species Scientific Committee, 2012).

On a general basis, the drying climate in southwestern Australia poses a great threat to seasonal wetlands (Sim et al., 2013), such as Little Darkin Swamp, due to reduced surface water from rainfall. Further declines in winter rainfall may lead to loss of specific plant and animal species that depend on specific hydrological regimes of the claypans, and it is predicted that the drying trend will continue (Bond, Lake, & Arthington, 2008; Strachan, 2016; Strachan et al., 2015). For Little Darkin Swamp, the changes in the climate will also likely change aspects of the hydroperiod such as the timing of first inundation and drying, the maximum water level and duration of inundation, and rate of drying, as well as frequency of inundation. What the consequences will be for the biota will be highly linked to the changes of the internal processes and functions, as well as changing the distribution of vegetation and macroinvertebrate communities.

For example, at Little Darkin Swamp, areas with a higher density of woody vegetation (i.e. *M. lateritia*) have higher organic matter content and shading, keeping temperatures cooler and retaining moisture for longer. These areas are therefore desirable for macroinvertebrates as they allow for preparation for desiccation. However, areas of open water with sparse vegetation dry more rapidly and are more exposed to high temperatures and UV light, which challenge macroinvertebrate survival. Management should, therefore, aim to maintain the high vegetation density areas by preventing practices within the catchment that would reduce runoff of rainfall into the wetland, and reducing the size of the current higher inundation zone in the centre of the wetland. This includes practices such as reducing weed growth, drainage and water storage in the surrounding area.

The interannual variability in water regime shown in Chapter 2 and the finely balanced metabolism shown in Chapter 3 suggests that the wetland functions of Little Darkin Swamp change from year to year. This implies a need for long-term monitoring of the wetland to identify inter-annual patterns in relation to changes in weather conditions and disturbances. So far it is not known how disturbances such as grazing and changes to hydrology have affected the wetland, mainly due to the lack of a baseline for comparison. This thesis has shown the ecological responses to annual drying and re-wetting of the sediments and overall wetland metabolism. This provides a baseline of ecological information for Little Darkin Swamp for monitoring, and allows future management to assess changes to the condition of similar wetlands of this type.

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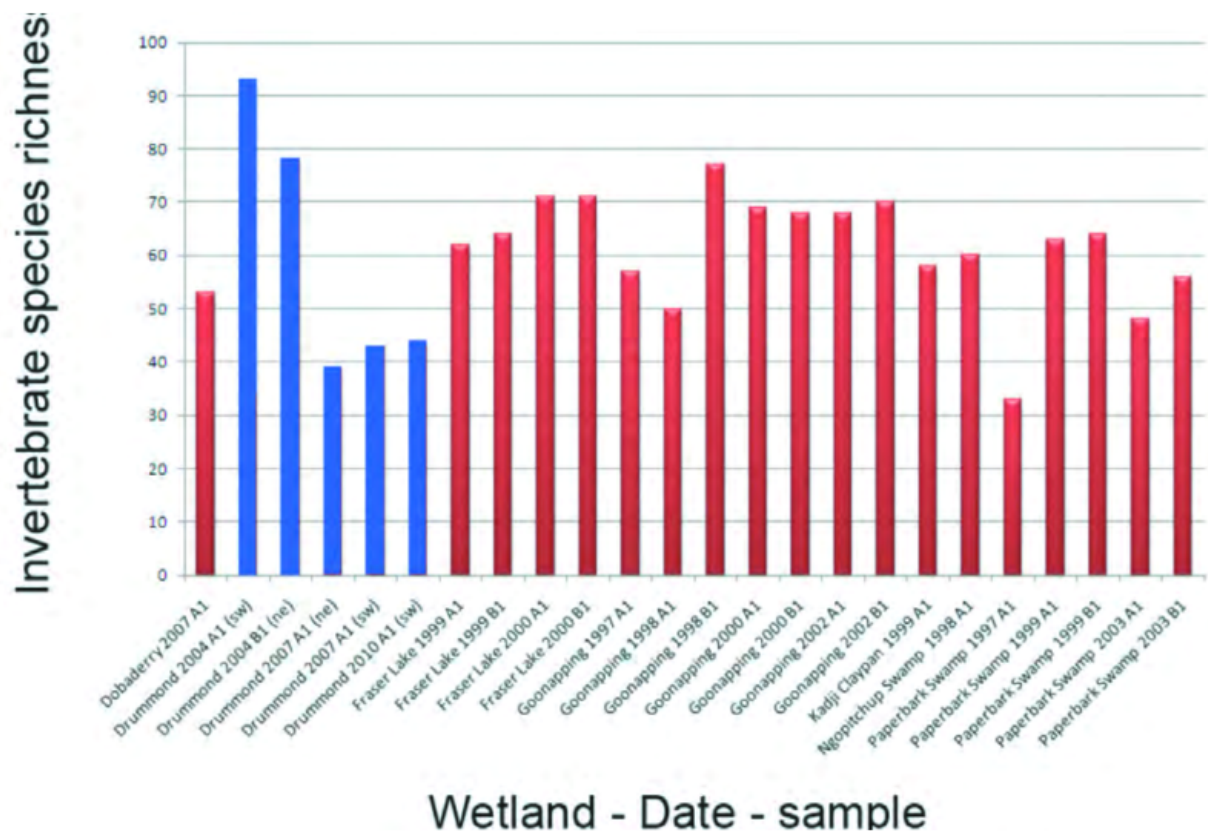


# 6 APPENDICES

## Appendix I – Macroinvertebrate richness at claypans of southwestern Australia.

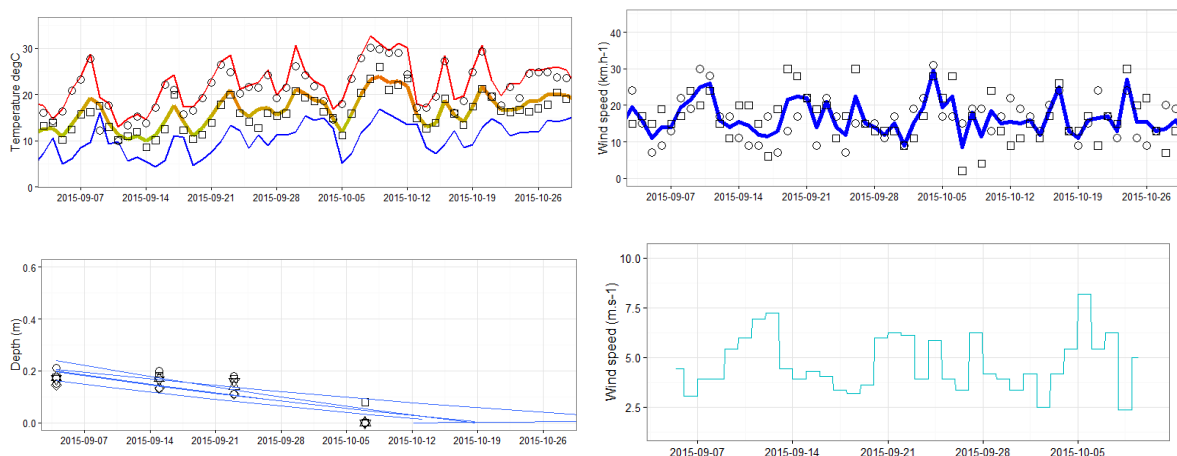
**Table 6.1.** Invertebrates collected at Goonaping Swamp, 1998 (Source: Pinder, Halse, McRae, & Shiel, 2004).

TAXA	1998	TAXA	1998
Turbellaria	✓	<i>Ilyodromus</i> sp. 255	✓
Nematoda	✓	<i>Cabonocypris nunkeri</i>	✓
ROTIFERA		COPEPODA	
Rotaria sp.	✓	<i>Calamoecia attenuata</i>	✓
<i>Brachionus quadridentatus</i>	✓	<i>Microcyclops varicans</i>	✓
<i>Lecane quadridentata</i>	✓	<i>Australocyclops palustrum</i>	✓
<i>Lecane</i> sp.	✓	<i>Mesocyclops brooksi</i>	✓
<i>Monommata</i> sp.	✓	<i>Canthocamptidae</i> sp. 5	✓
GASTROPODA		COLEOPTERA	
<i>Ferissia petterdi</i>	✓	<i>Halipius fuscatus</i>	✓
<i>Glyptophysa</i> cf. <i>gibbosa</i>	✓	<i>Alloessus bistriatus</i>	✓
ACARINA		<i>Sternopriscus</i> sp.	✓
<i>Eylais</i> sp.	✓	<i>Megaporus howitti</i>	✓
<i>Limnesia dentifera</i>	✓	<i>Lanceles lanceolatus</i>	✓
Oribatida	✓	<i>Onychohydus scutellaris</i>	✓
Mesostigmata	✓	<i>Hydrochus</i> sp.	✓
Trombidioidea	✓	<i>Berosus approximans</i>	✓
CONCHOSTRACA		<i>Paranacaena littoralis</i>	✓
<i>Eulimnadia</i> sp. ✓	✓	<i>Enochrus eyrensis</i>	✓
<i>Lynceus</i> sp.	✓	<i>Paracymus pygmaeus</i>	✓
CLADOCERA		DIPTERA	
<i>Latonopsis</i> sp.	✓	<i>Anopheles annulipes</i>	✓
<i>Alona affinis</i>	✓	<i>Culicoides</i> sp.	✓
<i>Alona setigera</i>	✓	Sciomyzidae	✓
<i>Alona macrocopa</i>	✓	<i>Paramerina levidensis</i>	✓
<i>Celsinotum</i> sp.	✓	Tanypodinae sp. C (nr <i>Tanypus</i> )	✓
<i>Chydorus</i> sp.	✓	<i>Corynoneura</i> sp.	✓
<i>Rak</i> sp.	✓	<i>Tanytarsus fuscithorax</i>	✓
<i>Ceriodaphnia</i> sp.	✓	<i>Chironomus</i> aff. <i>alternans</i>	✓
<i>Scapholeberis</i> cf. <i>kingi</i>	✓	<i>Polypedilum</i> nr. <i>convexum</i>	✓
<i>Simocephalus</i> sp.	✓	<i>Cladopelma curtivalva</i>	✓
<i>Macrothrix</i> sp.	✓	HEMIPTERA	
OSTRACODA		<i>Saldula brevicornis</i>	✓
<i>Limnocythere dorsicula</i>	✓	<i>Anisops thienemanni</i>	✓
<i>Limnocythere</i> sp. 447	✓	<i>Anisops hyperion</i>	✓
<i>Paralimnocythere</i> sp. 262	✓	ODONATA	
<i>Candonopsis tenuis</i>	✓	<i>Austrolestes analis</i>	✓
<i>Bennelongia australis</i>	✓	<i>Hemianax papuensis</i>	✓
<i>Candonocypris</i> sp.	✓	<i>Diplacodes bipunctata</i>	✓
<i>Cypretta baylyi</i>	✓	TRICHOPTERA	
<i>Cypretta</i> sp. 527	✓	<i>Triplectides australis</i>	✓
<i>Ilyodromus</i> sp. 566	✓		

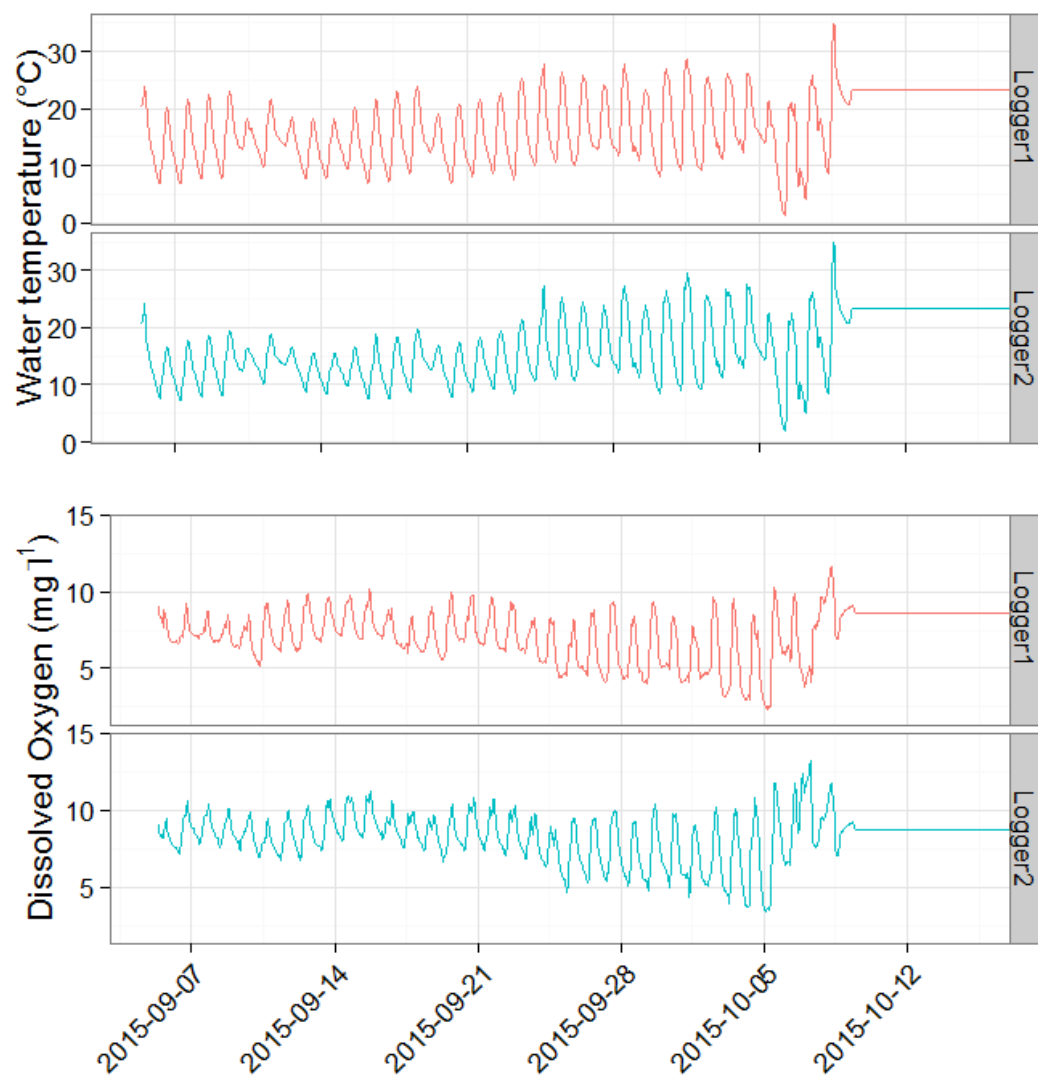


**Figure 6.1.** Invertebrate species richness in Drummond Nature Reserve claypan wetlands (blue columns) and a range of other clay-based wetlands (red columns) in the south-west of Western Australia (Source: Department of Parks and Wildlife, 2013).

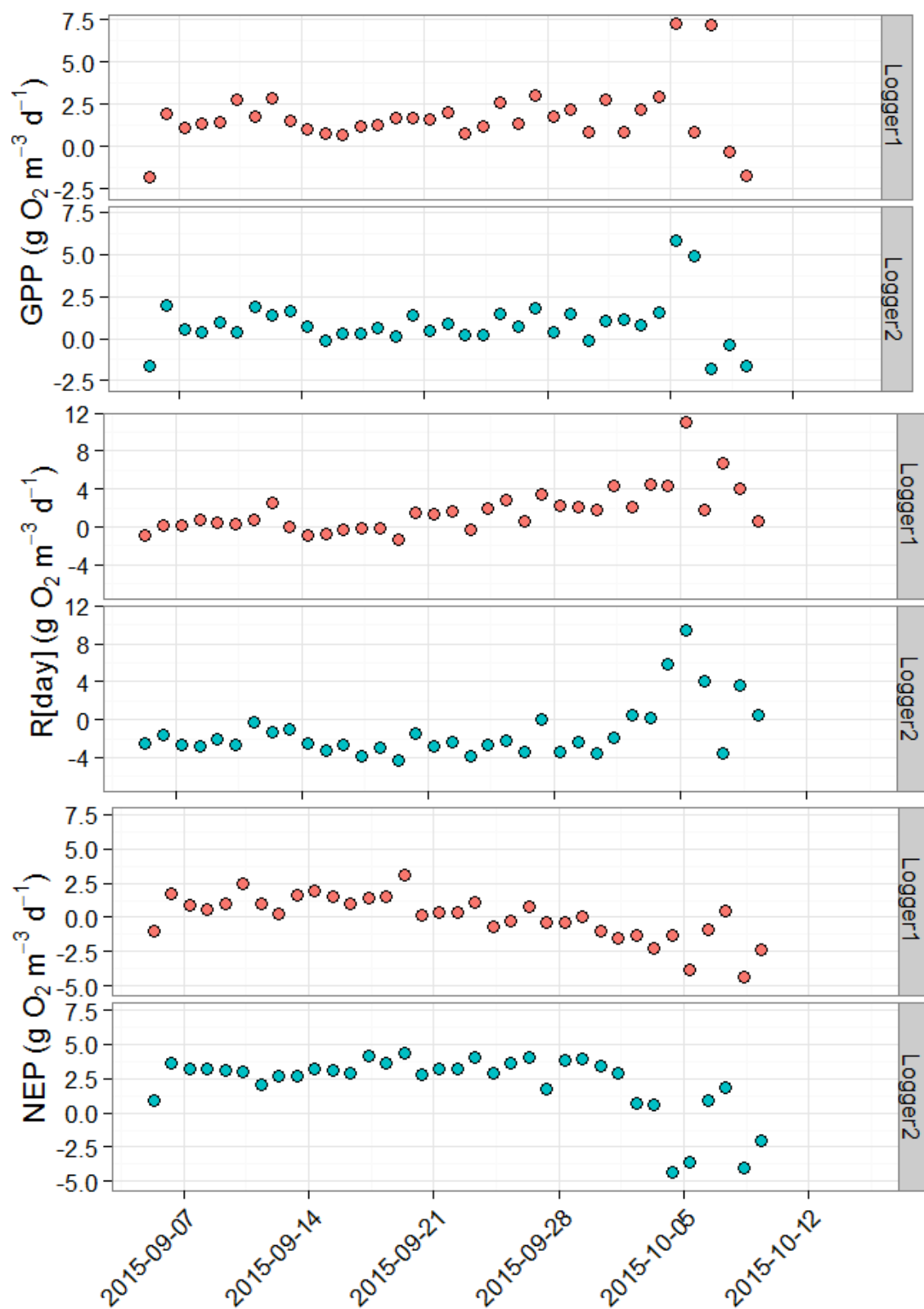
## Appendix II – Ecosystem metabolism winter 2015 and food chain



**Figure 6.2** Maximum, minimum and average temperature, wind speed, and water depth (taken at sites 1-7, see Chapter 2) for the period Sep-Oct 2015.



**Figure 6.3.** Water temperature and dissolved oxygen dynamics for two habitat types (Blue Log 2: open water, Red Log 1: vegetated) during winter 2015 (September- October).



**Figure 6.4.** Rates of GPP, R and NEP during the winter of 2015 (September- October) at two habitat types (Blue Log 2: open water, Red Log 1: vegetated).

## 6. Food chain

A total of 9 different species of consumers were collected for SIA at the 2015 sampling (Table 1), with 6 feeding strategies among them.

We found 4 trophic levels (primary producers, 1<sup>st</sup> and 2<sup>nd</sup> order consumers and top predator), which is relatively short for aquatic ecosystems (ref). This could be a result of the disturbance in the wetland, which can affect the length of the food web (ref: Warfe et al). The ecosystem size can also affect the food chain length. Little Darkin Swamp is a relatively small wetland and has a lower relative species richness, less basal resources and less functional trophic diversity, and therefore more omnivory (ref: Warfe). In addition, being a small wetland, disturbances will have larger effects compared to larger wetlands. The effects of disturbance on productivity are exacerbated in smaller systems leading to increased omnivory and shorter food chains (Warfe et al).

**Table 6.2.** Macroinvertebrate community composition at Little Darkin, late winter 2015, with their predicted feeding strategies.

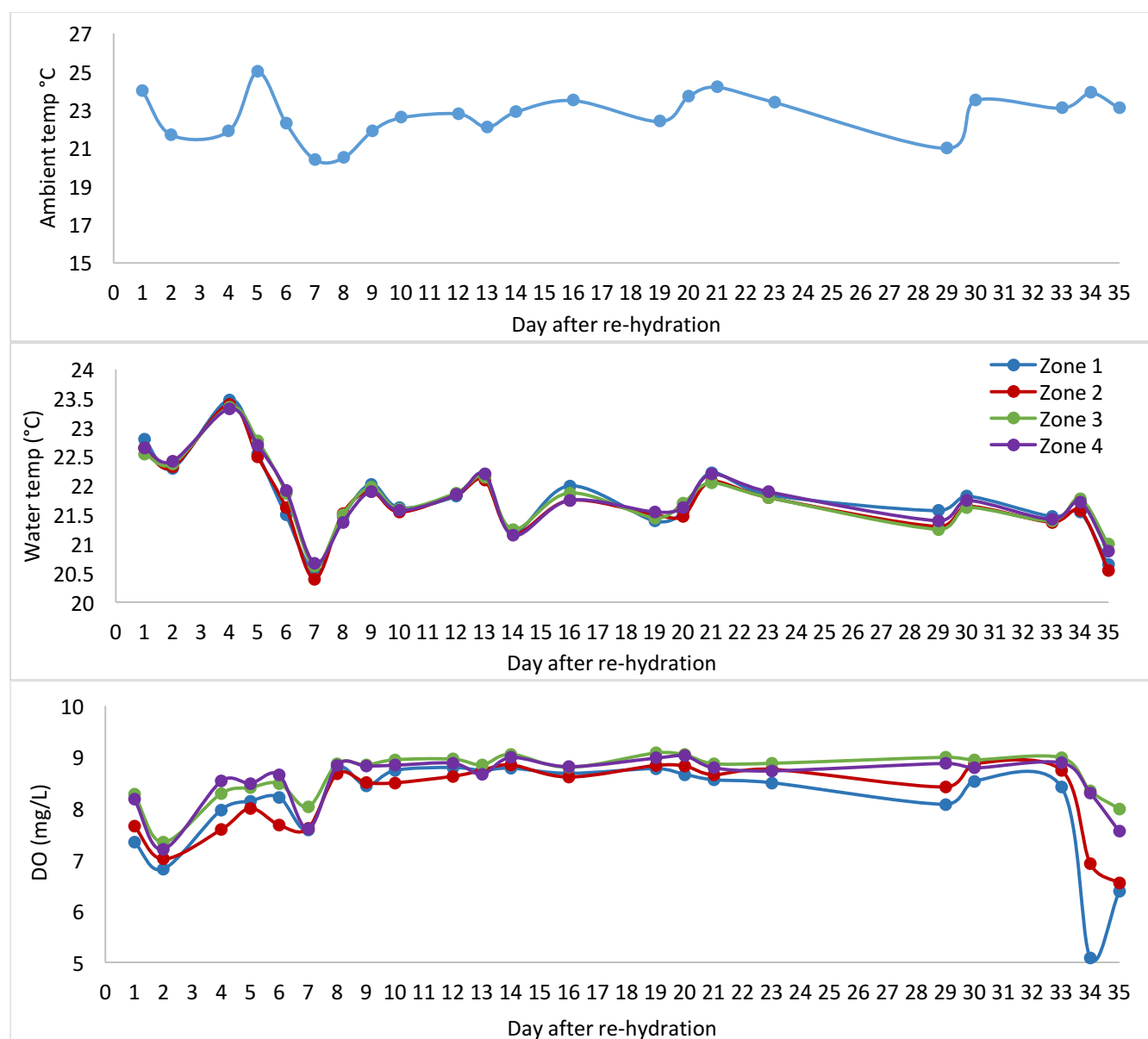
Order	Family	Description	Feeding strategy
Gastropoda		Aquatic snails	Grazer
Coleoptera	?Hydrochidae	Aquatic beetles	Grazer/shredder
		Aquatic weevils	Grazer/shredder
Araneae		Spider	Predator
Acarina		Aquatic mites	Predator/parasitic
Odonata		Damselfly larvae	Predator
Crustacea		Amphipods	Shredder/grazer/filterer
Ostracoda		Ostracods	Filter feeder
Amphibia		Tadpole	Predator/shredder

## APPENDIX III- Sediment re-hydration experiment additional data

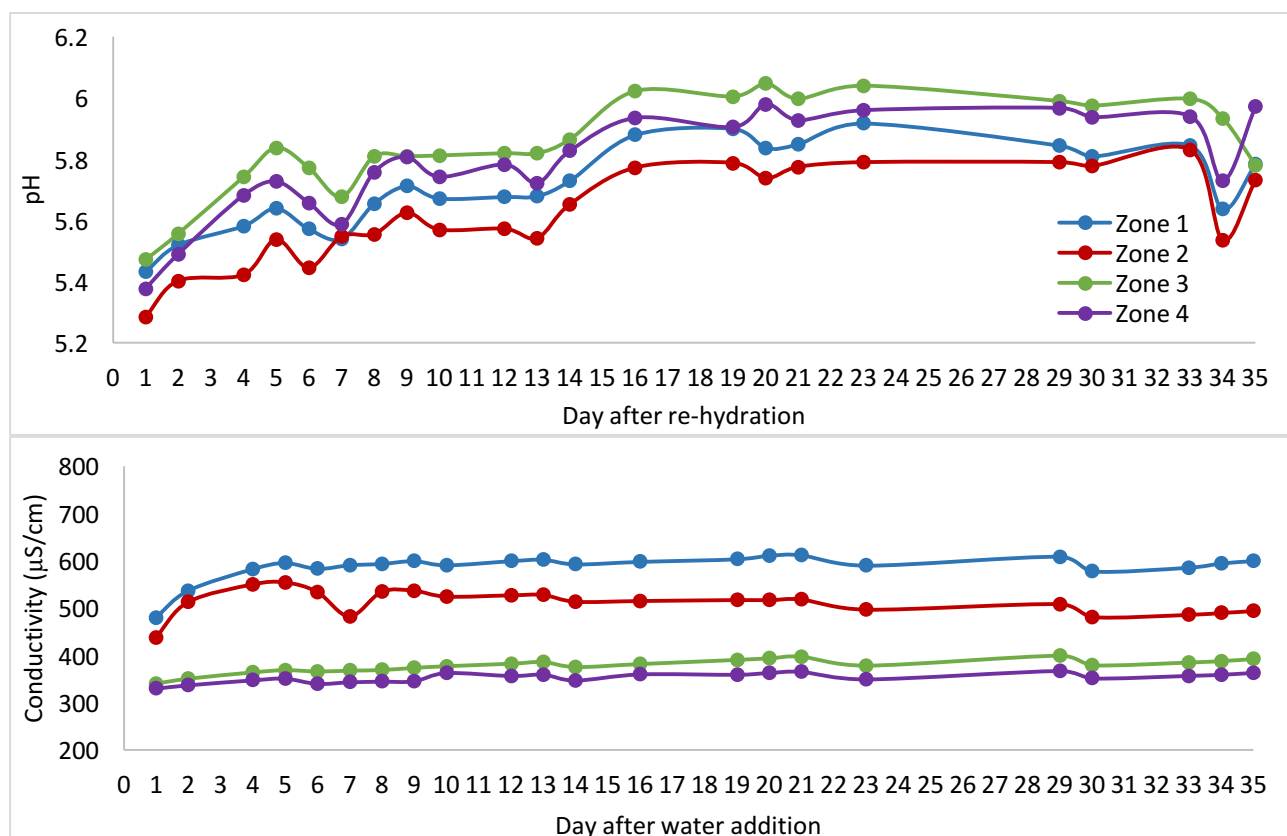
**Table 6.3.** Sample distributions in experimental aquaria and sample identifications for re-hydration experiment.

Elevation level	Vegetation zone	Aquaria #	Sample ID	Loggers
Level 1	V1a V1b	1	Z1V1-A	Logger 1
		7	Z1V1-B	
		11	Z1V1-C	
		15	Z1V1-D	
Level 2	V4	2	Z2V4-A	Logger 2
		6	Z2V4-B	
		10	Z2V4-C	
		14	Z2V4-D	
Level 3	V5	3	Z3V5-A	
		5	Z3V5-B	
		13	Z3V5-C	
		17	Z3V5-D	
Level 4	V6	4	Z4V6-A	
		8	Z4V6-B	
		12	Z4V6-C	
		16	Z4V6-D	
Control	-	9	-	

## Physicochemical response



**Figure 6.5.** Daily fluctuations in ambient (top) and water temperature (middle), and DO concentrations (bottom) in the four sediment zones over the course of 33 days.

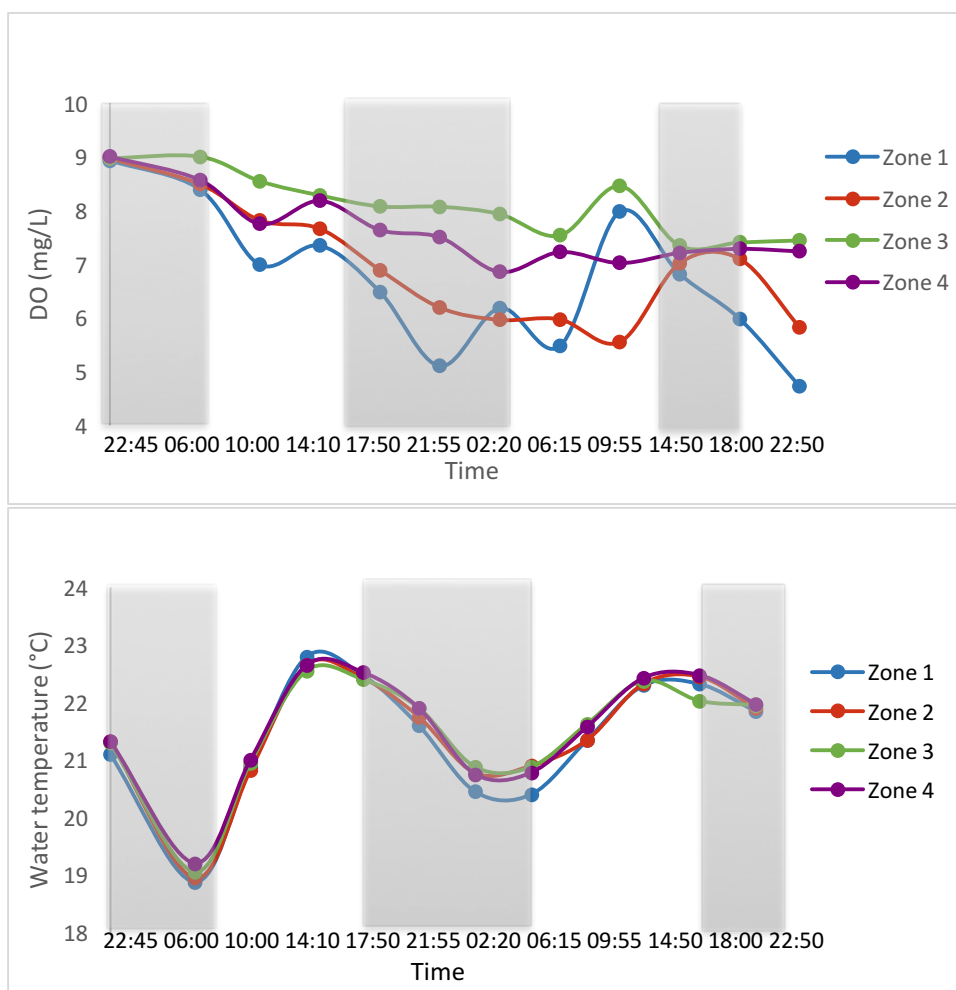


**Figure 6.6.** Daily fluctuations in pH and conductivity levels in the four sediment zones over the course of 33 days.

## Initial 48 hours: Temperature and DO

All zones had dissolved oxygen concentrations of 9 mg/L immediately after the addition of water. Once water was added and without any addition of oxygen, the DO levels of all zones steadily dropped with only slight rises in zones 1, 3 and 4 during daytime (Figure 3). This overall decreasing trend of DO in all zones indicates little or no primary production, and mostly respiration occurring during this time. This could possibly be due to decomposition of organic matter. At the end of the initial 48 hours, zones 1 and 2 had dropped down to 4.7 mg/L and 5.8 mg/L DO respectively, whereas zones 2 and 4 only dropped down to 7.2 mg/L and 7.4 mg/L respectively. Zone 1 had larger fluctuations in DO than any other zone, possibly due to more disturbance from handling of the sediments. The water temperatures were similar across the zones and showed diurnal fluctuations during the initial 48 hours as expected from the changing ambient temperatures caused by exposure of sunlight (Figure 3). The changes in water temperature did not have any effect on the DO concentrations during this time. There was no apparent pattern between water temperature and DO fluctuations, nor time of day and concentrations in DO in any zones.

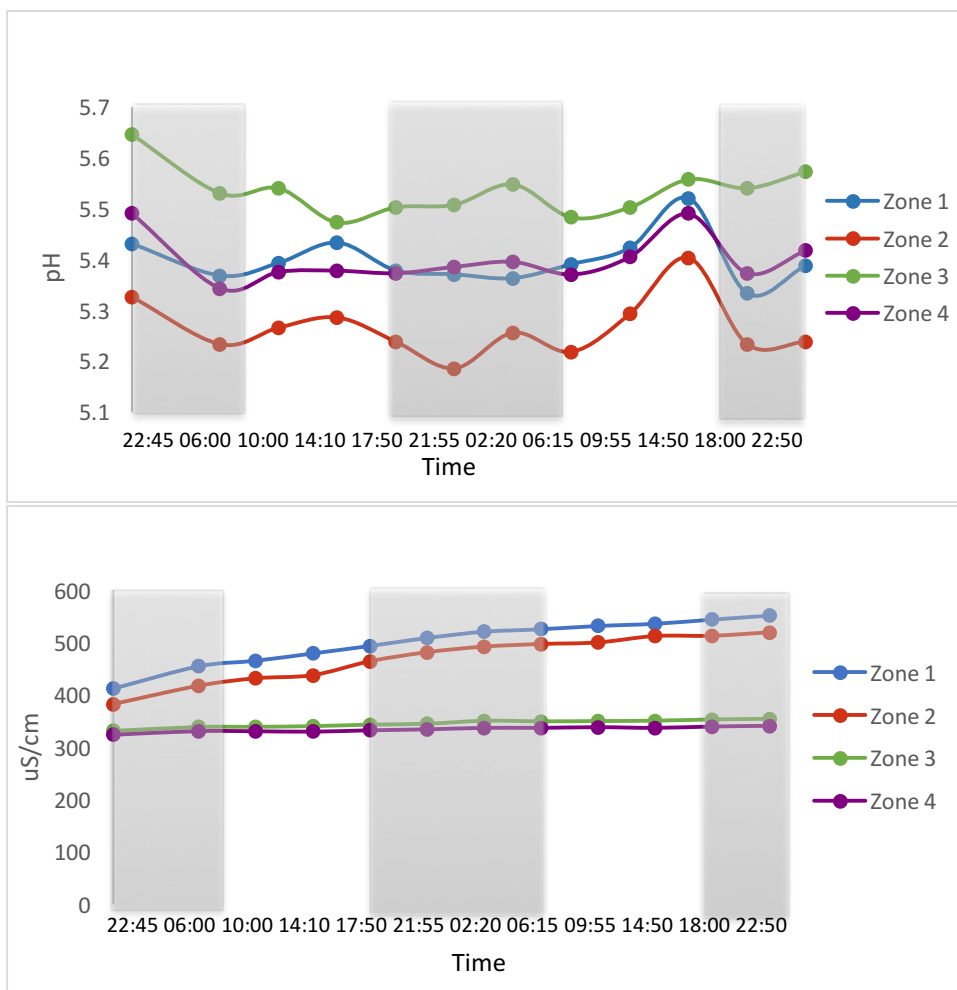




**Figure 6.7.** Fluctuations in average dissolved oxygen and temperature during initial 48 hours after the addition of water to sediments. Shaded areas represent night-time hours.

## pH and conductivity

The pH of the four zones only differed slightly and were all strongly acidic during the initial 48 hours of the experiment (Figure 4). The values ranged from pH 5.2-5.6, with zone 2 being most acidic and zone 3 being the least acidic. Zones 1 and 4 had similar pH levels and fluctuations during the first 48 hours. The fluctuations in pH seemed to be diurnal across all zones, with peaks during the day and drops during night-time, although this was more pronounced in some zones. Zones 1 and 2 had similar higher electrical conductivities compared to zones 3 and 4, which had similar lower conductivities. Again this could simply be caused by the differences in amounts of salt added to the aquaria, particularly at the start of the experiment before the release of any minerals from the sediments. The conductivities of zones 3 and 4 stayed constant at approximately 330  $\mu\text{S}/\text{cm}$  after the addition of water, while in zones 1 and 3 they steadily increased from 400  $\mu\text{S}/\text{cm}$  to approximately 550  $\mu\text{S}/\text{cm}$  (Figure 4).



**Figure 6.8.** Average levels of pH and conductivity across the zones during initial 48 hours after the addition of water to sediments.

## Macroinvertebrate emergence

**Table 6.4.** Macroinvertebrate presence/absence and species richness. X = presence, - = absence.

Order/Species	Zone 1	Zone 2	Zone 3	Zone 4
Nematoda	X	X	-	-
Platyhelminthes - Turbellarians	X	X	-	-
Platyhelminthes ?dugesia sp.	X	-	-	-
Platyhelminthes (green)	-	X	-	-
Platyhelminthes (Planarian)	-	-	-	X
Orange dot Platyhelminthes ?Mesostoma sp.	-	-	X	-
Acarina (red water mite)	X	-	-	-
Copepoda	X	X	X	-
Cladocera (water flea)	X	X	X	X
Cladocera ?Simocephalus sp.	X	-	-	-
Ostracoda (red/green)	X	X	X	X
Ostracoda (round green ball)	X	X	X	-
Amphipoda	X	-	-	-
Ceratopogonidae w/hair bristles	X	X	-	-
Ceratopogonidae (biting midges)	X	X	-	X
Chironomid larvae	X	X	X	-
Zygoptera (skin)	-	X	-	-
Philorheithridae ?Stick caddis	-	X	-	-
Hydroptilidae ?Micro caddis	-	X	-	-
?Plectrotarsidae ?Caddisflies	-	X	-	-
<b>Total number of taxa</b>	<b>12</b>	<b>12</b>	<b>6</b>	<b>4</b>

**Table 6.5.** Correlation matrix of environmental variables (-1 to 1). Correlation coefficient  $r > 0.5$  or  $r < -0.5$  indicates a correlation between variables.

	Cond 0-5	N	P	OM%	Plant mass	Gilvin	Chl <i>a</i>	Periphyton	Temp 8-33	DO 8-33	pH 8-33
Cond 0-5											
N	<b>0.5</b>										
P	-0.09	<b>0.5</b>									
OM%	<b>0.6</b>	<b>0.7</b>	0.2								
Plant mass	0.1	-0.009	-0.05	0.2							
Gilvin	-0.3	-0.2	0.2	0.3	-0.2						
Chl <i>a</i>	-0.2	-0.3	-0.2	-0.1	0.4	-0.3					
Periphyton	-0.1	0.012	0.2	-0.07	-0.1	-0.3	-0.1				
Temp 8-33	-0.1	0.2	0.2	-0.01	0.03	0.2	-0.3	0.09			
DO 8-33	<b>-0.5</b>	-0.3	-0.1	<b>-0.6</b>	-0.3	0.2	0.02	0.3	-0.4		
pH 8-33	<b>-0.5</b>	-0.4	0.2	<b>-0.5</b>	0.05	<b>0.6</b>	-0.3	-0.2	<b>-0.5</b>	-0.02	