

Report No. CEM2016-03

**BIODIVERSITY AND ECOSYSTEM FUNCTIONING OF  
THE NORTHERN PONDS, LAKE MACLEOD,  
WESTERN AUSTRALIA - FINAL REPORT**

**Lead Authors: Christopher R. J. Kavazos & Pierre Horwitz**



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FINAL REPORT**



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**July 2016**

**Centre for Ecosystem Management Report Number 2016-03**

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## ACKNOWLEDGEMENTS

This project would not have been possible without the logistical and financial support provided by Dampier Salt Ltd., and its staff, especially Brett Renton at the Lake MacLeod operations and in Perth: Chris McQuade, Steve Rusbridge, Star Gianatti and Brent Tobin.

We are immensely grateful to Rob Czarnik, the ECU School of Science Field Support and Safety Officer, for his hard work and invaluable help on almost every field trip. Mr Hugh Dunn and Mr Phillip Draber assisted with OH&S requirements in the early stages of the project.

Field assistance was provided by Dave Bauer, Michelle Newport, Pauline Hewitt, Rob Davis, Ben Jones, Luca Chiaroni, Emily Cole, Dylan Sharpe, Alison Sampey, and Aldo Turco.

Simon Judd produced the final report for publication.



## EXECUTIVE SUMMARY

This report provides a summary of the research conducted by Edith Cowan University (ECU) in a research collaboration with Dampier Salt Ltd. (DSL): a *Quantification of the Biodiversity of the Lake MacLeod Northern Ponds*. The research set out a number of projects that were to address knowledge gaps for the system.

### Hydrology and Sediments of permanent ponds

The Northern Ponds of Lake MacLeod represent a wetland system where the climate and hydrology permit the establishment of permanent inland waters dominated by marine processes, and differentiated by size. Because the system lies in the arid subtropical regions of Australia's northwest, and is therefore subjected to very low levels of precipitation, the surrounding catchments which drain into the basin appear to have little influence on the composition of the habitats found within the ponds. In fact, because of the rather constant supply of marine water and low rainfall, the ponds resemble those of marine systems in that biogenic processes are determining the benthic/biofilm habitat found in them.

Most of the ponds are relatively small lakes, where in-lake production of calcium carbonates (mainly tests of foraminifera, and a green alga *Acetabularia*) dominate biogenic sediments. The largest, Cygnet Pond, has more terrigenous processes, characterised by higher levels of nutrients, potassium depletion, aragonite muds and seagrass meadows, superimposed on these biogenic signatures.

These patterns of hydrology and sediment formation demonstrate the unique nature of the Northern Ponds as permanent inland lakes embedded in an arid environment otherwise characteristic of an evaporite system.

### Microbial communities

Each of eight size-differentiated ponds studied contain unique assemblages of bacteria (benthic and planktonic), and to a lesser degree, ciliates, and these differences could not be attributable to changes in the measured environmental parameters nor immigration rates. This suggests that either an unaccountable variable, which is spatially independent but varies between ponds is driving these changes, or that a neutral processes operating independently of immigration/dispersion is driving the separation of the communities. However, the benthic bacterial and ciliate communities appear to be highly segregated in terms of taxa co-occurrences. These results suggest that populations of OTUs may be opportunistically reacting to stochastic events, such as flooding and temperature fluctuations.

The ponds are primarily composed of marine taxa, as well as those from deep oceans, freshwater, hypersaline and terrestrial ecosystems. Each pond assemblage appears to be on its own evolutionary trajectory, with ecological drift likely playing a role in structuring each community. The fact that each

pond contains unique microbial communities, not only highlights the conservation value of each pond, but also provides information on the functioning of the system.

### Invertebrates

Thirty-four macroinvertebrate families were found from littoral habitats at six vents in the Northern Ponds. The assemblages were numerically dominated by amphipods and gastropods.

Invertebrate communities are different in the Chirrida and Cygnet systems. These differences are related to different environmental conditions and the connectivity (or lack of it) within and between ponds found in each system. Nutrient levels in ponds are good determinants of invertebrate community structure.

Historical analysis shows that flooding in the Cygnet pond can temporarily change the invertebrate assemblage. It also appears that nutrient levels have increased in the Cygnet Pond since the 1990's, possibly associated with the 2000 flood.

### Fish

The Northern Ponds have few species of fish, and the assemblage itself seems largely unremarkable, except for the broad range of habitats used by few species, their sheer abundance and biomass available for fish eating waterbirds. Fish species recorded at Northern Ponds are shown below.

Common name	Scientific name	Comments
Flag-tailed (yellowtail) grunters	<i>Amniataba caudavittata</i>	Abundant and ubiquitous
Few-Ray Hardyheads	<i>Craterocephalus pauciradiatus</i>	Abundant and ubiquitous
Tilapia	<i>Oreochromis mossambicus</i>	Ubiquitous
Giant herring	<i>Elops hawaiiensis</i>	Northeastern vents of Cygnet pond only
Sea mullet	<i>Mugil cephalus</i>	Reported in the literature; unconfirmed.
Spangled perch	<i>Leiopotherapon unicolor</i>	Reported in the literature; unconfirmed.

Flood events in the years 2000 and 2010-11 have allowed populations of the invasive cichlid fish tilapia *O. mossambicus* to enter and become established in the Northern Ponds, now the species appears to have a wide distribution and high abundance throughout the Northern Ponds where they have a broad habitat utilisation.

This study aimed to quantify and describe the distribution and habitat use of the invasive tilapia and native fish species within the Northern Ponds, and determine if the invasive species is likely to be competing directly for the same food resources and/or predating on native fish species. The results suggest that these impacts appear unlikely, given the abundance of different food items, and the non-selective nature of benthic feeding of both tilapia and grunters; the hardyheads may be protected from

competition due to their different size structure and pelagic feeding strategies. Even though no direct effects of tilapia were observed on native fish, the feeding behaviours and complexity of this system demand further study to fully understand the effects of tilapia on benthic and littoral habitats in the Northern Pond ecosystem.

### **Shorebird Feeding**

This study reveals the remarkable value that Lake MacLeod has as a feeding ground for non-breeding migratory shorebirds. Although the study focused on two particular sites in the Northern Ponds system (Godwit Beach on Cygnet Pond and a spillsheet near Whistler Pond), it is likely that other similar or better feeding grounds may occur at Lake MacLeod, due to the complex heterogeneity of feeding habitat and extensive mudflats, especially on the south-eastern side of Cygnet Pond. This valuable area supports high numbers of shorebirds and is also an important non-breeding foraging site for migratory and non-migratory species, as it is strategically situated between the southern part of the continent and the northwest and its future protection is of high importance for shorebirds.

The maximum invertebrate prey densities at Lake MacLeod were found in the first 5 cm of the sediment, indicating that food was always available for small to medium-size shorebirds. Amphipods were the primary benthic prey for shorebirds at Lake MacLeod. Wind played a major role in shorebird feeding behaviour at Lake MacLeod, resulting in either the availability or exclusion of large areas of mudflats as foraging habitat.

Migratory and resident shorebirds both achieved higher intake rates (energy/min) at Lake MacLeod than at Dampier and Port Hedland. These differences appeared to be related to differences in prey density, prey type and prey availability. Lake MacLeod has high-quality feeding habitat for small to medium-size migratory shorebirds, enabling them, theoretically, to fulfil their daily energy requirements (theoretical Daily Energy Expenditure - DEE) both post and premigration. This finding explains previous observations of high concentrations of migratory shorebirds at the lake.

### **Mangroves**

This study investigated the distribution and structure of *A. marina* in relation to hydrological and sediment conditions existing at the semi-arid, inland mangrove system at Lake MacLeod. In addition, the productivity, water-use efficiency and morphological traits of the mangroves growing in three different hydrological regimes (inland, coastal and riverine) of the Gascoyne were compared.

The trees growing at Lake MacLeod had intermediate levels of water-use efficiency when compared to the coastal sites. Even though the main water supply at Lake MacLeod is saline, it is the constant supply of water that is influencing the water-use efficiency of these inland trees. Trees with highest productivity levels at Lake MacLeod were those growing close to permanent bodies of water, such as the ponds. These results suggest that distance from a permanent water source, and not salinity, is the key factor driving



short term and long-term production of mangroves at Lake MacLeod and coastal locations. There are a number of processes which influence water supply to plants in coastal, riverine and inland habitats. Coastal habitats, and to a lesser degree the riverine habitat, are delivered regular inputs of seawater via tidal movement, whilst the inland habitat at Lake MacLeod is delivered via seepage. However, the key difference between inland and coastal systems is the constant supply of seawater at the inland system, compared to the irregular but greater supply of freshwater in coastal habitats.

### **Sedimentary record**

The analysis of the two seagrass cores analysed here provide a record of the concentration and fluxes of chemical elements and biogeochemical dynamics throughout the last ~6000 years in the Cygnet Pond. The records show similar trends, with an abrupt increase in elemental concentration and decrease in carbonate concentration since ~200-250 years BP, consistent with the arrival of agriculture in the catchment areas. The rapid decrease in carbonate content and the increase in fine sediments during this period are consistent with higher levels of terrestrial inputs.

Regime shift analysis revealed four distinct shifts in the composition of the cores. The first period, only observed in one of the cores, represents the basin before it was disconnected from the Indian Ocean around 5800 years BP. The second and third periods both represent changes from 5800 years BP to approximately 1000 years BP, while the fourth period represents contemporary changes from 1000 years BP to present day.

Major shifts in ecosystem dynamics linked to changes in sea level and climate can be distinguished in the PCA analysis, and match the events hypothesised to have occurred during the evolution of the basin by Logan (1987) and Shepherd (1990). Recent increases in fine sediment content of the sediments, which may explain the increase in chemical elements of the sediments, indicates increased run-off from surrounding catchments which may be caused by climatic changes and/or agriculture. These results add to a growing body of literature demonstrating the role of sedimentary archives to reconstruct the trajectories of anthropogenic pressures and structural changes on aquatic ecosystems, which can improve management outcomes to better manage ecological change.

### **Synthesis – State of knowledge**

This project has broadly enhanced our knowledge of the spatial coverage, temporal changes, habitat descriptions and ecosystem functioning for the aquatic invertebrates, shorebirds, fish, mangroves, littoral vegetation, microbial benthic biofilm and bacterioplankton components of the Lake MacLeod Northern Ponds system. There are however, areas where further knowledge is needed. Although not directly quantified, ECU research at Lake MacLeod began after the flood event of 2010, and the subsequent boom and bust in productivity was noted by researchers. This cycle appears to be an important process in the functioning of the system. Other knowledge gaps exist in understanding the diversity and distributions of the extensive samphire communities, macro-algal communities and

microbial mats found in the extensive mudflat areas. All of these communities are likely to contain novel taxa and contribute to the biodiversity value of Lake MacLeod.

The research has confirmed that the Northern Ponds of Lake Macleod represent a wetland system of State, national and international significance for the following reasons:

- important and high-quality feeding grounds for small to medium-size migratory shorebirds both post and premigration,
- unique microbial and invertebrate assemblages in the permanent ponds themselves, and evidence for ecological drift of these communities;
- outstanding example of inland mangrove stands;
- significant example of living and fossil microbialite communities

Together these values indicate that it would be appropriate for the system to be granted further formal recognition. The Northern Ponds of Lake Macleod meet many of the criteria required to be considered a Wetland of International Importance under the Ramsar Convention.

# TABLE OF CONTENTS

<b>ACKNOWLEDGEMENTS .....</b>	<b>III</b>
<b>EXECUTIVE SUMMARY .....</b>	<b>IV</b>
<b>LIST OF FIGURES.....</b>	<b>XII</b>
<b>LIST OF TABLES .....</b>	<b>XIV</b>
<b>SCIENTIFIC RESEARCH CONDUCTED AT LAKE MACLEOD.....</b>	<b>1</b>
<b>LAKE MACLEOD: AN INTRODUCTION .....</b>	<b>5</b>
Geological formation and climatology .....	8
Geological evolution .....	8
Formation of the modern system .....	9
Cygnet marsh seepage face .....	10
Climate .....	10
Contemporary Seepage Environment.....	12
Brine sheet environment .....	12
Majana environment.....	13
Biological Significance of Area .....	13
<b>BIOGENIC OR TERRIGENIC? PERMANENT WATER BODIES OF THE NORTHERN PONDS IN THE LAKE MACLEOD BASIN OF WESTERN AUSTRALIA.....</b>	<b>15</b>
Methods.....	16
Results.....	17
Pond morphometry and hydrology.....	17
Water chemistry and sedimentology .....	20
Discussion .....	23
<b>MICROBIAL COMMUNITY STRUCTURE AND BIOGEOGRAPHIC PATTERNS .....</b>	<b>27</b>
Study One: Bacterial and ciliate biogeographic patterns.....	27
Methods.....	27
Results .....	28
Study Two: Bacterioplankton communities: structure and origin .....	30

Methods .....	31
Results .....	31
Discussion .....	33
<b>TEMPORAL AND SPATIAL VARIATION OF INVERTEBRATE COMMUNITIES .....</b>	<b>38</b>
Methods .....	40
Results .....	41
Community richness and diversity .....	41
Community variation .....	41
Community variation and environmental determinants .....	43
Discussion .....	43
<b>FISH COMMUNITY STRUCTURE AND FEEDING ECOLOGY .....</b>	<b>46</b>
Methods .....	48
Results .....	49
Community and size structure .....	49
Dietary analysis .....	51
Discussion .....	54
<b>SHOREBIRD FORAGING AND FEEDING ECOLOGY .....</b>	<b>58</b>
Methods .....	59
Biotic determinants of shorebird distributions .....	59
Abiotic determinants of shorebird habitat use .....	61
Habitat quality as a shorebird feeding ground .....	61
Results and discussion .....	62
<b>ROLE OF HYDROLOGY AND ENVIRONMENTAL CONDITIONS ON MANGROVE MORPHOLOGY AND PHYSIOLOGY .....</b>	<b>66</b>
Introduction .....	66
Methodology .....	68
Part 1: Identification of environmental parameters .....	68

Part 2: Ecophysiological condition and morphological features .....	68
Results and discussion - Identification of Environmental Parameters.....	69
Results and Discussion - Ecophysiological Condition and Morphological Features.....	73
Conclusion.....	74
<b>RECENT SEDIMENTS REVEAL PERIODS OF MARINE AND TERRESTRIAL INFLUENCE.....</b>	<b>76</b>
Introduction .....	76
Methods.....	76
Results.....	78
Discussion .....	82
<b>DERIVING A HABITAT CLASSIFICATION SCHEME FOR THE NORTHERN PONDS .....</b>	<b>84</b>
Background .....	84
Methodology.....	84
Research Design .....	84
Landsat 7 ETM+ Image pre-processing .....	85
Unsupervised classification.....	85
Accuracy assessment/Field ground-truthing .....	86
Habitat Classification Map - Supervised Classification.....	86
Results.....	87
<b>SUMMARY AND CONCLUSIONS .....</b>	<b>91</b>
Update on knowledge gaps .....	92
<b>REFERENCES.....</b>	<b>94</b>
<b>APPENDIX .....</b>	<b>107</b>

## LIST OF FIGURES

Figure 1: Locality map of the Lake MacLeod and brine bodies (blue) in Western Australia. ....	5
Figure 2: Locality map of some of the ponds and vents, as well as some landmarks of Lake MacLeod. ....	6
Figure 3: Map of the MacLeod basin showing the location of some of the ponds. Box A shows the location of the northernmost five ponds which include Pete’s Vent (1), Pete’s Pond (2) and Annie’s Pond (3; box c) as well as Whistler’s Pond (4) and Harjie’s Pond (5). Box B shows the locations of Donut Pond (6), Jana’s Vent (7) and Cygnet Pond (8). ....	7
Figure 4: Locality map showing Shark Bay and MacLeod Basin on the West Australian coast in relation to major barrier ridges and drainage systems. During Quaternary marine-phases, MacLeod Basin was a marine gulf connected southward to Shark Bay. After Logan (1987). ....	8
Figure 5: Mean monthly rainfall (columns) and maximum temperature (line) for the period 1981-2010 at Carnarvon Airport weather station. Data retrieved from the Bureau of Meteorology website. ....	11
Figure 6: Boxplots showing significant differences between ponds and ammonium (left), nitrite/nitrate (middle) and phosphate (right) concentrations. S: Pete’s Vent, D: Donut Pond, A: Annie’s Pond, P: Pete’s Pond, W: Whistler’s Pond, H: Harjie’s Pond, J: Jana’s Vent; C: Cygnet Pond. ....	21
Figure 7: Composition of sediments sampled throughout eight ponds. ....	22
Figure 8: Multi-dimensional scaling plot of Bray-Curtis similarities among samples based on T-RFLP analysis labelled by ponds, vent system and season. ....	32
Figure 9: Relative abundance and percentage of sequence similarity to publically available sequences of OTUs in ponds and seawater. OTUs shown are those that were amongst the 10 most abundant OTUs in at least one pond of seawater (beach sample). OTUs in bold have less than 97% sequence similarity to publically available sequences. ....	34
Figure 10: Location of ‘vent sites’ selected for the collection of invertebrate and water quality data. Three sites were selected from the Chirrida Ponds System, these were: Whistler Pond, Rattlesnake Pond and Neil’s Pond. Three sites were also selected from the Cygnet Pond system, these were: Second Vent, Jana’s Vent and Goat Bay Vent. ....	40
Figure 11: nMDS plot showing the variation between system groups (A) and pond groups (B) using Bray-Curtis similarities. ....	42
Figure 12: Scatterplots showing mean $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ mean with standard deviations for fish and source materials collected from a) Pete’s Pond, b) Neil’s Pond, c) Dave’s Pond and d) Jack’s Vent. ....	53
Figure 13: Location of Godwit Beach in relation to Cygnet Pond. ....	59
Figure 14: Composition of prey items which different species were observed feeding on in Godwit Beach. ....	62
Figure 15: Plot showing the characteristics of hydrological categories identified at Lake MacLeod based on distance from pond edge and elevation. ....	70

Figure 16: Mangrove and samphire vegetation characteristics for each hydrological categories. ....	71
Figure 17: Water-use efficiency values for plants from each hydrological category at Lake MacLeod compared to plants from coastal and riverine sites. Different letters represent significantly different means. ....	74
Figure 18: Archival history of carbonate content (%), fine sediments (%), organic matter (%) and $\delta^{14}\text{C}$ for CP6 core. ....	81
Figure 19: Archival history of selected elements in the CP6 core. ....	81
Figure 20: Habitat classification map of the Northern Ponds of Lake MacLeod. ....	88
Figure 21: Bathymetric map of Pete's Vent with depth contours in meters (left) and water temperature ( $^{\circ}\text{C}$ ; right). All measurements were taken in July 2013. ....	108
Figure 22: Bathymetric map of Donut Pond with depth contours in meters (left) and water temperature ( $^{\circ}\text{C}$ ; right). All measurements were taken in July 2013. ....	109
Figure 23: Bathymetric map of Annie's Pond with depth contours in meters (left) and water temperature ( $^{\circ}\text{C}$ ; right). All measurements were taken in July 2013. ....	110
Figure 24: Bathymetric map of Pete's Pond with depth contours in meters (left) and water temperature ( $^{\circ}\text{C}$ ; right). All measurements were taken in July 2013. ....	111
Figure 25: Bathymetric map of Whistler's Pond with depth contours in meters (left) and water temperature ( $^{\circ}\text{C}$ ; right). All measurements were taken in July 2013. ....	112
Figure 26: Bathymetric map of Harjie's Pond with depth contours in meters (left) and water temperature ( $^{\circ}\text{C}$ ; right). All measurements were taken in July 2013. ....	113
Figure 27: Bathymetric map of Jana's Vent with depth contours in meters (left) and water temperature ( $^{\circ}\text{C}$ ; right). All measurements were taken in July 2013. ....	114
Figure 28: Bathymetric map of Cygnet Pond. Goat Island can be seen on near the western shore. Contours are in meters. All measurements were taken in July 2013. ....	115

## LIST OF TABLES

Table 1: A synthesis of research gaps and risks and significant biological assets, organised according to the biotic and abiotic characteristics of the Northern Ponds. Four overall dimensions of research (where red indicates high priority for research, orange a medium priority for research and green a low priority for research). Ideally any proposal for research should address high priority issues. After Horwitz et al. (2010). ....	3
Table 2: Summary of research projects undertaken during the ECU-DSL collaboration at Lake MacLeod. ....	4
Table 3: Summary of design used to collect samples in the ponds. Each pond had four transects established in random directions, and a variable number of samples collected at set intervals along each transect. The length and number of samples collected per transect increased as the size of the ponds increased. ....	17
Table 4: Summary of morphometric and hydrodynamic characteristics of each pond. All temperature measurements were taken from approximately 10 cm below the water surface. ....	19
Table 5: Conductivity (mS/cm) and percentage composition coefficients of the major ions for each pond determined using LDA. ....	20
Table 6: Diversity indices for the bacterial and ciliate communities in each pond. ....	29
Table 7: Partitioning of variation in bacterial and ciliate communities at two levels of spatial scale using partial db-RDA. Amount of community variation explained by each model is given as R <sup>2</sup> <sub>adj</sub> (%). E: environmental model; S: spatial model constructed from MEM variables; Pond: model representing the individual ponds; E Pond: environmental model corrected for pond identity; Pond E: pond model corrected for environmental variation; Pond (E+S): Pond model corrected for environmental and spatial variation; E (Pond+S): environmental model corrected for pond identity and spatial patterns within ponds; E (Pond+S): environmental model corrected for pond identity and spatial patterns within ponds ....	29
Table 8: Results of testing if patterns of OTU co-occurrence (C-score) are random and if simulated neutral communities differ significantly from observed communities based upon $\beta$ -diversity. All values are effect sizes when comparing the observed values with those determined using simulation processes. P<0.05 denoted by *; P<0.01 denoted by **, P<0.001 denoted by ***; P<0.0001****. ....	30
Table 9: Summary of community diversity indices for each pond for entire invertebrate community. ....	41
Table 10: Partition of variation in the invertebrate communities at three levels of spatial scale using partial RDA on hellinger transformed abundance data. E: environmental model; Sys: model representing the two study systems (Chirida & Cygnet); Pond: model representing the 6 study ponds. R <sup>2</sup> <sub>adj</sub> (%): community variation explained by model, expressed in %; DF <sub>model</sub> : degrees of freedom of model; DF <sub>res</sub> : residual degrees of freedom ....	43
Table 11: Summary table of the sampling technique employed for the two stages of this study. ....	48
Table 12: Summary of the abundances (MaxN), mean body total length (mm) and range of total length (mm) for each species observed in each habitat in July 2012. ....	50



Table 13: Summary of the percentage contribution of stomach contents (>5% volumetric contribution of dietary item) for tilapia and grunters. ....	51
Table 14: Summary of daily energy intake rates for different species at Godwit beach post-migration and pre-migration. * indicate non-resident species that migrate to the northern hemisphere. All values are mean $\pm$ SE. ....	64
Table 15: Summary statistics for the different components of the sediment cores. ....	80
Table 16: Summary statistics of element concentrations in the two cores. All units are mg/kg. Coefficient of variation (CV) expressed as %. ....	80
Table 17: Final habitat classification categories for the Northern Ponds of Lake MacLeod. ....	89
Table 18: An updated synthesis of research gaps and risks and significant biological assets, organised according to the biotic and abiotic characteristics of the Northern Ponds. Four overall dimensions of research (where red indicates high priority for research, orange a medium priority for research and green a low priority for research). Ideally any proposal for research should address high priority issues. ....	93

## SCIENTIFIC RESEARCH CONDUCTED AT LAKE MACLEOD

Despite the significant biological assets found at Lake MacLeod, information regarding the lake, and specifically the ecology of the Northern Ponds, is vague. Apart from the various publications and reports of Dr Brian Logan on the geology and hydrology of the basin, there is minimal data available on the functioning of the basin as a whole. Birds Australia have conducted extensive bird surveys near the Northern Ponds, mostly from 1999 to 2006 as part of a monitoring program facilitated by Rio Tinto Dampier Salt Ltd. The report of Horwitz et al. (2010) highlights significant gaps in research and sets a framework for future research programs to align with current knowledge.

Phillips et al (2005) recognised biological assets at Lake MacLeod to include: 1) aquatic invertebrates and phytoplankton, 2) waterbirds, waders and shorebirds, 3) mangroves, 4) fish and 5) the unique salt marsh habitats. Horwitz et al. (2010) suggest another two other assets; the seagrass and algae that comprise aquatic habitats and the cyanobacterial mats which are common on the mudflats. Hydrology, geomorphology and climate are the drivers of the ecological character of the Northern Ponds.

The analysis done by Horwitz et al. (2010) highlights where knowledge gaps exist in our understanding of the ecology of Lake MacLeod. In particular, the gap analysis focused on the significant biological assets which make the system unique, which are fundamental in its functioning and contribute to its conservation value. The gap analysis sought to provide a framework to determine where future research and management initiatives should be directed. The analysis found that the hydrology, geomorphology, sediments/stratigraphy and climate are relatively well researched for the vents and ponds.

In general, the vents in Cygnet Pond are the best visited and studied sites (i.e. Jack's Vent, Cygnet Pond). Horwitz et al. (2010) suggest that there is probably sufficient information at these sites to provide a historical context of the fish, invertebrate, water chemistry, mangroves, and to a certain extent, shorebird communities. For instance they provide several reasons why continued research on the Northern Ponds is necessary: the recent flooding event in 2000 was observed to have introduced novel fish species into the communities; goats have continued to have some sort of impact on mangrove, riparian and littoral vegetation; some sites have endured certain levels of regular human visitation; and microbial communities remain poorly known.

A data audit lead Horwitz et al. 2010) to recommend that future studies should be designed to: 1) recognise and characterise other significant biological assets, 2) conduct research to address knowledge gaps and 3) to gain the ability to understand and mitigate environmental risks. Further, they recommended a research framework which covers four dimensions of the Northern Pond ecosystem:

1. Spatial coverage – to understand the differences between vents and ponds, and between ponds in the system
2. Temporal coverage – to understand and monitor environmental change, the influences of events like flooding, processes like human interference, the effects of other identified risks

3. Habitat structure and mapping – to understand the distribution of communities and habitats
4. Ecosystem function – to understand how drivers, such as geology and hydrology, determine ecological processes like sedimentation, decomposition, productivity and respiration.

This report provides a summary of the research conducted by Edith Cowan University (ECU) in a research collaboration with Dampier Salt Ltd. (DSL) that emerged from this data audit (Table 2). Two main research efforts were funded under the collaboration:

1. *Quantification of the Biodiversity of the Lake MacLeod Northern Ponds and*
2. *Dampier Salt sites and ponds and their importance for migratory and other shorebirds.*

These two projects were initiated in 2010 and were finalised in 2016. Reporting for the latter project was also accomplished in a separate report titled *Shorebird foraging ecology in northwestern Australia salt works* (Estrella et al. 2016); it included an investigation into shorebird feeding at all three of DSL's salt works in Northwestern Australia.

As part of the quantification of the biodiversity at Lake MacLeod, a number of research projects were funded, including: (1) *Temporal and spatial variation in aquatic invertebrate communities* (McLure 2011); (2) *Influence of hydrological and environmental conditions on mangrove vegetation* (Dunham 2014); (3) *The potential effects of the invasive tilapia on the native fish assemblages* (Cameron-Caluori 2014); (4) *Small-scale biogeographic patterns of benthic bacterial and ciliate communities* (Kavazos 2016). As well as these projects, a number of additional projects were conducted on the microbial communities, spatial distribution of mudflat habitats and sediment archive history.

The collaboration between Edith Cowan University and Dampier Salt Ltd. represents the first major scientific program into the functioning of the Lake MacLeod basin since the work of Logan and Shepherd was conducted in the 1980's, and Streamtec undertook a series of invertebrate investigations in the late 1990s. Although research into Lake MacLeod's biodiversity has been commissioned by Dampier Salt Ltd. in the past (see Bertzeletos et al. (2012), Ellison (2003), Phillips et al. (2005), and Horwitz et al. (2010) for reviews of ecological studies at Lake MacLeod), this collaboration marks the first time a major ecological study has been conducted at the Northern Ponds, and addresses the knowledge gaps identified by Horwitz et al. (2010). This report will present an overview of the geomorphology, hydrology and ecological character of the Northern Ponds, as well as summaries of the ECU research projects, before an update on the research gaps and areas for future work are provided.

**Table 1: A synthesis of research gaps and risks and significant biological assets, organised according to the biotic and abiotic characteristics of the Northern Ponds. Four overall dimensions of research (where red indicates high priority for research, orange a medium priority for research and green a low priority for research). Ideally any proposal for research should address high priority issues. After Horwitz et al. (2010).**

Fields			Northern Ponds			
			Spatial Coverage	Temporal Change	Habitat Description	Ecosystem Function
Biotic	Invertebrate Fauna	Aquatic Invertebrates				
		Terrestrial Invertebrates				
	Vertebrate Fauna	Mammals				
		Terrestrial Birds				
		Waterbirds				
		Reptiles				
		Amphibians				
		Fish				
		Littoral Vegetation, Mangroves & Aquatic Habitats	Littoral vegetation			
	Mangroves					
	Riparian					
	Seagrass/macro algae					
	Diatoms/phytoplankton					
	Algae (mats & benthic)					
	Cyanobacterial mats					
Microbial	Fungi					
	Stromatolites					
Social & Cultural		Cultural History				
		Significant sites				
Abiotic	Water Quality	Physiochemistry				
		Nutrients				
		Ions				
	Hydrology	Vent systems				
		Surface water				
		Aquifers				
		Sinks				
		Floods				
		Geology	Geology			
	Geomorphology					
	Sediments					
	Climate	Temperature				
		Rainfall				
		Cyclones				
		Evaporation				

**Table 2: Summary of research projects undertaken during the ECU-DSL collaboration at Lake MacLeod.**

Project	Level	Personnel	Data period	Study Sites	Data Collected
Aquatic Invertebrates	Under-graduate Honours	– <u>Neisha McLure</u> Pierre Horwitz Kathryn McMahon	July 2011	Whistler's Pond Rattlesnake Pond Neil's Pond Second Vent Jana's Vent Goat Bay	Invertebrate inventory of the littoral communities found in each of the sampled ponds
Mangrove and littoral vegetation	Post-graduate Masters	– <u>Natasha Dunham</u> Ray Froend Kathryn McMahon Pierre Horwitz	Feb & July 2012	Goat Bay Whistler's Pond Neil's Pond Pete's Pond	Mangrove distribution and the role of environmental factors influencing their morphology and physiology
Fish assemblages	Post-graduate Masters	– <u>Hannah Cameron</u> <u>Calouri</u> Glenn Hyndes Pierre Horwitz	2012-2013	Pete's Pond Annie's Pond Whistler's Pond Harjie's Pond Jack's Vent Dave's Pond Neil's Pond Jana's Vent	Distribution and size structure of each fish population. Feeding ecology and trophic implications of the invasive tilapia.
Microbial ecology	Postgraduate – PhD	<u>Christopher Kavazos</u> Megan Huggett Ute Mueller Pierre Horwitz	2012-2014	Pete's Pond Annie's Pond Whistler's Pond Harjie's Pond Donut Pond Jana's Vent Cygnet Pond	Spatial patterns and assembly processes of pond benthic bacteria and ciliate communities
Shorebird feeding ecology	Post-doctorate	<u>Sora Marin-Estrella</u> Robert Davis Pierre Horwitz	2013-2014	Godwit Beach Whistler's Pond	Feeding ecology and habitat use by shorebirds
Habitat mapping	Research	<u>Dave Blake</u> Holly Winkle Pierre Horwitz	2014-2015	Northern Ponds	Classification and distribution of habitats - Northern Ponds
Bacterio-plankton ecology	Research	<u>Megan Huggett</u> Christopher Kavazos Pierre Horwitz	2014-2015	Pete's Pond Annie's Pond Whistler's Pond Harjie's Pond Donut Pond Jana's Vent Cygnet Pond	Composition, seasonal variation and origin of bacterioplankton communities within ponds
Seagrass sediment archives	Research	<u>Oscar Serrano-Gras</u> Pierre Horwitz Christopher Kavazos	2014	Cygnet Pond	Analysis of the sediment archives collected in seagrass meadows

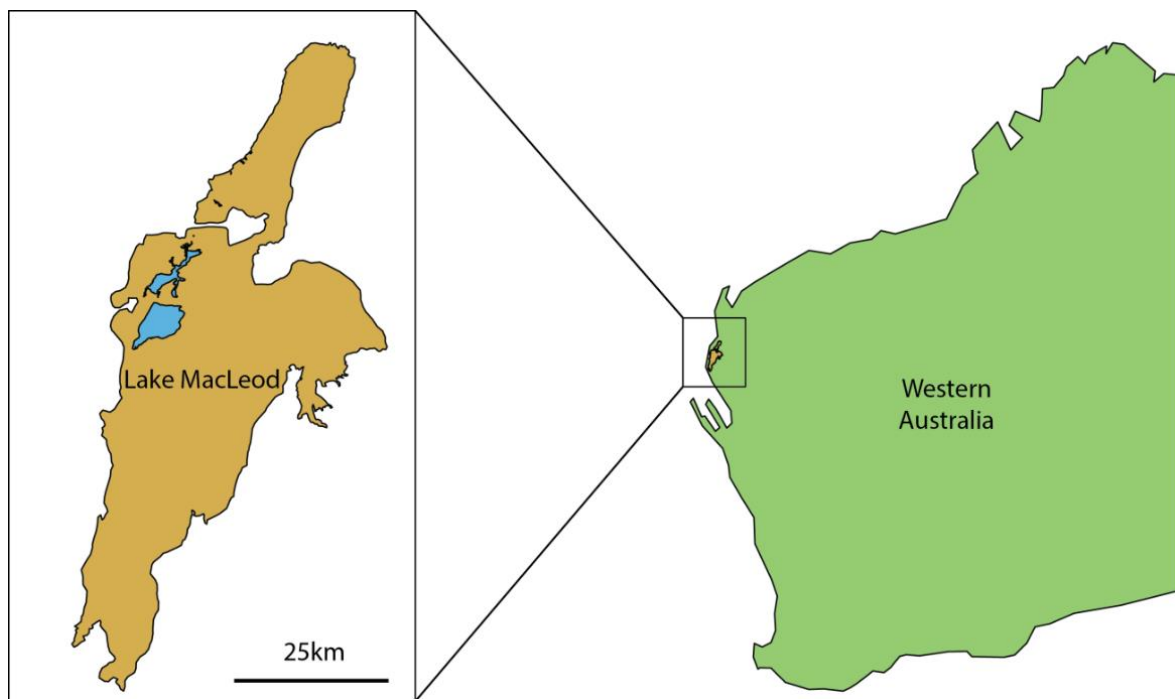
## LAKE MACLEOD: AN INTRODUCTION

This section serves as the state of knowledge prior to the commencement of the current research programme, starting in 2010. It draws extensively from the work of Logan (1987) and Shepherd (1990). Unless otherwise stated, the source documents for this section are:

Logan, B. 1987. *The MacLeod evaporite basin, Western Australia*. The American Association of Petroleum Geologists, Tulsa, Oklahoma

Shepherd, M. J. 1990 *Hydrologic environments and sedimentation, Cygnet Seepage Face, Lake MacLeod, Western Australia*, University of Western Australia

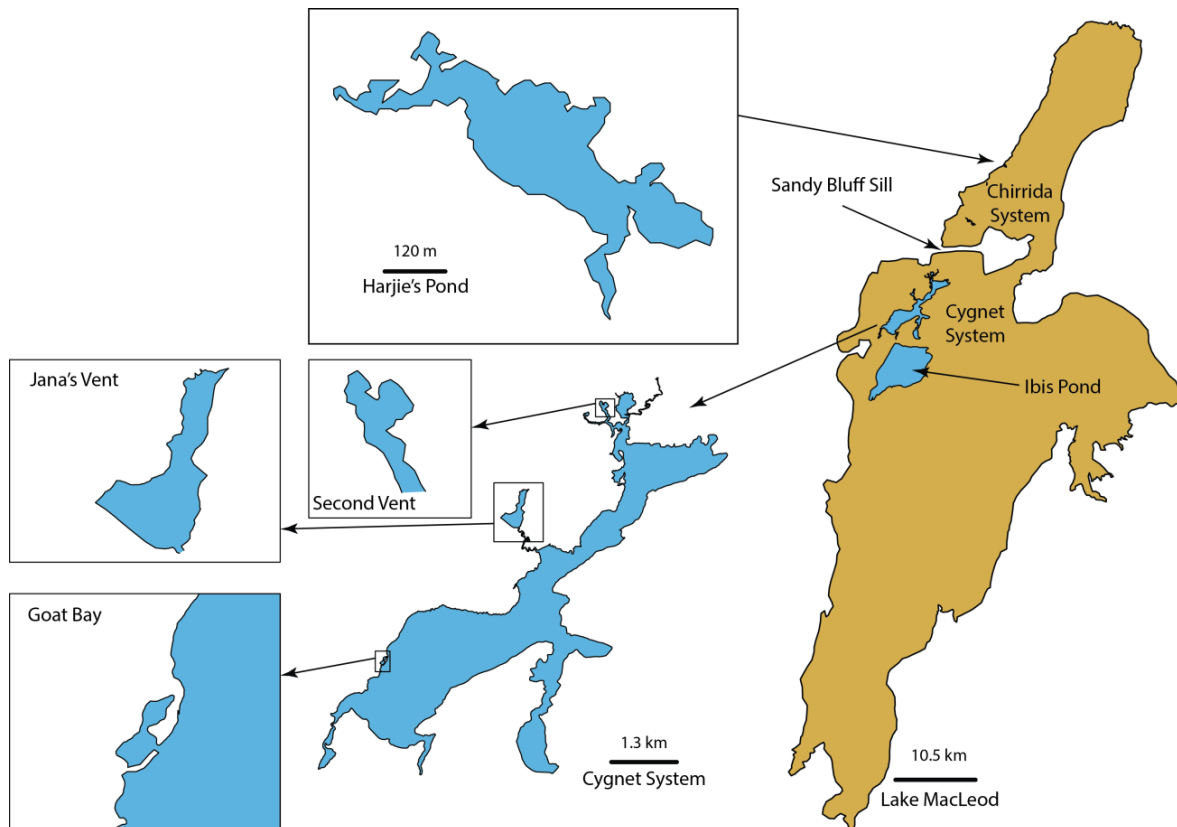
Lake MacLeod is a large inland saline lake located 20 km inland of the Carnarvon-Ningaloo coastline in Western Australia (Figure 1). The MacLeod lakebed is predominantly dry and covers an area of 2000 km<sup>2</sup>, 60 km<sup>2</sup> of which is covered by numerous permanent bodies of brine. The brine bodies persist due to a marine-water seepage-face that sustains brine inflow along the lake's north-western edge despite the huge evaporative outflow. Due to the continual seepage of marine water into the lake, Lake MacLeod contains areas of permanent saline wetlands and unique mangrove swamps (Ellison and Simmonds 2003), and it provides a major feeding habitat for migratory birds (Phillips et al. 2005, Bertzeletos et al. 2012).



**Figure 1: Locality map of the Lake MacLeod and brine bodies (blue) in Western Australia.**

Brine seepage occurs along seepage faces, of which there are five at Lake MacLeod, with four being active (Logan 1987). The Cygnet seepage face is the only one to discharge brine (originating from the Indian Ocean) freely to the surface to form the extensive ponds and brine sheets that are characteristic of the area (Logan 1987). The

active part of the Cygnet seepage face covers 113 km<sup>2</sup>, runs approximately 30 km along the north-western barrier base and extends up to 5 km eastward towards the basin interior (Shepherd 1990). The seepage face can be divided into two distinct systems, the northern Cygnet seepage face, or the Chirrida System, and the southern Cygnet seepage face, or the Cygnet System (Figure 2). The two systems are separated by an extension of the impermeable coastal barrier that extends eastward into the basin, known as Sandy Bluff Sill. Seepage is maintained by a hydrostatic head caused by the seepage points being up to 3.5m below sea level, and very high evaporation rates.

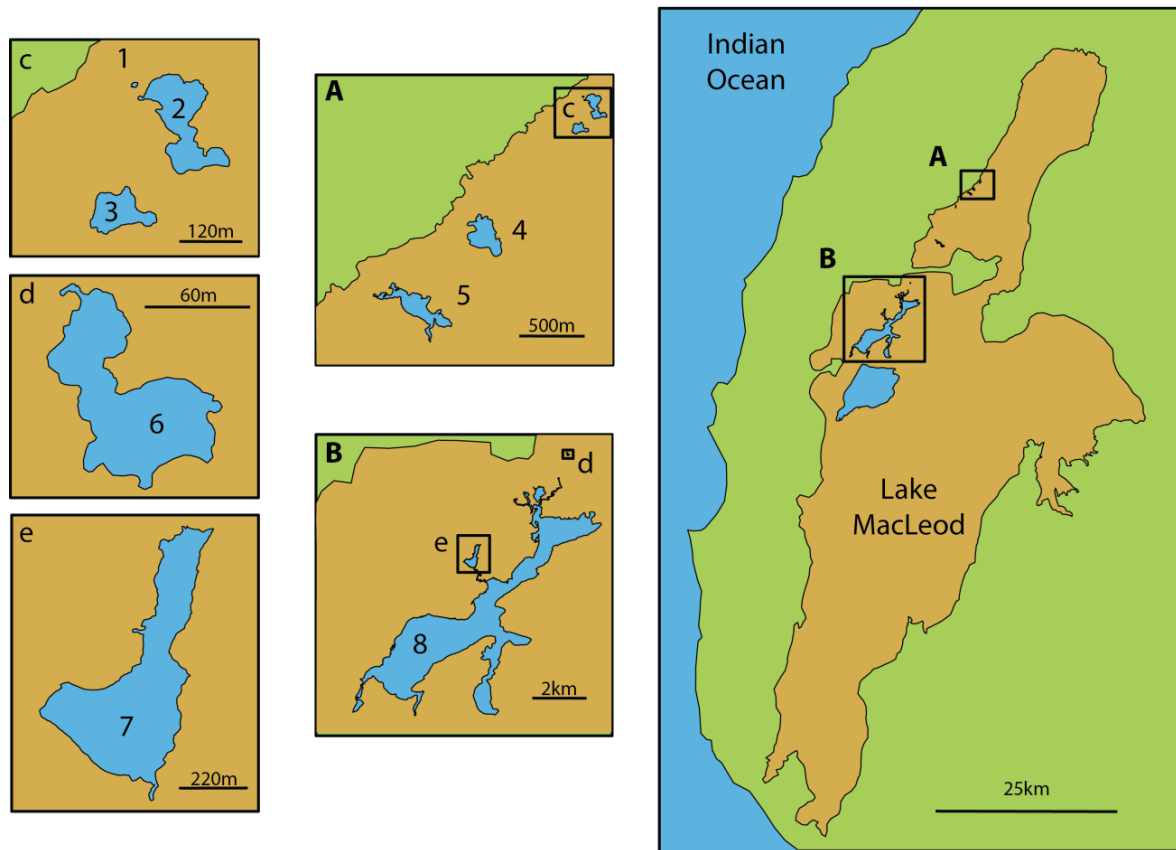


**Figure 2: Locality map of some of the ponds and vents, as well as some landmarks of Lake MacLeod.**

In the Cygnet System, seawater is discharged at numerous points along the western edge, flowing directly into Cygnet Pond and continuing southwards into Ibis Pond (which are called 'ponds' but are really very large permanent salt lakes). Some of the larger vents, like Second Vent and Jana's Vent, consist of 1 to 2m deep ponds out of which water flows through channels into Cygnet Pond. Other vents, like those at Goat Bay, open directly into the pond. Cygnet Pond is approximately 12 km<sup>2</sup> in area and has an average depth of 0.4m (Logan 1987). Brine flows south-east and over the south-eastern pond margin into Ibis Pond. Ibis Pond is the largest body of water in the basin, with possible depths of 1.5m and a surface area between 32 and 260km<sup>2</sup>, depending on evapotranspiration.

The Chirrida System contains eight main discrete ponds, with each pond having a large vent and numerous smaller millimetre sized vents (Shepherd 1990). The largest of these ponds, Harjie's Pond, is over 300m long, whilst the smaller ponds are less than 10m in diameter (see Figure 3 for location and size of some of these

ponds). The ponds are mostly confined by mangrove roots and pneumatophores and accumulated sediment, preventing water from spilling over the banks. The ponds are shallow, rarely deeper than 1 m, and usually spill out over the eastern edges to form spill sheets. These spill sheets are extremely shallow and cover extensive cyanobacterial mats, which tend to flow south-east, although they can be moved throughout the basin by the wind.



**Figure 3: Map of the MacLeod basin showing the location of some of the ponds. Box A shows the location of the northernmost five ponds which include Pete's Vent (1), Pete's Pond (2) and Annie's Pond (3; box c) as well as Whistler's Pond (4) and Harjie's Pond (5). Box B shows the locations of Donut Pond (6), Jana's Vent (7) and Cygnet Pond (8).**

This section will discuss the geological evolution of Lake MacLeod since the early Holocene and how its development has resulted in the evaporite system that it is today. As the greatest biological assets are found at the seepage faces, the different environments found there, and what defines them, will be introduced. Much of the information on the Lake MacLeod geology and hydrology has been produced by Logan (1987) as part of the Lake MacLeod Research Program, supported by the University of Western Australia's Geology Department, Dampier Salt Ltd., the Australian Research Council, Texada Mines Pty. Ltd. and Logiden Pty. Ltd. The mechanisms which sustain the input of seawater into Lake MacLeod, as well as detailed information on the seepage face environment, has been given by Shepherd (1990).



## Geological formation and climatology

### Geological evolution

Lake MacLeod (MacLeod Basin) lies in the MacLeod Graben behind the Quobba Barrier, which prevents the free inflow of seawater from the Indian Ocean (Figure 4). The basin currently lies at 3 to 4m below sea level and, because some parts of the barrier contain permeable rocks, seawater seeps into the basin at a sufficiently high volume to sustain a 2000km<sup>2</sup> marine evaporite system. However, the contemporary environment has evolved from a marine system to the evaporite system it is today through a series of changes which began in the early Holocene. The MacLeod basin was opened along its southern perimeter during the Pleistocene marine phases and early into the Holocene, and was analogous to the modern Freycinet Basin found today in Shark Bay. It evolved into the modern evaporite system via gradual process of sill closure from encroachment of surrounding dunes.

The account of Logan (1987) describes the evolution of the MacLeod basin during the Pleistocene and Holocene. A brief summary of Logan's description of the development of the modern system during since the last glaciation event will be given here. The Holocene transgression and closure of the Texada sill were important factors which influenced the evolution of the modern day MacLeod Basin.

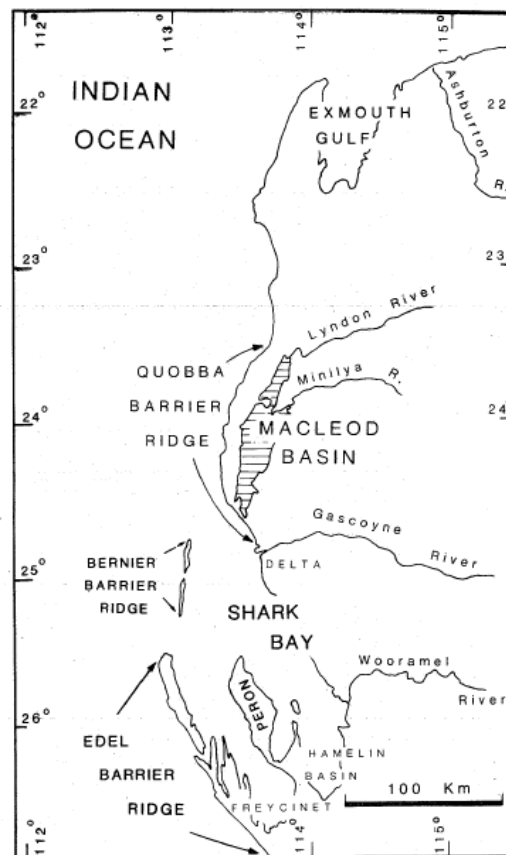


Figure 4: Locality map showing Shark Bay and MacLeod Basin on the West Australian coast in relation to major barrier ridges and drainage systems. During Quaternary marine-phases, MacLeod Basin was a marine gulf connected southward to Shark Bay. After Logan (1987)

Prior to the Holocene transgression (7900 – 6500 years BP), the basin floor lay between 10 and 12m below sea level, and the system was open to the Indian Ocean via the 5m deep Texada sill along its southern perimeter. From about 5800 YBP, seawater inflow over the Texada sill became restricted to a few narrow channels, because of sedimentation and dune encroachment, before becoming totally cut-off by 5100 years BP. During this period, the seepage intake areas on the seaward face of the barrier were at maximum submergence, and there was seawater discharge along all the seepage faces (Bejaling, Quobba, Cygnet Marsh, Gnarlaloo and Warroora seepage faces). Shortly after the closure of the Texada sill, basin-wide cementation of the upper parts of the sediment column from the crystallisation of gypsum, lead to the development of an impermeable barrier, or hydroseal, which isolated the basin from surrounding aquifers. The formation of the hydroseal allowed for the retention of high order, dense brines and salts. It is at this point that the MacLeod basin switched from a meta-haline marine system to the evaporite environment it is today.

However, dropping sea levels and a rising sediment table continued to evolve the system over the next 5000 years. Particularly during the last 4000 years when sea levels dropped by approximately 2m, resulting in the reduction of seepage and extinction of many seepage faces. In particular, seepage from the Warroora face stopped, and Cygnet Marsh had seepage reduced by half. Sedimentation processes that began filling the basin with evaporative products from about 5800 YBP, caused the basin floor to rise from 10m below sea level to the present day levels of about 5m below sea level. The rise of the sediment surface had consequences on seepage as brine surfaces were forced upwards, hydrostatic head and inflow decreased and seepage faces narrowed and brine flow dropped as gradients dropped as discharge points were plugged by precipitated sediment.

### **Formation of the modern system**

The present basin system is seepage based. Inflow occurs along seepage faces, which represents an interface across which there is potential for seawater discharge and creation of water bodies on the basinal slope. These seepage faces are tens to hundreds of kilometres long, and seawater inflow is driven by hydrostatic pressure through the permeable Quobba barrier, which is maintained by the huge evaporative loss of seawater that occurs in the basin.

The modern system has a sedimentation surface found at roughly 4.3m below sea level, and therefore inflow is driven by hydrostatic pressure through the barrier. Because the inflow volume is small compared to the evaporative outflow, the brine surface is mostly suppressed below the sediment surface. These vast expanses of permanently damp sediment, called majanna, characterise the modern MacLeod system. Where there is high, localised brine inflow, particularly at the Cygnet seepage face, permanent (and temporary) ponds have been established. Brine outflows from the basin through brine sinks located along the hinterland (eastern) margin, and it is thought that only 2% of the precipitated products are retained within the system.

There is potential for seawater inflow at all points in the basin which are below sea level, but delivery is focused around seepage faces located at the barrier base. These faces are found at about 3.5m below sea level, and are

above the hydroseal. There are five segments to the seepage zone, although presently, only the Cygnet seepage face is active where inflow locally exceeds outflow and brine is freely discharged into the basin.

### **Cygnet marsh seepage face**

Because the Cygnet marsh is the region of Lake MacLeod that has the greatest biodiversity value, and is the location of the studies being presented and discussed in this report, this discharge domain will be introduced. The other seepage faces, although they are active, transmit relatively small amounts of seawater into the basin, which is all suppressed into majanna. Cygnet marsh is the locus for the most seawater inflow into the basin, and is the only location where permanent brine sheets form. The active face lies between 0 and 3.5m below sea level, although earlier in the Holocene, the face was active up to 2m above today's sea level. The intake zone is a 30km long sector of the Trealla Limestone which reaches 5m above sea level on the barrier sea face and dips to 4m below sea level at Cygnet marsh.

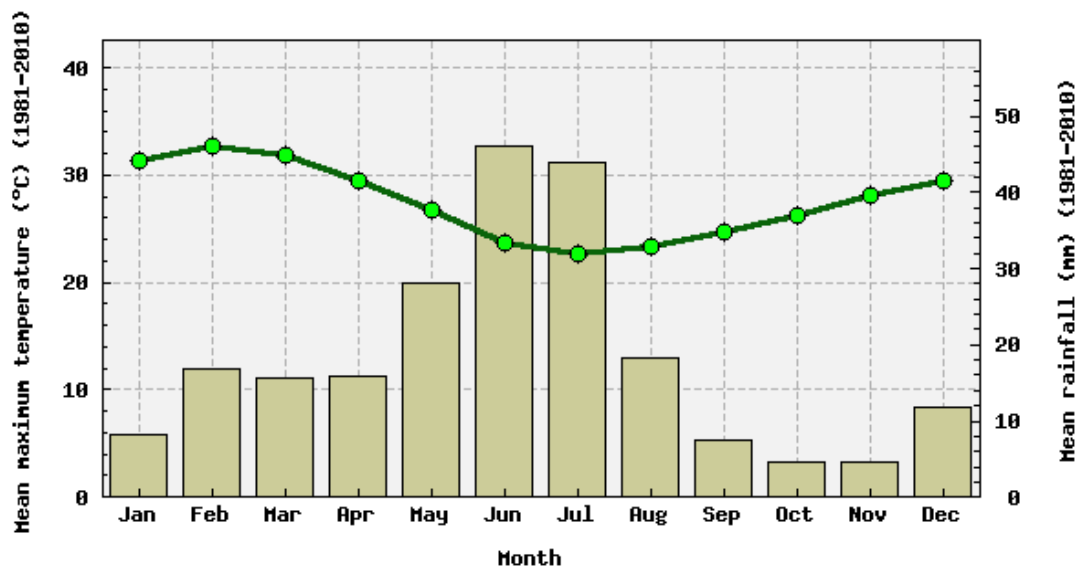
Although there is continuous free discharge of brine along the Cygnet seepage face, brine tables are mostly suppressed between 10cm and 1m below the sediment surface. The sediments remain damp as there is continuous flow via capillary action which replaces evaporative losses. There is also free discharge from point sources (vents) into depressions called ponds and channels. Vents can vary from being large, cavernous openings that are meters in diameter, to small holes that are only centimetres in diameter. In some vents, seawater flows freely through the vents, but many have become choked with detritus and sediment, and have slow seepage rates. These choked vents are distinguished as sub-circular to circular mounds with up to 1m relief. Seawater continues to be released by these mounds and flows away in radial patterns into inter-mound depressions. Larger, more active vents are usually surrounded by 1 to 2m deep ponds, which seawater often flows out of into adjacent ponds or into spill sheets. The decreased evaporation rates in winter are reflected by the rise in the brine table and growth of the brine sheets throughout the seepage face.

### **Climate**

The MacLeod basin lies between 23° and 26° south and because of the high solar radiation and the winds found there, the region experiences up to 2.6m of evaporation per year which is 10 times greater than annual precipitation. There is minimal surface water in such arid environments, and as a result, both marine and terrestrial waters tend to become concentrated and precipitate salts. Wind is the major energy source, in that it promotes brines to flow throughout the basin, causes circulation within the larger water bodies, generates wave action, causes deflation of sediment and is a major contributor to evaporation. Evaporation rates are greatest in summer (10.0mm/day) and lowest during winter (4.2mm/day), which have been attributed to the southerly gale season experienced in summer and the low barometric pressures and northerly winds in winter.

The basin experiences prevailing southerly winds because of its location in the southeast tradewind belt. The southerlies are distorted by strong sea-breezes during summer and by winds associated with depressions in the southern Indian Ocean during winter. The typical pattern is for south to southwest winds in the mornings with

stronger south to southeast winds in the afternoons. Average wind velocities in summer are 26.6km/hour, with mean maximum velocities of 113km/hour. In winter, mean wind velocities are 16.5km/hour, with mean maximum velocities of 102km/hour. Similar seasonal variations in rainfall are also seen (Figure 5), although the amounts and distribution of rainfall can be very erratic. According to Logan (1987), up to 50% of the annual rainfall can be attributed to less than 5% of the rainfall events. Similarly, wet years can have annual rainfalls up to three times the annual average, and dry years can have annual rainfalls that are only a fraction of the average. Nonetheless, mean monthly rainfall is highest in winter (32.7mm/month) and lowest in summer months (12.5mm/month).



**Figure 5: Mean monthly rainfall (columns) and maximum temperature (line) for the period 1981-2010 at Carnarvon Airport weather station. Data retrieved from the Bureau of Meteorology website.**

Cyclones are characteristic of the region, although they rarely cross the coast south of Exmouth. However, even though the cyclone system may not directly cross over Lake MacLeod, they often bring heavy rains to river catchments and subsequently cause runoff into the basin. Cyclones generally form between November and April, and moving southwest to south-southeast at 10 – 30km/hour. This results in strong north to northeast winds with approach, followed by northwest to southwest winds as the cyclone moves down the coast. Wind velocities range between 60 km/hour to more than 180 km/hour. They usually bring heavy rains, but some are dry or only bring small amounts of rain. During the period 1990 to 2016, there have been at least 6 tropical cyclones to impact the Carnarvon/Gascoyne region. They include Cyclone Frank (1995), Cyclone Steve (2000), Cyclone Alistair (2001), Cyclone Glenda (2006), Cyclone Nicholas (2008) and Cyclone Olwyn (2015). In addition, the area can also be significant influenced by rainfall from tropical low events such as the system which caused significant flooding during 2010. During floods, freshwater can enter the basin from the north east via the Lyndon River and Cardabia Creek, from the east from the Minilya River and in the south-east from distributaries of the Gascoyne River. Flooding from the Gascoyne River is infrequent, probably a decadal event, but it can cause the lake to be extensively inundated. Such events have occurred in 1960, 1961, 1980, 1995, 2000 and 2010 (see

Phillips et al. 2005). The Minilya River floods less than once every second year (Halse et al. 2000, Phillips et al. 2005).

## **Contemporary Seepage Environment**

The Cygnet Marsh is the primary point of seawater inflow into the MacLeod Basin, and the hydrology, hydrochemistry and sedimentology of this environment has been described by Shepherd (1990). As stated above, seawater enters the basin along the Cygnet seepage face, with the discharge environment being primarily determined by the hydrologic balance of inflow rate and evaporation rate. Shepherd (1990) defined two main environmental units that occur because either evaporative loss is greater than seawater inflow (*majana* environment), or seawater inflow is greater than evaporative loss (*brine sheet* environment). The *majana* environment can then be divided into two sub-environments: gypsite *majana* and carbonate *majana*. Similarly, the *brine sheet* environment can be divided into four sub-environments: the marginal sheets, ponds, deep sheets and spill sheets. According to Shepherd (1990), each of the environmental units has unique biological communities and habitat characteristics which define the overall ecology of Lake MacLeod.

### **Brine sheet environment**

The *brine sheet* environment is defined as any area along the seepage face which has a prevailing net positive water balance (Shepherd 1990). *Brine sheets* represent a variety of habitats, some of which are permanent, and others which are ephemeral. Similarly *brine sheets* can cover an area of tens of square kilometres and have depths of over one meter, or can have surface areas less than a square meter and depths of only a single centimetre. The features of a *brine sheet* are determined by the inflow volume and local topography. Shepherd (1990) divided the *brine sheet* environment into two domains, the discharge domain and the spillover domain. In the discharge domain, the water bodies remain connected to the seepage point, whilst in the spillover domain, the water body is derived from spillover of brine from the discharge domain.

The discharge domain contains the marginal sheet and pond sub-environments. The marginal sheet sub-environment are flat areas located at the highest points of the seepage face and are usually covered by a 1 - 10cm deep *brine sheet*. They are formed by the discharge from small vents and *brine seepage* from the sediment, and because they lie on flat surface, they are relatively immobile. They are usually found bordering the barrier side of the ponds. Ponds, on the other hand, lie in deep depressions and are the result of long-term net positive water balance. High amounts of *seepage* usually occur through the vents, and is balanced by evaporative loss and spillover loss. The ponds are often greater than one meter deep, but usually deeper than 50cm. The ponds are usually located down slope of the marginal sheets, but above the spillover and *majana* environments.

In the spillover domain, there are the deep sheet and spill sheet sub-environments. The deep sheets are expansive, partially confined depressions that are between 20cm and 50cm deep. There is no free discharge directly into these ponds, and inflow is from spillover from the topographically higher ponds and marginal

sheets. The spill sheet sub-environment is also located below the discharge domain, although they are often found between the pond sub-environments. However, the brines are derived from higher parts of the seepage face, and because these sheets are shallow (rarely exceeding 10cm in depth) and not topographically enclosed, they are mobile. Spill sheets tend to move down slope towards the basin center, but they are also influenced by prevailing winds.

### **Majana environment**

Majana environments are areas which have a negative water balance, and thus brine is suppressed to sub-surface levels. This occurs because evaporative loss greatly exceeds the seepage inflow. Gypsite majana lies in low parts of the seepage face and occupies most of the basin surface, and consists of gypsum-field brines. They are typically not inundated by surface waters, and the brine surface lies between 0 and 1m below the surface. Carbonate majana instead is found higher up in the seepage face, and is usually surrounded by brine sheet environments. Carbonate majana contains carbonate-field brines because they have an interaction with seepage which maintains them below the gypsum threshold. As a result, brines are usually suppressed to levels 0 and 0.5m below the surface.

### **Biological Significance of Area**

The region of wetlands associated with the Cygnet seepage face is known as the Northern Ponds. The biological significance of the Northern Ponds is that they contain a series of permanently inundated ponds in an otherwise arid environment, where inland water is scarce, and usually ephemeral, thus presenting unique environmental conditions for the formation of diverse plants and animal communities (Ellison 2003). These wetlands support the largest inland populations of *Avicennia marina* (Grey Mangrove) in the world, significant populations of shorebirds and unique invertebrate assemblages (Ellison 1997). The ponds are usually fringed with Grey Mangroves, although they are found along the margins of channels and scattered throughout the majana environment. Samphire shrubs (probably belonging mostly to the *Halosarcia* genus) are also found along the margins of the ponds, and scattered throughout the mudflats. The mangroves provide habitat for at least four species of mangrove birds, *Butorides striatus* (Green Heron), *Gergone tenebrosa* (Dusky Flyeater), *Zosterops lutea* (Yellow White-eye) and *Artamus leucorhynchus* (White-breasted Woodswallow Johnstone 1990).

Lake MacLeod is also an important habitat for shorebirds and waterbirds, some of which are vagrant, and others that are migratory visitors. The wetlands provide an important feeding ground for shorebirds. These birds mostly inhabit the shallow waters and mudflats associated with the ponds. Some estimates of bird numbers have exceeded 50,000 at a given site. Up to 70 species of birds have been identified around the ponds, 28 of which are listed under Australia's bilateral treaties on migratory birds with Japan and China. Six of these species have abundances at Lake MacLeod that regularly exceed 1% of the world population, therefore making the wetlands internationally important.

There are two species of fish which are usually found around the vents of the ponds, where the brine is similar in composition to that of sea water. These species include *Amniataba caudavittus* (Flag-tailed grunter) and *Craterocephalus pauciradiatus* (Estuarine hardyhead). A third species, *Mugil cephalus* (sea-mullet), are also thought to be found around the vents in Cygnet Pond, particularly Jack's Vent (but see Fish Community Structure and Feeding Ecology section of this report). *Leiopotherapon unicolour* (Spangled Perch) and *Lutjanus argentimaculatus* (Mangrove Jack) have been irregularly recorded in the ponds. (After flood events, *Oreochromis mossambicus* (tilapia) has inhabited many of the ponds, and probably entered the lake from the flooded tributaries of the Gascoyne River; see Fish Community Structure and Feeding Ecology section of this report.)

The shorebirds and fish populations are sustained, however, by the invertebrate communities found within the sediments and ponds. These communities are thought to consist of salt tolerant freshwater species and marine species. Lake MacLeod is significant for invertebrates because the area is thought to contain a number of Gondwanan relics (Halse et al. 2000). Examples of invertebrates inhabiting Lake MacLeod include *Marginella* and *Hydrococcus* gastropods, *Capitella* and *Leitoscolopsis* polychaetes, ostracods, copepods, amphipods and foraminifera.

## BIOGENIC OR TERRIGENIC? PERMANENT WATER BODIES OF THE NORTHERN PONDS IN THE LAKE MACLEOD BASIN OF WESTERN AUSTRALIA

Source document:

Kavazos, C. R. J. 2016. *Small-scale biogeographic patterns of benthic bacterial and ciliate communities in the saline ponds of Lake MacLeod, north-western Australia*. PhD thesis. Edith Cowan University

Bodies of water that are salty are usually categorised into either those that are marine, where there is an obvious connection to the ocean (i.e. estuaries) or those that are inland, epicontinental, and lack any such connection to the marine environment. For the latter, athalassohaline lakes are almost always endorheic (internally draining), and can be either permanent or ephemeral, depending upon the nature of the hydrological inputs, the temperature and evaporation rate (De Deckker 1983). In any global region, as a general rule, the lakes become more ephemeral as the mean annual temperature increases, the mean annual precipitation declines, and the net evaporation rate increases (Williams 2002). These water bodies dry annually, or interannually, once evaporative processes overwhelm inundation, and as they dry, salinities can dramatically increase.

De Deckker (1983) based his categorisation of salt lakes on geomorphological grounds: i) large closed basins with (often) extensive internal drainage areas; ii) small closed basins with small internal drainage areas; and iii) crater lakes. A fourth category, coastal dune barrier lakes, is excluded because they don't conform to the notion of athalassohaline lakes. The term salt lake, or evaporite lake (also known as a salina), refers to these landlocked water bodies which have concentrations of salts and other dissolved minerals that are considerably higher than most lakes, and often higher than seawater (De Deckker 1983).

A rarer class of salt water bodies contravene most of these general categorisations of salt lakes, under the following circumstances seen in the Northern Ponds at Lake MacLeod in Western Australia. In arid (hot and dry) subtropical coastal regions, where marine water travels through karst (Tertiary and Pleistocene age barriers; Wyrwoll et al. 2000), and upwells into a basin that is slightly below sea level, inland lakes can exist in a state with permanent seawater, and with relatively constant salinities. These salt lakes overflow into the basin itself, where the water is subject to evapoconcentration but can also be flushed by large flood events carried by rivers that drain into the basin and caused by relatively rare tropical low pressure (cyclonic) events. Such a salt lake system is either: i) marine but inland (athalassohaline), or ii) neither marine nor characteristically inland.

Like other wetland systems, understanding the influence of geomorphology, climate and hydrology, is important when determining ecological processes (Mitsch and Gosselink 2000). The chemical and physical compositions of the ponds, which are influenced by these drivers, define the habitat and determine the nature of the biological communities that inhabit them (De Deckker 1983, Boggs et al. 2006, Timms 2009). In turn, the biological communities can also act to determine the physical structure of the sediments (Schnurrenberger et al. 2003) and possibly even the water chemistry at the sediment-water interface (Woodruff et al. 1999, Gainswin 2004).



The inner wetlands have different physical attributes, hydrodynamics and, although they are all seawater derived saline wetlands, are also likely to vary on a theme by having slightly different hydrochemistries and nutrient levels. Differences in habitat structure are likely to influence the habitats and biological communities found in them (Radke et al. 2002, Radke et al. 2003, Boggs et al. 2006, Boggs et al. 2007, Long et al. 2009). Morphometric and hydrodynamic characteristics of the ponds are expected to cause variation in the water chemistry, primarily because of differences in brine residency time and evaporation. Water chemistry parameters that are likely to differ between ponds include concentrations of major ions and nutrients, both of which have been shown to alter the biological communities of salt lakes (Radke et al. 2003, Casamayor et al. 2013).

This study describes the physical (sediments) and chemical (salinity and nutrients) characteristics of the habitats at the sediment-water interface in eight of the permanent water bodies in the Northern Ponds system of Lake MacLeod. These habitats cover a large area of the Northern Ponds, and as benthic processes are fundamental components of aquatic ecosystem functioning, are likely to contain important biological diversity (Alongi 1994, Cotner and Biddanda 2002). The aim of the work is to relate these physical and chemical characteristics to the morphometric and hydrological parameters of the water bodies and to determine whether biogenic processes more characteristic of marine environments can over-ride terrigenous processes in an inland sedimentary basin.

## **Methods**

Eight ponds were used in this study, including Pete's Vent, Donut Pond, Annie's Pond, Pete's Pond, Whistler's Pond, Harjie's Pond, Jana's Vent and Cygnet Pond (Figure 3). This group of ponds represents ecosystems ranging in size from tens of square metres to square kilometres which are located in two distinct regions of the basin and represent the variability of the pond habitats found there. The Lowrance HDS-5 Fish Finding Sonar and GPS unit was used to log water depth, temperature and location throughout each pond. Measurements were made in July 2013 by attaching the sonar device to a body board and towing it at slow speed behind a boat. Water outflow rates from the ponds were measured using a calibrated portable flowmeter (Marsh-McBirney, Inc. FLO-MATE™ Model 2000) during November 2013.

In each pond four transects were established, with each transect originating from a randomly selected point and orientated in a random direction. The length of each transect ranged from 3m in Pete's Vent to 2000m in Cygnet Pond (Table 3). Samples for analysing water chemistry and sedimentology were collected during November 2012 at predefined distance along each transect, ranging from separation distances of 0.1m to the length of each transect. Samples were collected using 70 mL sterile jars. The jars were first opened underwater, in proximity to where the scoop was to be taken. The scoops were taken to include only the top layer of the sediment, which included as much as possible, the biofilm and the water immediately above it. In some of the deeper ponds, SCUBA was required for sampling, however, most samples were collected by snorkelling when the sediment was shallower than arms reach. Care was taken to not disturb the sediment. The samples were transported on ice to a field laboratory on the day of collection, then kept cold in a fridge for 24 hours to allow particles in the water to settle. After 24 hours, water overlying the samples was decanted and remaining sediment was frozen in the

collection jar. The decanted liquid was stored at -20°C and used for water quality measurements. Water conductivity was measured using a field calibrated Orion multimeter probe (Orion, Thermo Scientific).

**Table 3: Summary of design used to collect samples in the ponds. Each pond had four transects established in random directions, and a variable number of samples collected at set intervals along each transect. The length and number of samples collected per transect increased as the size of the ponds increased.**

Pond	Samples per transect	Length of transect (m)
Pete's Vent	12	3
Donut Pond	12	15
Annie's Pond	12	15
Pete's Pond	12	15
Whistler's Pond	16	30
Harjie's Pond	16	30
Jana's Vent	16	30
Cygnat Pond	22	2000

## Results

### Pond morphometry and hydrology

The bathymetric maps for the eight study ponds and their basic morphometric features are summarised in Table 4 (see appendix for bathymetric and temperature profile maps). Surface area of the ponds varied from 95m<sup>2</sup> to 7km<sup>2</sup>, and water volume from 98,000L to over 4.4GL. Water volume in the ponds was directly proportional to surface area. The vents are almost exclusively found in the north-western region of each of the ponds, and the water bodies generally extend in a south or south-east direction and become shallower. Most of the ponds have a defined boundary around the perimeter, although the shore is generally steeper along the western and northern edges of the ponds. These vent areas are easily identified when in the ponds by looking for depressions in the pond floor.

The vent regions were all found to be more than 1.5m deep, with the deepest one at Jana's Vent (2.6m). The shallower reaches of the ponds, where depth measurements were difficult to make as the sonar device can only measure greater than 0.30m, were generally found in the south to south-eastern margins of the ponds. For example, Annie's Pond, Pete's Pond and Donut Pond do not have definite shorelines along their south-eastern margins. In these ponds, the water bodies typically reach a depth of less than 0.30m and mangrove pneumatophores become dense and impede water flow along their eastern margins.

The ponds either had a large outflow channel, where water flowed out of the pond into adjacent spill sheets, or were confined water bodies with little or no surface outflow. In the former scenario where there were outflow channels, water inflow through the vents must be sufficiently great enough, and evaporative loss low enough,

to cause a conspicuously net positive water balance. Alternatively, in the other ponds with no outlet channels, water inflow must be in equilibrium with evaporative (including evapotranspiration) loss, hence the constant water levels despite the constant inflow from the vents. These ponds have a more neutral water balance.

There was a weak but non-significant, increase in conductivity with pond size ( $R^2=0.51$ ,  $P=0.07$ ). The lowest conductivity was measured in Pete's Vent (54.9 mS/cm) and the highest at Cygnet Pond (75.8 mS/cm). The hydrochemistry of the ponds are summarised in (Table 5). The most abundant cation in the brines was  $\text{Na}^+$ , accounting for 36 to 43% of the total equivalent ions. The second major cation was  $\text{Mg}^{+2}$  which accounted for 7 to 11% of the total ionic content. The major anion was  $\text{Cl}^-$  which accounted for 41 to 48% of the total ionic content, followed by  $\text{SO}_4^{-2}$  which accounted for 4 to 6% of ionic content. All the nutrient concentrations showed high variability among ponds (Figure 6).



Plate: Measuring outflow channel dimensions, Whistler Pond . Photo: P.Horwitz

**Table 4: Summary of morphometric and hydrodynamic characteristics of each pond. All temperature measurements were taken from approximately 10 cm below the water surface.**

Pond	Volume (m <sup>3</sup> )	Surface Area (m <sup>2</sup> )	Perimeter (m)	Mean depth (m)	Maximum depth (m)	Outflow Rate (m <sup>3</sup> /day)	Evaporation (m <sup>3</sup> /day)	Total Water loss (m <sup>3</sup> /day)	Residence Time (days)	Mean Temp. (°C)	Min Temp. (°C)	Max Temp. (°C)
Pete's Vent	98	95	40	1.03	1.63	17	1	18	5	27.3	27.2	27.4
Donut Pond	3,848	5,978	500	0.64	1.67	0*	41	41	95	23.6	23.4	24.0
Annie's Pond	4,826	6,712	400	0.72	2.40	1,094	46	1,140	4	22.2	22.0	23.3
Pete's Pond	7,390	14,880	810	0.50	0.82	0*	101	101	73	22.3	21.1	25.4
Whistler's Pond	47,470	46,544	1,250	1.02	2.11	11,214	316	11,530	4	22.1	21.5	25.2
Harjie's Pond	53,655	65,069	2,570	0.82	2.82	0*	442	442	1,198	22.5	21.5	25.7
Jana's Vent	136,528	187,509	2,950	0.73	2.57	27,173	1,273	28,446	5	19.5	17.5	24.3
Cygnets Pond	4,436,004	7,147,702	22,650	0.62	2.18	49,926	48,528	98,454	45	15.7	12.3	24.0

\*These systems had no defined channel for outflow and it is likely that wind direction has a huge influence on water outflow OR water inflow = evaporative loss.

**Table 5: Conductivity (mS/cm) and percentage composition coefficients of the major ions for each pond determined using LDA.**

	Conductivity	Na <sup>+</sup>	Mg <sup>+2</sup>	Ca <sup>+2</sup>	K <sup>+</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-2</sup>
Pete's Vent	54.9	37.7	7.6	1.6	0.7	47.7	4.7
Donut Pond	57.2	41.2	8.6	1.7	0.8	43.4	4.3
Annie's Pond	64.9	36.7	7.5	1.6	0.7	48.0	5.5
Pete's Pond	59.3	42.9	8.7	1.7	0.7	41.0	5.0
Whistler's Pond	56.1	41.0	8.6	1.9	0.8	43.7	4.0
Harjie's Pond	56.1	39.6	8.2	1.8	0.8	45.4	4.3
Jana's Vent	61.3	41.3	9.1	1.8	0.8	42.6	4.5
Cygnat Pond	75.8	38.4	10.5	2.4	0.2	44.7	3.8

### Water chemistry and sedimentology

There were significant differences in log transformed nutrient concentrations for ammonium ( $F_{7,402}=30.08$ ,  $P<0.001$ ), nitrite/nitrate ( $F_{7,402}=91.58$ ,  $P<0.001$ ) and phosphate ( $F_{7,402}=35.25$ ,  $P<0.001$ ) between ponds (Figure 2). Donut Pond, Pete's Pond and Harjie's Pond were found to have low concentrations of ammonium, while Cygnat Pond had the highest concentration. Cygnat Pond and Whistler's Pond also had the highest levels of nitrite/nitrate with Harjie's Pond having the lowest concentration. The lowest concentrations of phosphates were found in Donut Pond, Pete's Pond, Whistler's Pond and Harjie's Pond. Similar to the other nutrients, the highest phosphate concentrations were found in Cygnat Pond. Approximately 95% of the samples had  $PO_4^{3-}$  in the mesotrophic range ( $10 - 35 \mu g L^{-1}$ ).

The most common sediment components, in order of abundances, were pellets, aragonite mud, skeletal fragments, diatomaceous material and organic detritus. These components are mostly autochthonous, being derived from biological and/or chemical processes operating within the lake itself. Few allochthonous particles were found in the biofilm and sediments immediately beneath; these particles consisted of mostly sand grains, probably transported into the seepage face from the surrounding Quobba sands formation. It was found that there are six types of sediments found within the ponds (Figure 7). The different sediment groups were different in their relative composition of pellets, aragonite mud, skeletal fragments, diatomaceous material and organic detritus. The most common sediment types were Pelletal Sand (48%), Pelletal Sand and Aragonite Mud (20%) and Pelletal-Skeletal Sand (13%). The remaining sediment types consisted of Pelletal-Diatomaceous Sand (9%), Pelletal-Aragonite Mud (7%) and Aragonite Mud (3%).

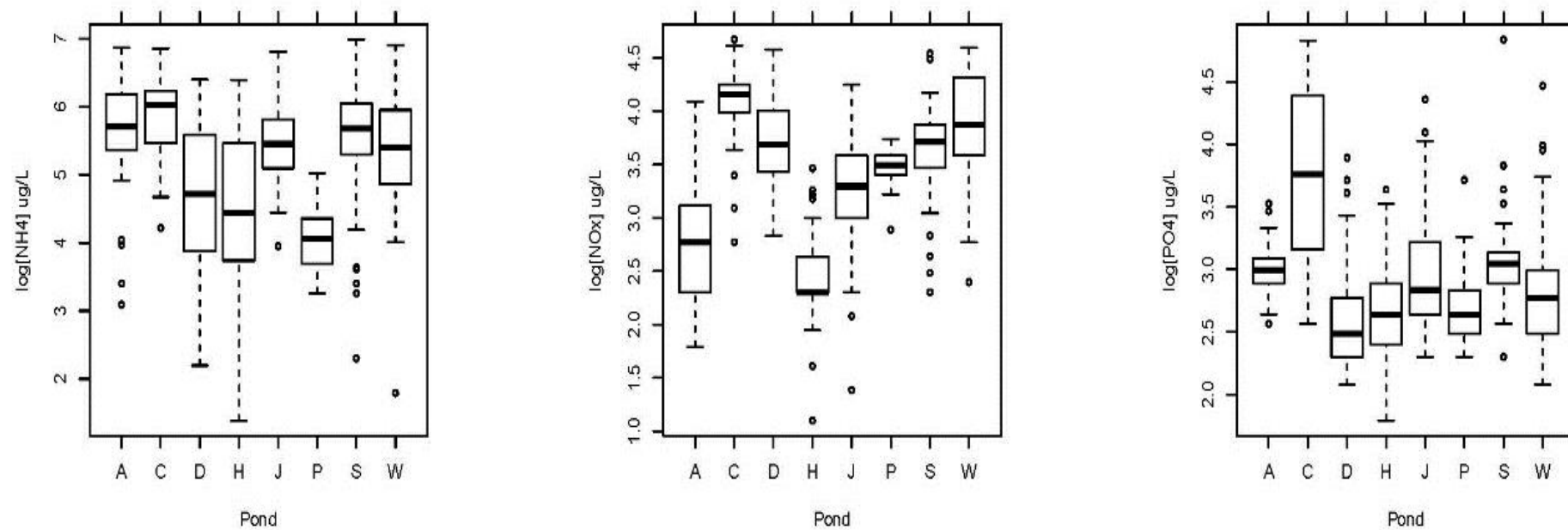


Figure 6: Boxplots showing significant differences between ponds and ammonium (left), nitrite/nitrate (middle) and phosphate (right) concentrations. S: Pete's Vent, D: Donut Pond, A: Annie's Pond, P: Pete's Pond, W: Whistler's Pond, H: Harjie's Pond, J: Jana's Vent; C: Cygnet Pond.

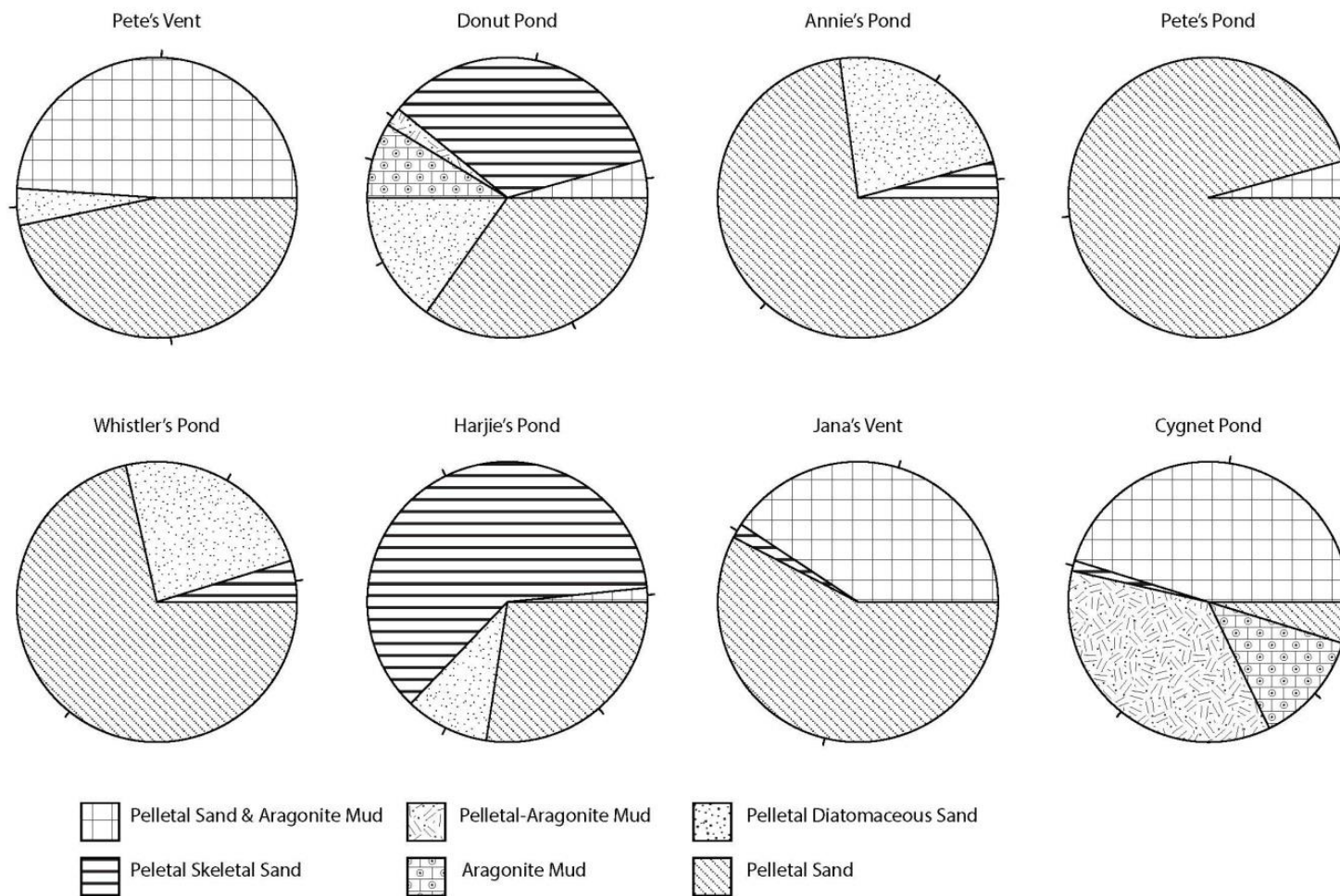


Figure 7: Composition of sediments sampled throughout eight ponds.

## Discussion

The ponds seem to maintain conditions within them that facilitate marine processes, although Cygnet Pond differed from this theme. The results show that the extensive benthic habitats of the Northern Ponds consist of predominantly biogenic sediments formed by the post-mortem accumulation of foraminifera and the green algae, *Acetabularia caliculus*. The foraminiferan population is thought to be dominated by miliolid forms belonging to the *Miliolinella*, *Peneroplis* and *Spirolina* genera (Shepherd 1990). There appears to be little influence of terrestrial inputs in the sediments because of the very low amounts of alluvial and wind-blown sediment particles detected. However, the Aragonite muds and high salinities found within Cygnet Pond suggest that physical and chemical processes occurring in this pond are different to the other ponds.

Although the Northern Ponds, as a system, has terrestrial attributes which are associated with its context in the landscape, including pond morphometry, influence of floods and diurnal cycles of pH and dissolved oxygen concentrations, a dominant marine signature persists. Despite the high amounts of evaporation, flushing rates of brine through the ponds is great enough that the hypersaline conditions often associated with inland salt lake do not occur. Instead, the ponds have relatively stable ionic compositions, which do not seem to vary greatly throughout the ponds. This stability in ionic conditions, which is a characteristic of the marine environment (Millero et al. 2008), allows marine organisms, especially *A. caliculus* and foraminiferans, to thrive. The remains of these organisms allows for the formation of biogenic sediments dominated by pelletal sands. This dominant marine signature, however, was not evident in Cygnet Pond. In fact, conditions which would be expected to occur in a typical Australian salt lake where terrigenous processes dominate were found. The sediment forming processes which are occurring in the other ponds through the post-mortem accumulation of organism remains is not occurring in Cygnet Pond, and the water body is large enough that evapotranspiration of the water increases the salinity to levels much higher than is found in marine environments.

The salinities within most of the ponds did not differ greatly from marine water, which suggests that the water turnover rates are high enough that the ponds are flushed before evapotranspiration can increase the ionic concentrations significantly. The salinity in Cygnet Pond was higher than the other ponds, and that of marine water, and provides another example of the different environmental conditions found in this water body. The chemical properties of each pond (i.e. dominance of  $\text{Na}^+$  and  $\text{Cl}^-$  ions) is typical of Australian salt lakes (Radke et al. 2002, Timms 2009) and was expected as the vents are primarily delivering marine-water in to the basin (Shepherd 1990). The ionic concentration at Cygnet Pond was different to the other ponds, mostly because it was enriched with magnesium and depleted in potassium ions. The reason for the decrease in potassium concentration when the other ions increased in concentration in Cygnet Pond is unknown, but could either be due to potassium deposition, or biological uptake, in particular, by the extensive *Ruppia* sp. and *Halodule* sp. meadows found there. Potassium depletion has been recorded in some Australian saline lakes, where it is thought potassium can be taken up by reactive surfaces, such as an interaction with clay minerals in the sediment column (Herczeg and Berry Lyons 1991, Radke et al. 2002). The aragonite mud dominated sediments



found in Cygnet Pond, which were not well established in any of the other ponds, may therefore be important in suppressing potassium levels in the Cygnet Pond water column.



Plate: Microbialite communities, Pete's Pond. Photo: P.Horwitz

A detailed account of the morphology, hydrodynamics, physicochemistry and sedimentology of a unique Australian saline wetland is reported here. Features of this system are similar to those reported for the Monegros saline wetlands (Mees et al. 2011, Casamayor et al. 2013, Castañeda et al. 2013) and for the salt lakes of the Eyre Peninsula (Timms 2009) and Yarra Yarra basin (Boggs et al. 2006) in that the water bodies are typically shallow and occur because of subsurface geomorphological features. Unlike these systems, the ponds at Lake MacLeod are permanently inundated because of the constant seepage inflow of marine-water, which seems to be a rare feature of saline systems. An analogue might exist for the four saline lakes located on the Eyre Peninsula of South Australia, where seawater is delivered via marine springs, and are also permanently inundated (Timms 2009, 2010). The closest analogues in northwestern Australia are the “birradas” of Shark Bay, and Mandora Marsh which is inland of Eighty Mile Beach in the Pilbara. Halse et al. (2000) state “Most of the birradas contain gypsum and, although they may dry intermittently, anecdotal information suggests their water levels show subdued response to oceanic tides.” Indeed they liken Lake MacLeod, as a whole to be an example of a very large birrida. The Northern Ponds themselves, some 200 km north of Shark Bay, are clearly part of this analogous regional system as permanent water bodies fed by sea water in which the tidal signal appears to be completely, or almost so, obliterated by the long passage through the karst. Mandora Marsh is also a permanent arid zone

wetland in north-western Australia, contains waters that range from fresh to saline, however they are not of marine origin (Storey et al. 2011).

The Northern Ponds of Lake MacLeod represent a wetland system where the climate and hydrology permits the establishment of permanent inland waters dominated by marine processes, and differentiated by size. Most of the ponds are relatively small lakes, where autochthonous production of calcium carbonates (mainly tests of foraminifera, and *Acetabularia*) dominate biogenic sediments. The largest, Cygnet Pond, has terrigenous processes, characterised by higher levels of nutrients, potassium depletion, aragonite muds and seagrass meadows, superimposed on these biogenic signatures. Because the system lies in the arid subtropical regions of Australia's northwest, and is therefore subjected to very low levels of precipitation, the surrounding catchments which drain into the basin appear to have little influence on the composition of the habitats found within the ponds. In fact, because of the rather constant supply of marine water and low rainfall, the ponds resemble those of marine systems in that biogenic processes are determining the benthic/biofilm habitat found in them. The pond sediments, caused by the accumulation of deceased marine organisms, is evidence of the strong marine influence of this system, and future studies should investigate how the biotic assemblages found in these ponds reassemble those of marine systems.



Plate: Dense pneumatophores in shallow water, Whistler Pond. Photo: P.Horwitz

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## MICROBIAL COMMUNITY STRUCTURE AND BIOGEOGRAPHIC PATTERNS

Source documents:

Kavazos, C. R. J. 2016. *Small-scale biogeographic patterns of benthic bacterial and ciliate communities in the saline ponds of Lake MacLeod, north-western Australia*. Edith Cowan University.

Huggett, M. J., C. R. J. Kavazos, R. Bernasconi, R. Czarnik, and P. Horwitz. *Origin, environment, and novel hydrology shape distinctive inland saline bacterioplankton communities in northwestern Australia*. [Submitted Microbial Ecology 2016].

Two studies were conducted in Lake MacLeod investigating the microbial communities found within the pond habitats. The first, Kavazos (2016), details the biogeographic patterns of benthic bacterial and ciliate communities. The second, Huggett et al. (2016), provides an analysis of the taxonomic composition of the bacterioplankton communities, and their novelty. These two studies will be summarised in this section of the report.

### Study One: Bacterial and ciliate biogeographic patterns

Microbes play important roles in the Earth's biogeochemical cycles (Falkowski et al. 2008) and functioning of ecosystems (Bell et al. 2005, Reed and Martiny 2007). Because of their overwhelming abundance and diversity (Whitman et al. 1998, Torsvik et al. 2002) understanding how these communities are spatially structured, and the mechanisms that create these patterns can provide useful insights into the functioning of ecosystems. By identifying the distribution of bacteria and ciliate taxa across the Northern Ponds, the contribution of environmental heterogeneity and spatial variation on the patterns of community structure can be disentangled. This will allow for comparisons of the spatial patterns and assembly processes of two groups of microbes, prokaryotic bacteria and eukaryotic ciliates to be made. The role of species-sorting and neutral processes on the communities will be investigated by accounting for variation attributable to environmental and spatial factors (Borcard et al. 1992).

### Methods

Different sized ponds were studied in order to investigate taxa-area relationships. For this reason, eight ponds were chosen that represent the change in spatial scales of the ponds found at Lake MacLeod. The ponds chosen for this study were (from smallest to largest): Pete's Vent (95 m<sup>2</sup>), Donut Pond (6,000 m<sup>2</sup>), Annie's Pond (6,700 m<sup>2</sup>), Pete's Pond (14,900 m<sup>2</sup>), Whistler's Pond (46,500 m<sup>2</sup>), Harjie's Pond (65,100 m<sup>2</sup>), Jana's Vent (187,500 m<sup>2</sup>) and Cygnet Pond (7,147,700 m<sup>2</sup>; Figure 3). The sampling design was adapted from Horner-Devine et al. (2004) with samples collected from each pond along transects. In each pond four transects were established, with each transect originating from randomly selected points and orientated in a random direction. The length of transects ranged from 3m in Pete's Vent to 2000m in Cygnet Pond. Samples were collected at predefined distance along

each transect, ranging from separation distances of 0.1m to the length of each transect. Hence, this spatially explicit sampling design can account for biotic and abiotic structures at scales ranging from 0.1m to thousands of metres. Further, this method allows for the creation of a data set where the location of each sample point in space is known, and the distances between samples can be determined.

Samples were collected in unused 70 mL sterilised jars that were opened underwater where the sediment scoop was to be taken. The scoops were taken to include only the top layer of the sediment. In some of the deeper ponds, SCUBA was required for sampling, however, most samples were collected by snorkelling when the sediment was shallower than arms reach. Care was taken to not disturb the sediment prior to collection. Water and sediment subsamples were used for analysis to determine sediment, nutrient and ionic parameters (see Kavazos 2016 and previous section), while DNA community fingerprinting methods, namely ARISA for bacteria and T-RFLP for ciliates, were used to measure community composition (see Kavazos (2016) for details of community fingerprinting methods). These methods do not strictly define these organisms to taxonomic groups, but instead use the proxy, operation taxonomic units (OTUs), to determine community composition.

Using Bray-Curtis dissimilarity matrices, distance-based redundancy analysis (db-RDA) was done to constrain the community datasets to environmental and spatial variation. Environmental data used to constrain the db-RDA were derived from Kavazos et al (2016). Null model analysis of OTU co-occurrence was performed using EcoSim700 software (Gotelli and Entsminger 2004), using the C-score index (Stone and Roberts 1990) to compare patterns of co-occurrence in the real datasets with random matrices. If the observed C-score is higher than expected, the community displays significant segregation of OTUs, and significant aggregation if the observed C-score is lower than expected. The neutral diversity ( $\theta$ ) and immigration ( $I$ ) parameters were derived using a maximum likelihood approach and the sampling formula for multiple samples developed by Etienne (2009). Based on those parameters, 4999 simulated communities were predicted by the neutral model of Etienne (2007). The observed  $\beta$ -diversity (calculated following Legendre and De Cáceres 2013) of the observed communities were compared to those of the simulated data sets using the method developed by Maaß et al. (2014). If the observed  $\beta$ -diversity was significantly higher or lower than 95% of the simulated datasets, the observed community was considered to have  $\beta$ -diversity different to what would be expected under neutral assembly processes.

## Results

In total, 463 samples were analysed for bacterial OTUs, with 171 OTUs detected, and 330 samples were analysed for ciliates and 30 OTUs were detected (Table 6). Five bacterial OTUs were present in all of the ponds, whereas only a single ciliate OTU was detected in every pond. A maximum of 127 and 22 different bacterial and ciliate OTUs were detected within Cygnet Pond, respectively. The least number of bacterial OTUs (56) were found in Whistler's Pond and ciliate OTUs (6) found in Annie's Pond.

**Table 6: Diversity indices for the bacterial and ciliate communities in each pond.**

	Bacteria			Ciliates		
	OTU richness	Shannon diversity	Number of samples	OTU richness	Shannon diversity	Number of samples
<b>Pete's Vent</b>	85	37.5	46	18	8.4	32
<b>Donut Pond</b>	95	31.1	47	15	5.4	32
<b>Annie's Pond</b>	81	19.0	48	6	1.7	19
<b>Pete's Pond</b>	94	16.6	48	14	2.7	39
<b>Whistler's Pond</b>	56	6.9	64	11	3.6	52
<b>Harjie's Pond</b>	65	20.1	61	14	4.6	52
<b>Jana's Vent</b>	105	43.9	63	17	3.3	54
<b>Cygnat Pond</b>	127	48.3	86	22	4.9	50
<b>Entire dataset</b>	171	44.1	463	30	6.3	330

Environmental variation between the samples explained 15.8 and 8.6% of the variation in bacterial and ciliate communities, respectively (Table 7). However, the role of environmental variation was significantly reduced when other factors, including the pond in which the samples were taken and the location of the samples within each pond. The most important factor in determining community structure was pond identity, largely because of the unique communities found within each of them. For bacteria, pond identity explained 33.1% of the community variation and 18.8% of variation in the ciliate communities. Environmental variation within each pond explained non-significant amounts of variation for both communities, whilst location within each pond accounted for 5.3 and 2.8% of community variation for the bacteria and ciliate communities, respectively.

**Table 7: Partitioning of variation in bacterial and ciliate communities at two levels of spatial scale using partial db-RDA. Amount of community variation explained by each model is given as R<sup>2</sup>adj (%). E: environmental model; S: spatial model constructed from MEM variables; Pond: model representing the individual ponds; E|Pond: environmental model corrected for pond identity; Pond|E: pond model corrected for environmental variation; Pond|(E+S): Pond model corrected for environmental and spatial variation; E|(Pond+S): environmental model corrected for pond identity and spatial patterns within ponds; S|(E+Pond): spatial model corrected for environmental variation and pond identity.**

	Bacteria	Ciliate
<b>Inter-pond scale</b>		
E	15.8 <sup>a</sup>	8.6 <sup>b</sup>
Pond	33.1	18.8
E Pond	0.8	0.0
Pond E	18.0	10.1
<b>Within-pond Scale</b>		
Pond (E+S)	19.6	11.5
E (Pond+S)	0.4	0.0
S (E+Pond)	5.3	2.8



Highly significant segregation in bacterial OTUs was found in all eight ponds, suggesting that patterns of bacterial OTU co-occurrence are not consistent with a random pattern (Table 8). Similar results were found in Whistler's Pond, Jana's Vent and Cygnet Pond for the ciliate communities, however the remaining 5 ponds displayed patterns similar to what can be predicted to occur randomly. The observed levels of  $\beta$ -diversity were all lower than the expected values using the data sets generated by the neutral models of Etienne (2007, 2009) for both communities (Table 8). These results suggest that the communities are more diverse than what can be expected based on immigration rates alone.

**Table 8: Results of testing if patterns of OTU co-occurrence (C-score) are random and if simulated neutral communities differ significantly from observed communities based upon  $\beta$ -diversity. All values are effect sizes when comparing the observed values with those determined using simulation processes.  $P < 0.05$  denoted by \*,  $P < 0.01$  denoted by \*\*,  $P < 0.001$  denoted by \*\*\*,  $P < 0.0001$  \*\*\*\*.**

	Bacteria		Ciliates	
	C-Score effect size	$\beta$ -diversity effect size	C-Score effect size	$\beta$ -diversity effect size
Pete's Vent	99.0****	-21.3****	1.2	-6.8**
Donut Pond	39.5****	-17.3****	0.0	-3.9*
Annie's Pond	14.3****	-9.5****	-0.7	-2.5*
Pete's Pond	11.6****	-9.8****	-1.2	-2.8*
Whistler's Pond	6.1****	-5.0***	1.9*	-14.2****
Harjie's Pond	31.0****	-13.0****	-0.9	-3.0*
Jana's Vent	29.0****	-24.4****	2.4*	-4.1**
Cygnet Pond	44.5****	-28.4****	5.9****	-3.5**
Entire data set	19.1****	-4.0***	0.4	-2.1*

## Study Two: Bacterioplankton communities: structure and origin

The assemblages that constitute microbial communities in well-defined habitats are, to a certain extent, predictable based on both the functional capability of an ecosystem (Hooper et al. 2005), and the relative contribution of its various external influences and inputs. For example, the microbial communities that typically dominate marine, freshwater and saline ecosystems are known to be distinct from one another (Rappé et al. 2000, Lindström et al. 2005). The identity of microbial taxa can provide insights into the types of factors that influence the characteristics of an ecosystem and extract information regarding the origin, inputs and function of the wider landscape. Community structure and diversity can also act as a barometer for the status of an ecosystem's health (Rapport et al. 1998, Folke et al. 2004). Shifts in diversity and species composition have been shown to be determined by external stressors, such as disturbances including invasions of alien species, disease or events like fire and flood (Chapin et al. 2000).

Little is known about the bacteroplankton communities in the Northern Ponds. This study tests the hypotheses that bacterioplankton communities within the ponds are influenced by a mix of factors, and that given the unique environmental conditions found there, will harbour unique, but predominately marine assemblages. By determining the identity of the microbes and identifying their potential origins, key factors which are important in determining the ecosystem functioning of the Northern Ponds can be discerned.

## Methods

Surface waters were collected in 1 L containers along transects moving away from the vents within each of the ponds. Separate water samples were collected for water chemical analyses. Samples were also collected from the nearby Indian Ocean to compare microbial communities in the ponds with the ocean. T-RFLP was done using general bacterial primers for every sample. Selected samples (three per pond) were used for a subsequent sequencing analysis. These sequences were further explored using BLASTn to determine nearest match sequences and the habitat in which they have been recorded. Analysis of similarities (ANOSIM) was used to examine the differences between pond communities and seasons, and non-metric multidimensional scaling (n-MDS) used to graphically represent these patterns.

## Results

Microbial community composition differed between ponds (ANOSIM  $R=0.63$ ) and to a lesser degree, between seasons (ANOSIM  $R=0.15$ ; Figure 8). There was also differences between communities found in the Cygnet and Chirrida systems (ANOSIM  $R=0.398$ ).

A total of 873,422 high quality sequences were recovered from the 39 samples, ranging from 85,295 to 1,697 per sample. This included over 20 phyla/classes, including 45 bacterial orders. Seawater samples were dominated by Alphaproteobacteria (38.1%, including sequences identified as SAR116 (16.3%) and SAR11(11.1%)), Bacteroidetes (28.9%) and Gammaproteobacteria (15.6%, including SAR86 (10.4%)). Some ponds, including Donut Pond, Harjie's Pond and Jana's Vent were also dominated by these groups, although Harjie's Pond and Jana's Vent had high proportions of Gammaproteobacteria (27.8 and 41.2%, respectively). Annie's Pond was dominated by Alphaproteobacteria (76.0%) and had higher amounts of Deltaproteobacteria (2.9%) than all other ponds. Cygnet and Pete's Ponds both had high abundance of Actinobacteria (27.7 and 31.4%, respectively). Several taxa were detected only in the ponds and not in the seawater, such as sequences from Candidate Division TM6, Caldithrix, Acidobacteria, Lentisphaerae, Planctomycetes, Spirochaetes and Tenericutes.



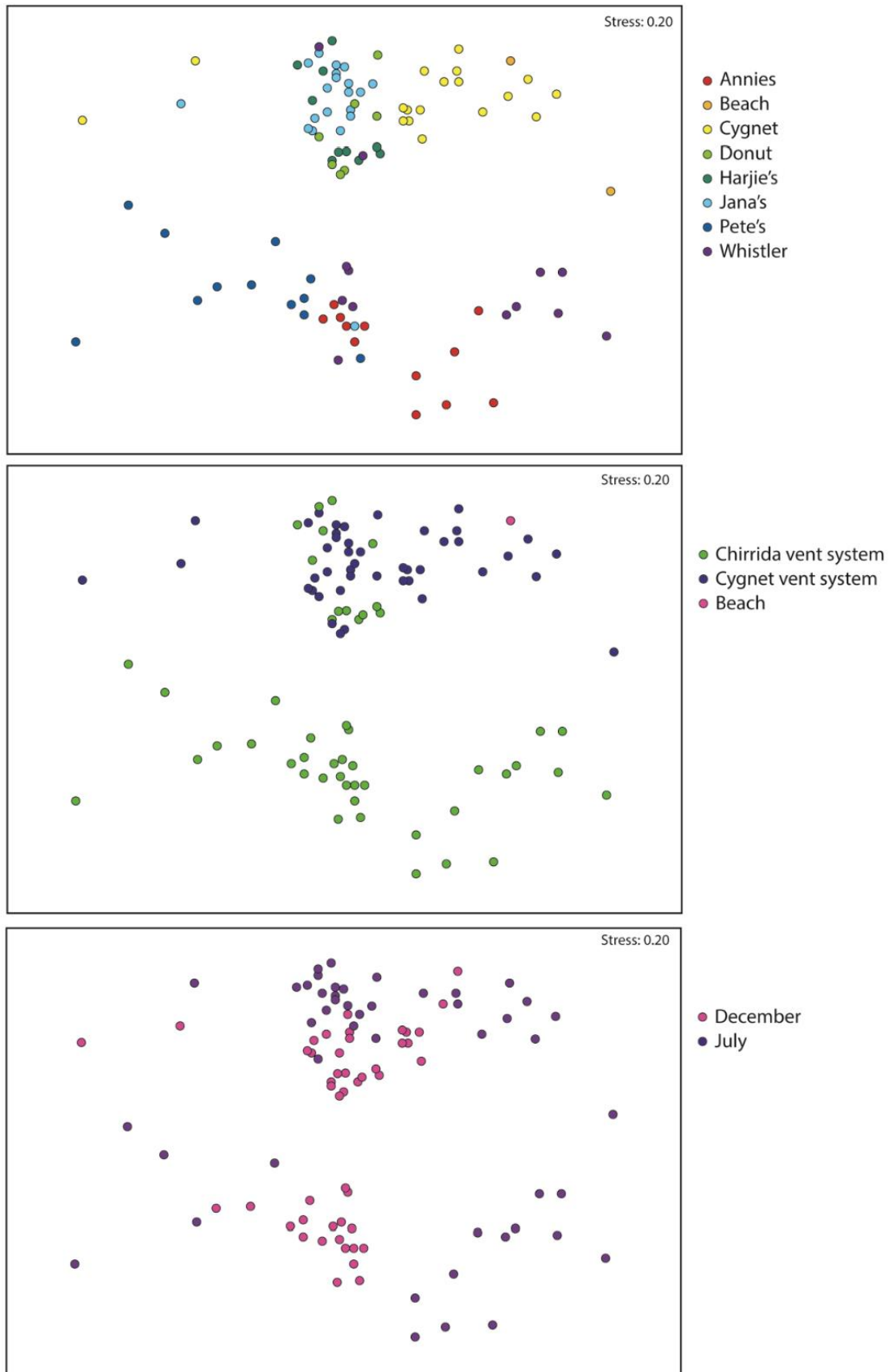


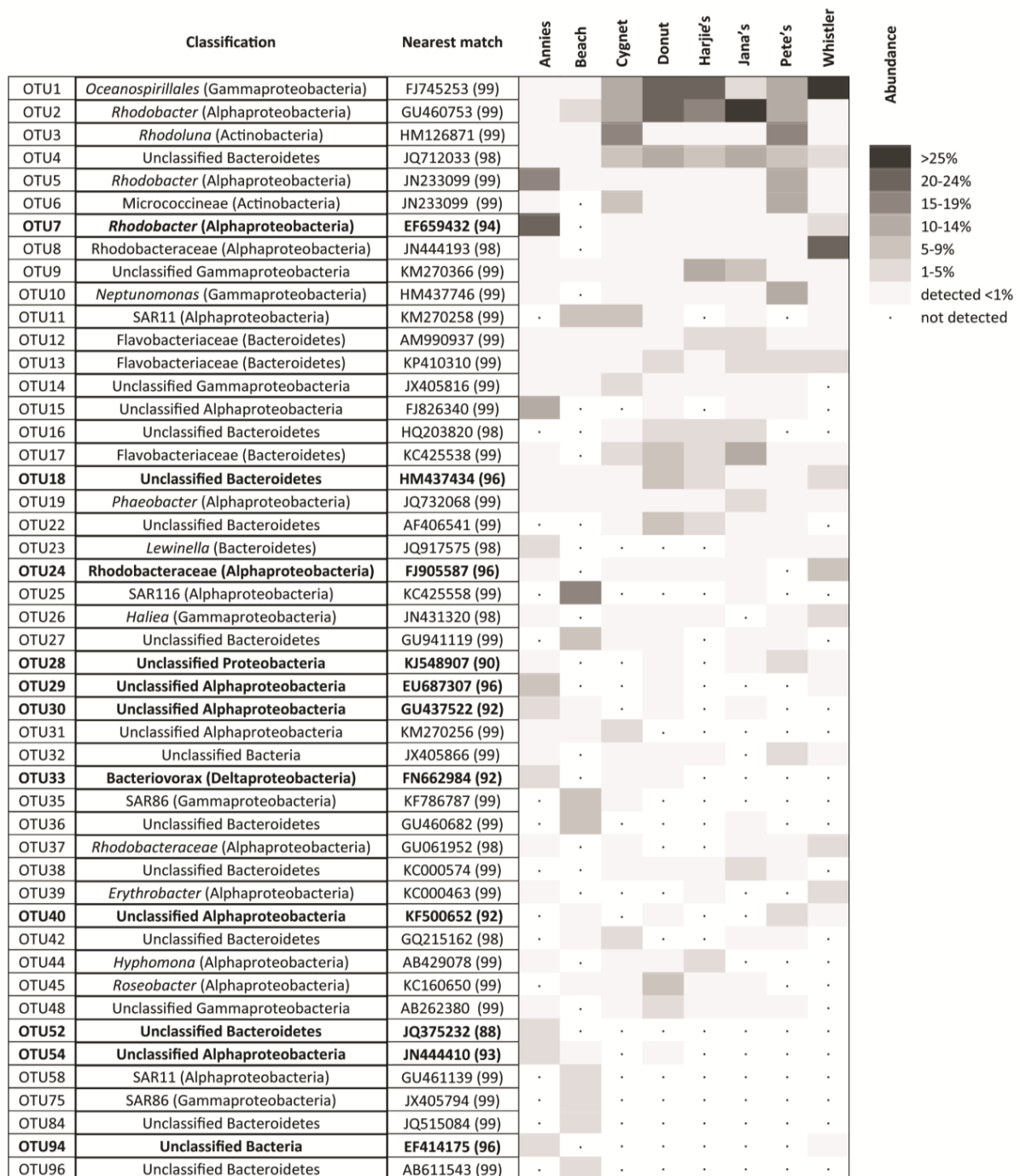
Figure 8: Multi-dimensional scaling plot of Bray-Curtis similarities among samples based on T-RFLP analysis labelled by ponds, vent system and season.

Previously unknown bacterial diversity was detected across the ponds (Figure 9). Annie's Pond had an average 32.8% of OTUs with low sequence similarity (<94%) to publically available sequences, and OTU52 (3.2% of all OTUs in Annie's Pond) was <89% similar to previously known sequences and may therefore represent a novel Family within the Bacteroidetes. Pete's Pond, Harjie's Pond, Whistler's Pond and Donut Pond each contained between 2.2 and 5.9% of OTUs with <96% sequence similarity to known sequences, and are therefore likely to represent new species. In contrast, samples from Cygnet Pond, Jana's Vent and seawater were all dominated by OTUs that matched (>98% sequence similarity) publically available sequences.

Over 84% of OTUs from the seawater samples were closely related to sequences previously derived from the marine environment, including seawater (47.5%), sediment (15.1%), a variety of hosts (corals, sponges, echinoderms and ascidians; 16.3%), fish (1.4%) and the deep ocean (3.6%). Jana's Vent had comparable number of sequences from the marine environment (81.6%) as the seawater samples. All other ponds had smaller abundances of sequences (between 55.7 and 75.0%) matched to marine sources. All ponds, except Annie's Pond, were enriched (between 8.7 and 7.4%) in sequences closely matched to those from the deep ocean. Ponds were also enriched in sequences most closely matched to those from high saline environments. Ponds also had between 1.9 and 98.1% of sequences which have been derived from terrestrial soils. Some ponds, but not seawater, had a small number of sequences (up to 1.9%) which resemble sequences derived from other wetlands, salt marshes or estuaries, while all sample had low abundances of sequences found from mangrove areas (between 0.3 and 1.9%).

## Discussion

Each of the ponds contain unique assemblages of bacteria (benthic and planktonic), and to a lesser degree, ciliates, and these differences could not be attributable to changes in the measured environmental parameters nor immigration rates. This suggests that either an unaccountable variable, which is spatially independent but varies between ponds is driving these changes, or that a neutral processes operating independently of immigration/dispersion is driving the separation of the communities. However, the benthic bacterial and ciliate communities appear to be highly segregated in terms of taxa co-occurrences. These results suggest that populations of OTUs may be opportunistically reacting to stochastic events, such as flooding and temperature fluctuations. These patterns have been described in bacterial communities before (Caruso et al. 2011). The process of divergence in ecological communities due to stochasticity instead of immigration and environmental drivers has been termed 'ecological drift' (Hubbell 2001), and is thought to have the strongest influence in communities where environmental determinants are weak drivers of community structure. Identifying ecological drift signals in communities is difficult (Volkov et al. 2007), but it is introduced here as a possible explanation for the patterns found in the microbial pond communities because of the weak role of environmental parameters and the clear distinction of pond communities (Ruokolainen et al. 2009).



**Figure 9: Relative abundance and percentage of sequence similarity to publically available sequences of OTUs in ponds and seawater. OTUs shown are those that were amongst the 10 most abundant OTUs in at least one pond of seawater (beach sample). OTUs in bold have less than 97% sequence similarity to publically available sequences.**

In all of the ponds, the planktonic microbial communities are dominated by OTUs that are most closely related to marine taxa. Also of interest is the enrichment of OTUs who share close relatives from deep sea marine environments. In addition to the deepsea lineages, the ponds were also higher in OTUs most closely related to terrestrial, hypersaline and freshwater OTUs, indicating these influences within the system, each of them being

expected in higher proportions given the overall lacustrine and terrestrial setting of the Northern Ponds. The high amounts of novel taxa found within the ponds indicates that analogous systems to Lake MacLeod are relatively unknown (or unsampled), thus adding to the site for unique, and possible endemic, taxa. Novel microbial taxonomic diversity has been argued to be the basis for considering certain areas for conservation value (Andrei et al. 2015).



Plate: Pete's Vent. Photo: P.Horwitz

Two OTUs were detected in all the samples and can therefore be considered the 'core microbiome' of the bacterioplankton community. The first is identified as a member of the marine lineage that likely represents a novel family within the Gammaproteobacteria Order *Oceanospirillales*. This OTU has previously been associated with tunicates (Dishaw et al. 2014) and seawater (Poretsky et al. 2010). The second member of the core microbiome is identified as a member of the genus *Phaeobacter* within the Alphaproteobacteria, and has previously been detected in intertidal sediments (Decleyre et al. 2015). There is a persistent and viable population of SAR11 within Cygnet Pond, whereas in all other ponds, this lineage was rare or not established. The Alphaproteobacteria SAR11 clade has been demonstrated to be one of the most abundant microbial groups in ocean and lake environments (Rappé et al. 2000). High abundances of SAR11 have been documented in high saline marine environments, such as the Red Sea (Ngugi et al. 2012). Cygnet Pond had the highest salinities, reaching over 51ppt, suggesting that members of this key marine lineage may be able to tolerate, and even flourish, in high salinities substantially different to coastal marine ecosystems.

Overall, the ponds are primarily composed of marine taxa, as well as those from deep oceans, freshwater, hypersaline and terrestrial ecosystems. Each pond assemblage appears to be on its own evolutionary trajectory, with ecological drift likely playing a role in structuring each community. The fact that each pond contains unique



microbial communities, not only highlights the conservation value of each pond, but also provides information on the functioning of the system.

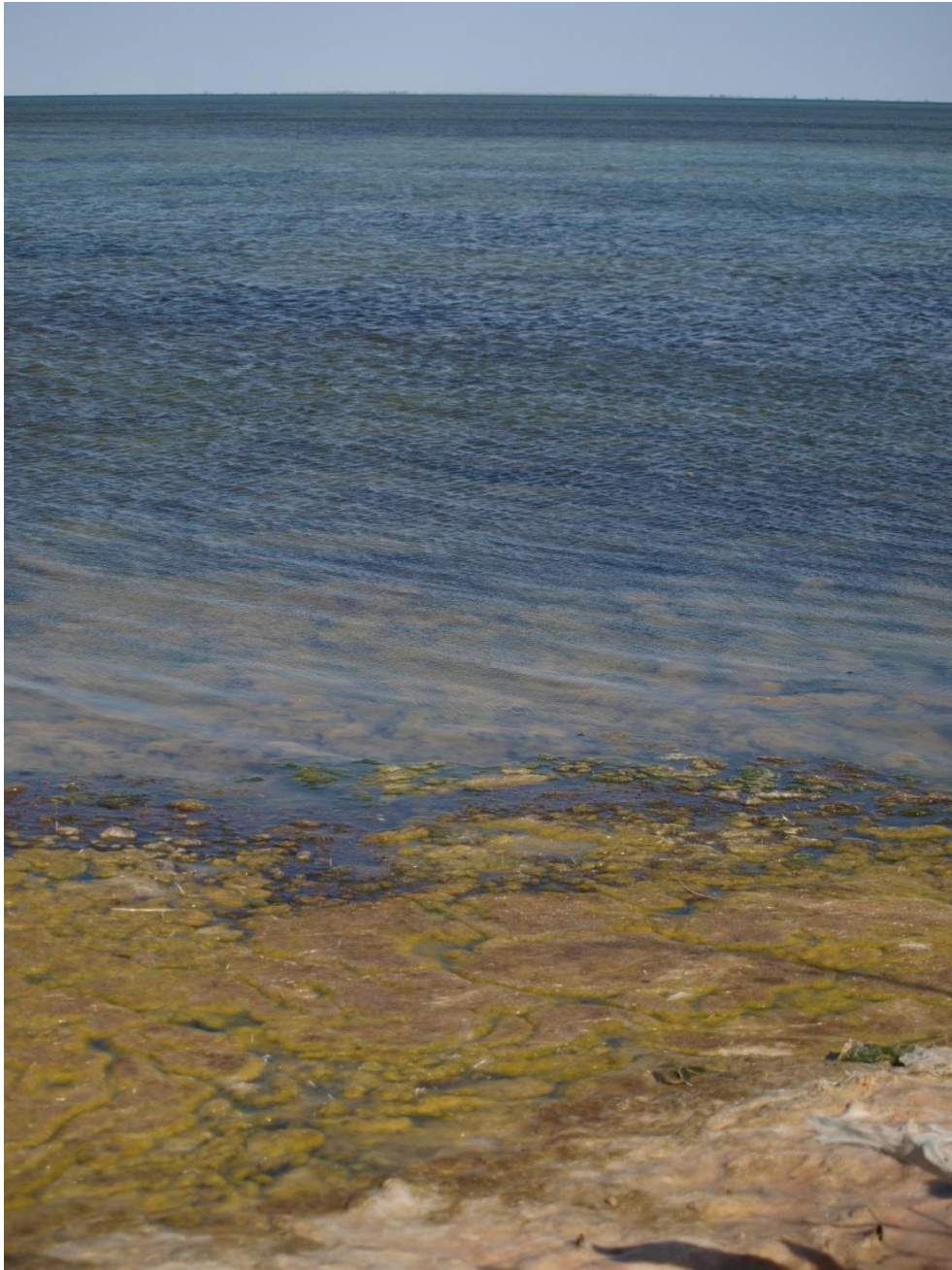


Plate: Cast algal mats on the western edge of Cygnet Pond, at Goat Bay. Photo: P.Horwitz



## TEMPORAL AND SPATIAL VARIATION OF INVERTEBRATE COMMUNITIES

Source document:

McLure, N. 2011. *Temporal and spatial variation in aquatic invertebrate communities at Lake MacLeod, northwestern Australia*. Honours Thesis, School of Natural Sciences, Edith Cowan University.

Valued in their own right, and for the important role they play in food webs, aquatic invertebrates are recognised as one of the 'Significant Biological Assets' of the Lake MacLeod system (Phillips et al. 2005). As a key element of food webs, aquatic invertebrates are fed upon by fish, which in turn are consumed by waterbirds such as pelicans, cormorants, gulls and terns (Phillips et al. 2005). The Northern Ponds invertebrate community is dominated by marine affiliated invertebrates and invertebrates more commonly associated with inland waters (Timms 2009). The high proportion of marine taxa is likely associated with the marine vents which feed the system and maintain permanent water.

Invertebrates contribute to the biodiversity of the Northern Ponds with surveys undertaken at Lake MacLeod in 1994 and 1995, as part of the Southern Carnarvon Basin (SCB) study, recording over 50 invertebrate taxa (Halse et al. 2000). The results of this survey found the Northern Ponds invertebrate assemblages to be significantly different to other wetlands in the region, with less than half of the taxa recorded at Lake MacLeod also recorded in the surrounding wetlands. Other studies commissioned by DSL in the late 1990's and early 2000's to look at aquatic invertebrates in the Northern Ponds include the Inland Aquatic Integrity Resource Condition Monitoring (IAI RCM) project and a biomonitoring programme undertaken by Streamtec Ecological consultants on behalf of Dampier Salt Limited. The IAI RCM project collected 16 invertebrate taxa (excluding ostracods and copepods) and Streamtec recorded 20 taxa.

Floods, as disturbance events, cause changes in physicochemical characteristics and link parts of the Northern Ponds that would otherwise remain separate (or might be separated by chemical barriers); they could also affect invertebrate communities producing long or short term changes in assemblages. The large flood event of March 2000, documented only at Cygnet Pond (there are no records of this event from the Chirrida Ponds), increased water depth and produced changes to salinity, nutrient levels and pH (Streamtec 2000). During this flood event the salinity gradients between vents and the rest of the ponds are thought to have been reversed with vents remaining saline while other parts of the pond were 'relatively fresh' (Streamtec 2001). Such events could remove species unable to cope with such changes in water quality. Flood events could also homogenise invertebrate communities by temporarily connecting vent systems and allowing invertebrates to move around the wetland. Furthermore, flooding could alter composition by introducing species from the surrounding catchment.

McLure (2011) provides an insight into the temporal changes of the invertebrate assemblages by re-analysing the invertebrate datasets generated by the Streamtec monitoring program. This dataset draws on data from five reports and contains water quality and invertebrate sampling dataset taken from a range of sites across Cygnet

Pond between 1998 and 2001. It was found that changes in water quality between sites over time were linked to changes in invertebrate communities. In particular, nitrogen levels, ionic concentration and dissolved oxygen were the most important determinants of community structure. The strong effect of the time of sampling was associated with a large flood event which occurred in the middle of the study and altered conditions in Cygnet Pond. For habitat type, the main pattern detected was that assemblages associated with the littoral margins were different to assemblages collected from other habitats.

McLure (2011) also set out to conduct a field study to investigate differences in vent assemblages associated with connectivity and water quality. The study was designed to test the following hypotheses:

- 1) Vents located north and south of Sandy Bluff Sill will support different invertebrate communities
- 2) Invertebrate assemblages are influenced by differences in water quality experienced between and within systems.

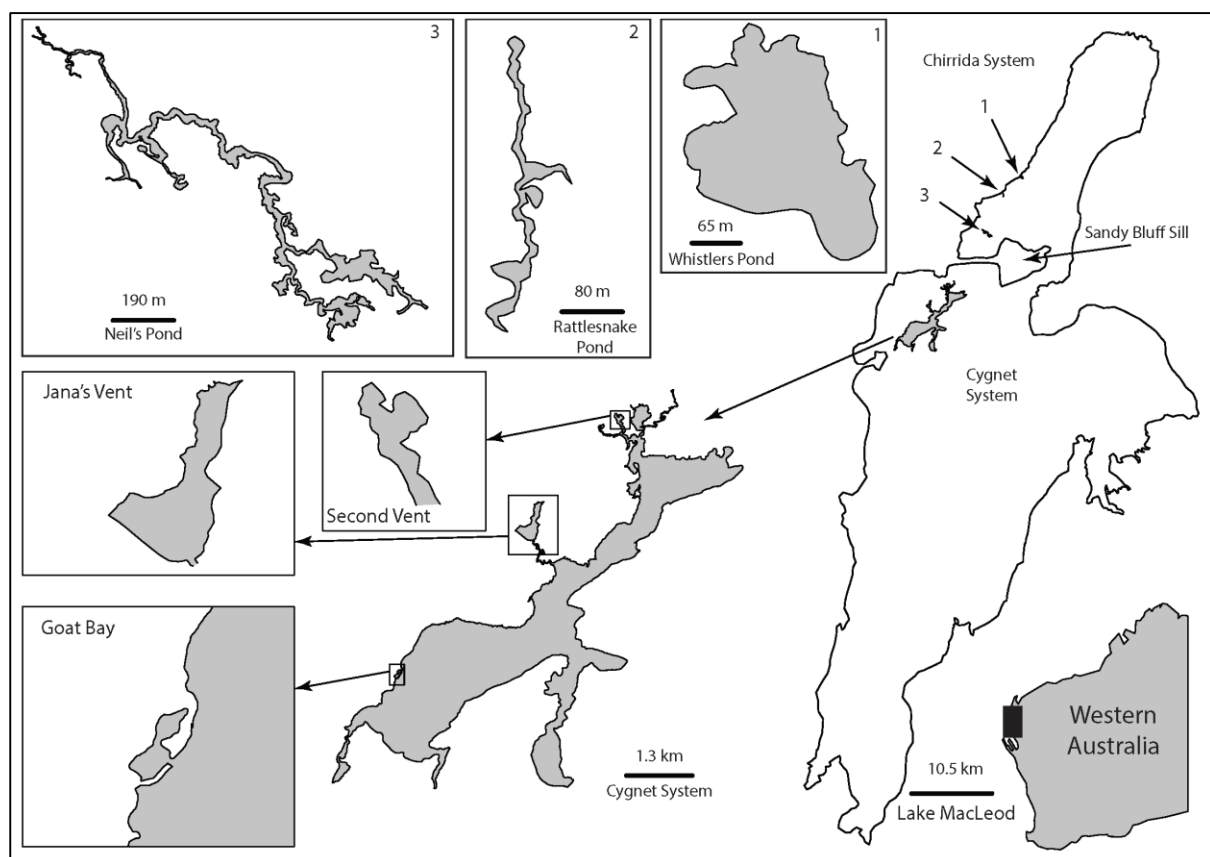


Plate: Establishing invertebrate sampling site in a channel at Neil's Pond. Photo: P.Horwitz



## Methods

Invertebrates and water quality data were collected from six ponds in the Northern Ponds of Lake MacLeod (Figure 10). Three sites were selected from the Chirrida System and three selected from the Cygnet System. The Chirrida System sites included Whistler Pond, Rattlesnake Pond and Neil's Pond, whilst the Cygnet System sites were Second Vent, Jana's Vent and Goat Bay Vent. At each site 5 replicate samples were collected. Invertebrates were collected using a D-frame pond net with a 250µm mesh size net. Each replicate sampling occurred along a 10m transect along the shoreline. The net was swept up and down through the water column and under any overhanging ledges. A vigorous zigzag motion was used through areas of epiphyte coated mangrove roots and pneumatophores and under overhanging vegetation. At 1 or 2 points along each transect, the bottom sediments were disturbed and the net was swept back and forward a few times through the water. In most cases sampling was undertaken from the shore, rather than from in the water. This approach limited disturbance of the very soft and fine sediments and limited trampling of delicate structures. The contents of the nets were emptied into 1 litre jars filled with ethanol before being transported back to the laboratory for sorting. Preserved invertebrate samples were sieved, washed and picked in the laboratory using 1mm mesh sieves. Any invertebrates smaller than 1mm were not identified nor included in analyses. Individuals were identified to at least family level.



**Figure 10: Location of 'vent sites' selected for the collection of invertebrate and water quality data. Three sites were selected from the Chirrida Ponds System, these were: Whistler Pond, Rattlesnake Pond and Neil's Pond. Three sites were also selected from the Cygnet Pond system, these were: Second Vent, Jana's Vent and Goat Bay Vent.**

## Results

### Community richness and diversity

In total, 5,111 organisms were found, belonging to 34 taxonomic families (Family Chironominae was divided into the two Tribes: Chironomini and Tanytarsini). Most of the taxa were uncommon, being found in less than 25% of the samples. There were also a few common taxa found occurring in more than 80% of the samples. The most frequently occurring taxonomic group was the Corophiidae family (Order: Amphipoda), which were found in every sample. Cylichnidae (Class: Gastropoda) and Paracalliopiidae (Order: Amphipoda) were found in more than 80% of the samples. The amphipods, Paracalliopiidae and Corophiidae, were the most abundant families with 1747 and 1432 individuals, respectively. The gastropod, Cylichnidae, was also present in high abundance, with 843 individuals found. The highest number of families was found in Neil's Pond, with 29 families, and lowest in Jana's Vent, with 17 families found (Table 9). Similarly, Shannon Diversity was greatest in Neil's Pond and lowest in Jana's Vent.

**Table 9: Summary of community diversity indices for each pond for entire invertebrate community.**

		Family Richness	Shannon Diversity	Shannon Evenness
<b>Chirrida System</b>	Whistlers	24	7.98	0.33
	Rattlesnake	21	4.32	0.21
	Neil's	29	8.26	0.28
	Total	31	6.96	0.22
<b>Cygnnet System</b>	Second Vent	21	7.72	0.37
	Jana's	17	3.23	0.19
	Goat Bay	20	4.07	0.20
	Total	24	6.14	0.26
<b>Total Community</b>		<b>34</b>	<b>7.24</b>	<b>0.20</b>

### Community variation

Community composition between the Cygnnet and Chirrida systems differed significantly ( $P < 0.001$ ), although there was significant variation in dispersion between the two groups (Figure 11A). There was also significant differences in community composition between the ponds ( $P < 0.001$ ; Figure 11B). The most abundant family found in the Cygnnet system were the Corophiidae, whilst in the Chirrida system, Paracalliopiidae were the most abundant. The Opisthibranch, Cylichnidae, was common throughout both systems, although more abundant in the Cygnnet system. Some families, including Marginellidae, were found in the Chirrida system but were absent from the Cygnnet system. The Corophiidae were most abundant in Jana's Vent whilst the Paracalliopiidae were most abundant in Rattlesnake Pond.

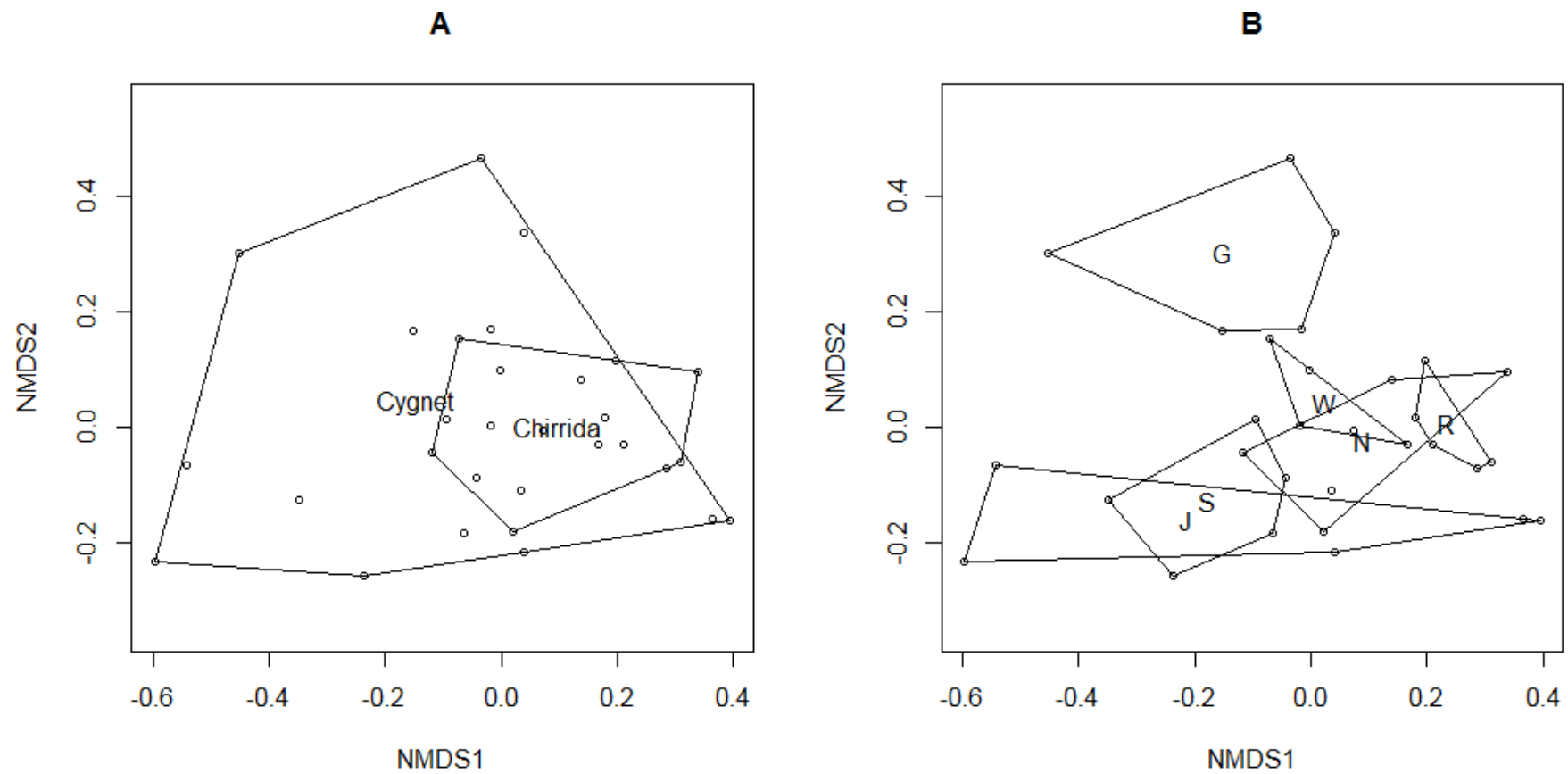


Figure 11: nMDS plot showing the variation between system groups (A) and pond groups (B) using Bray-Curtis similarities. Initials represent the different sampling areas. G: Goat Bay; J: Jana's Vent; N: Neil's Pond; R: Rattlesnake Pond; S: Second Vent; W: Whistler's Pond. Stress = 0.15

## Community variation and environmental determinants

The invertebrate communities were significantly different between the Chirrida and Cygnet systems (Sys component in Table 10), with system identity explaining up to 10.3% of the community variation. The environmental model included PO<sub>4</sub> concentration, NH<sub>4</sub> concentration, turbidity and dissolved oxygen concentration (based on a forward selection process). The environmental conditions were significant determinants of the invertebrate communities, with the environment accounting for up to 30.4% of community variation (Table 10).

**Table 10: Partition of variation in the invertebrate communities at three levels of spatial scale using partial RDA on hellinger transformed abundance data. E: environmental model; Sys: model representing the two study systems (Chirrida & Cygnet); Pond: model representing the 6 study ponds. R<sup>2</sup><sub>adj</sub> (%): community variation explained by model, expressed in %; DF<sub>model</sub>: degrees of freedom of model; DF<sub>res</sub>: residual degrees of freedom**

	R <sup>2</sup> <sub>adj</sub> (%)	DF <sub>model</sub>	DF <sub>res</sub>	F	P
<b>E</b>	30.4	4	25	4.15	0.005
<b>Sys</b>	10.3	1	28	4.31	0.005
<b>Total</b>	31.8	5	24	3.69	0.005
<b>Residual</b>	68.2				

## Discussion

The invertebrate communities were found to be different for the Chirrida and Cygnet systems, with the Chirrida ponds supporting a richer and more diverse invertebrate assemblage than the Cygnet system. The Chirrida system also had the greatest number of taxa which were unique to the system; of the 34 families recorded during sampling, 21 families were found in both systems, 11 were found only in the Chirrida Ponds and only 2 were confined to Cygnet Pond. An assessment of the number of taxa supported by each vent system also showed vent systems within the Chirrida Ponds to be consistently richer than vent systems within the Cygnet Pond system.

The effect of 'pond' was found to be a significant predictor of assemblages, and in most instances different sampling locations within a vent site produced more similar assemblages than sampling locations from different vent sites. This was linked to the tendency for separate vent systems to support different taxa, as demonstrated by the high proportion of taxa only recorded from one site. Differences between individual vent systems were most obvious in the Chirrida Ponds. These sites formed quite distinct groups on the ordination plot and the invertebrate dataset indicated that it was the Chirrida Ponds vent systems which had a high proportion of 'unique' taxa only recorded from one vent. Neil's Pond really stood out in this respect.

The patterns in invertebrate assemblages observed in this round of sampling are similar to those seen in the historical dataset. The amphipods, Paracalliopiidae and Corophiidae, were found to be the most important discriminating families between the two systems, with the former being more abundant in the Chirrida system and the latter more abundant in the Cygnet system. The differences in the composition of assemblages, between and within the North and South, appear to be correlated with varying levels of connectivity between and within the two regions. The Chirrida Ponds and the Cygnet Pond are a considerable distance apart, and under most circumstances, they are not connected by surface water. Whilst some of the sites sampled may be connected during large flood events, aquatic invertebrate groups which disperse via surface water would likely have had a limited number of opportunities to migrate between the two regions. Similarly, when looking at connectivity within the two regions the Chirrida Ponds vent systems are less connected than the Cygnet Pond vent systems. Interestingly, the assemblages collected from vent systems associated with the Chirrida Ponds were more different to one another in their composition than assemblages collected from vents within the Cygnet Pond system. These findings fit with other studies that propose assemblages in ponds or pools are differentiated by distance, with assemblages in water bodies located closer together and more regularly linked by floods being more similar (Marshall et al. 2003, Leigh and Sheldon 2009).

The environmental variables, phosphate concentration, ammonia concentration, turbidity and dissolved oxygen concentration were the best determinants of the invertebrate assemblages. These variables could account for over 20% of the variation in invertebrate assemblages between the Chirrida and Cygnet systems. The environmental variables were less important at the between pond scale though, accounting for only 6.7% of the community variation. Nonetheless, these results suggest that the nutrient concentrations are the most important determinants of invertebrate assemblages. Nutrient levels varied significantly between sites, and there was a surprising amount of variability within sites. Total nitrogen ranged from 0.273mg/L to 0.569mg/L, NO<sub>x</sub> ranged from 0.023mg/L to 0.18mg/L and NH<sub>4</sub> ranged from 0.013mg/L to 0.61mg/L. Total nitrogen levels were within a range similar to that recorded by Halse et al. (2000) and to levels recorded by Streamtec prior to the March 2000 flood event. Interestingly, total nitrogen levels recorded in July 2011 were much lower than many of the levels recorded in the three rounds of sampling that followed the March 2000 flood (October 2000, March 2001 and November 2001) (Streamtec 1999, 2000, 2001, 2003). The other nutrients assessed this time around (NH<sub>4</sub>, NO<sub>x</sub>, PO<sub>4</sub>) were not recorded in previous rounds of invertebrate sampling; as such there are no background levels with which to compare results with. Concentrations of chlorophyll-a reached 3.2mg/L, much higher in this round of sampling than they were in 1994 and 1995 when the highest reading recorded for chlorophyll a was 0.02mg/L (Halse et al. 2000). Some of the variability in the composition and abundance of assemblages could be linked to differing levels of turbidity supporting different proportions of invertebrate taxa with collector/filter feeding strategies, which seems to be the case at Rattlesnake Pond. Nitrogen as ammonia/ammonium also appeared to influence invertebrate composition with higher NH<sub>4</sub> concentrations being associated with lower richness. This is particularly evident when comparing Neil's Pond and Second Vent. Neil's Pond recorded the lowest concentration of total nitrogen and also supported the richest invertebrate assemblage; whereas Jana's Vent recorded the highest NH<sub>4</sub> concentration, as well as second highest total

nitrogen concentration, and had the lowest invertebrate richness. This is not that surprising considering the toxicity of high concentrations of  $\text{NH}_4$  (Spiels and Mitsch 2000, Beutel 2001, Alonso and Camargo 2003).

The results of this study indicate that vent systems associated with the Northern Ponds Cygnet Seepage Face support significantly different aquatic invertebrate assemblages. Patterns in assemblages within and between the Chirrida and Cygnet Pond vent systems do appear to show some correlation with connectivity. The two regions shared approximately half of the total number of taxa collected during surveys, suggesting that the physical distance between vent systems and the low frequency of connectivity by surface water may well be contributing to differences in assemblages between the Chirrida Pond vent systems and the Cygnet Pond vent systems. Within these regions the individual vent systems also appear to support different assemblages. This was particularly evident in the Chirrida Ponds where vent systems not connected by surface water supported a high proportion of taxa associated only with one vent system.

The key findings from this thesis are:

1. Thirty-four macroinvertebrate families were found from littoral habitats at six vents in the Northern Ponds. The assemblages were numerically dominated by amphipods and gastropods.
2. Invertebrate communities are different in the Chirrida and Cygnet systems. These differences are related to different environmental conditions and the connectivity (or lack of it) within and between ponds found in each system.
3. Nutrient levels in ponds are good determinants of invertebrate community structure.
4. It appears that nutrient levels have increased in the Cygnet pond since the 1990's.



Plate: Tilapia killed by bird predation but probably too large for digestion (Jana's Vent).

## FISH COMMUNITY STRUCTURE AND FEEDING ECOLOGY

Source Document:

Cameron-Caluori, H. 2014. *A preliminary investigation of the potential effects of the invasive Mozambique tilapia Oreochromis mossambicus on the native fish assemblages of Lake MacLeod*, Western Australia. Master of Science Thesis. Edith Cowan University.

Various invasive fish species have established wild populations in Western Australia which compete with native fish and other aquatic animals for food and habitat. Exotics often exhibit aggressive behaviour or predation towards native species, causing the native species to decline in abundance, and in extreme cases, become locally extinct. The Mozambique tilapia *Oreochromis mossambicus* (Peters 1852), a species of Tilapiine fishes of the Cichlidae family, is one of the most successful marine and freshwater invasive fish species worldwide (Courtenay 1997, Maddern et al. 2007, Martin et al. 2010, Russell et al. 2012). This species is native to, and generally very common, in West Africa, along with many other Tilapiine species (Bradford et al. 2011). *Oreochromis mossambicus* has been listed as one of the top 100 worst invasive species across the globe and has successfully spread to over 90 countries on 5 continents (Russell et al. 2012), forming wild populations in every country they have been introduced (Canonico et al. 2005).

Tilapia is used worldwide in aquaculture because of their high fecundity, fast growth rates and generalist diet (Doupé et al. 2010, Martin et al. 2010). They have the ability to withstand high fluctuations in salinity, temperature and dissolved oxygen levels which allows them to survive in a wide variety of environments (Scholfield et al. 2001, Canonico et al. 2005, Martin et al. 2010), which has ultimately facilitated their success as invaders (Martin et al. 2010, Russell et al. 2012). The importation of *O. mossambicus* has been banned in many countries around the world, including Australia since 1963; however, its populations have continued to expand throughout Queensland and Western Australia (Russell et al. 2012).

Although *O. mossambicus* is omnivorous, it is generally herbivorous and/or detritivorous (Maitipe and De Silva 1985), but they display high dietary plasticity by feeding opportunistically on invertebrates, zooplankton, larval fish and eggs (Maitipe and De Silva 1985). The potential for dietary competition between this species and native fish is likely, as well as the potential for the direct predation of small or juvenile fish. By invading a new system, *O. mossambicus* may disrupt the trophic processes of a community by causing changes that can propagate throughout the food web.

The *Oreochromis* genus is distinct from other tilapia genera in their breeding and maternal mouthbrooding behaviours (Bradford et al. 2011). Males build and aggressively protect breeding areas (called leks) during the spawning season, while females mouthbrood the fertilised eggs (Maddern et al. 2007, Doupe et al. 2009). This behaviour has the potential to affect the movements and activities of native fish species (Doupe et al. 2009). Other species in the presence of breeding *O. mossambicus* have shown a decline in egg production and fertilisation success, which may be due to the behavioural interactions and/or a possible chemical/hormonal

release from the breeding males (Doupe et al. 2009). Additionally, the construction of these leks, which can be up to 80cm in diameter (Maddern et al. 2007), affect the habitats and available food sources of the native fish communities because of the removal of macrophytes and periphyton. Lek construction also disrupts the sediment which is the habitat of other fauna (Maddern et al. 2007, Doupe et al. 2009).

*Oreochromis mossambicus* has the potential to cause drastic damage to an ecosystem in which it has become established. However, there is little empirical or direct evidence to support these ideas (Maddern et al. 2007, Doupe and Burrows 2008). Since it was first sighted in Western Australia in 1978, *O. mossambicus* has invaded the Gascoyne River, Lyons River, Minilya River, Lyndon River and Chapman River, where it can now be found in high abundance (Morgan and Gill 2004, Morgan et al. 2004, Maddern et al. 2007). Despite the continually increasing presence of invasive tilapia, and specifically *O. mossambicus*, in Australia, there has been little evidence of the ecological influences of this invasive species and quantifiable data of its effect on native fish communities is limited (Doupe and Burrows 2008, Doupe et al. 2009).

*Oreochromis mossambicus* has been observed multiple times in the Northern Ponds for over a decade, but its presence has never been quantified (see Phillips et al. 2005). During major flood events, the Gascoyne, Minilya and Lyndon rivers can overflow into the Lake MacLeod basin, thus enabling riverine fish species to reach the ponds. It is likely that such an event occurred during the flood event in the year 2000, which has already been hypothesised to have allowed the first populations of *O. mossambicus* to enter the lake. Another major flood event occurred in late December 2010 and January 2011, during which *O. mossambicus* were observed entering Lake MacLeod from distributaries of the Gascoyne River and from which individuals from Jana's Vent were identified as *O. mossambicus* by the Western Australian Museum (McLure 2011).

Native fish communities in Lake MacLeod are abundant but the species richness is low (Phillips et al. 2005). Little quantitative work has been conducted on the fish communities of Lake MacLeod, but the native species that have been recorded are limited to yellowtail grunters *Amniataba caudavittata* (Richardson, 1845) and hardyheads *Craterocephalus pauciradiatus* (Gunther, 1861), along with occasional sightings of spangled perch *Leiopotherapon unicolor* (Gunther, 1859) and seamullet *Mugil cephalus* (Linnaeus, 1785). This preliminary work suggests that the fish communities within Lake MacLeod are potentially the top order consumers within the ponds, although they are a food source for the bird populations, and little quantitative evidence exists. The most widely recorded species, *A. caudavittata* and *C. pauciradiatus* are both known to inhabit estuaries and other euhaline environments along Australia's west coast (Prince et al. 1982, Morgan and Gill 2004). Their ability to live in the dynamic and variable habitats found within estuaries likely contributes to their success in surviving the Lake MacLeod environment. *A. caudavittata* generally exhibits a fairly omnivorous diet (Wise et al. 1994, Young et al. 1997, Molony and Parry 2006, Davis et al. 2012) and atherinids such as *C. pauciradiatus* generally consume a range of small invertebrates, plankton and detritus (Allen et al. 2005).

The overall aim of this study is to determine if the invasive *O. mossambicus* is likely to have an effect on the native fish communities of Lake MacLeod through competition for key resources. Specifically, this study aims to determine the abundances and size structures of *O. mossambicus* and native species (mainly *A. caudavittata*



and *C. pauciradiatus*) in representative habitats of the Northern Ponds of Lake MacLeod to establish if there is overlap in the habitat distribution of these species and the potential for competition with native species. Secondly, this study aims to determine if *O. mossambicus* might be affecting the most common native species through trophic processes, either through similar food resource use and/or through direct predation. These aims will help to understand the potential effects of *O. mossambicus* on Lake MacLeod's native fish communities.

## Methods

Three main habitat types occupied by the fish communities within the Northern Ponds were identified: 1) pneumatophore beds; 2) nearshore habitats; and 3) open vents. The pneumatophore bed habitat is shallow (depth <0.5 m) with dense pneumatophore roots. This habitat extends from the beginning of the fringing vegetation along the shoreline to the edge of the pneumatophores in the pond, covering a width up to 10 m. The nearshore habitat is classified as the sloping littoral zones with soft sandy sediment containing few pneumatophores or the vertical drops around the pond edges with over-hanging mangrove roots. These nearshore habitats are typically between 0.5-1 m in depth. The open vent habitat is classified as the deepwater vents (depth >2 m), where the seawater enters the ponds from the underground karst system. These vents typically have a high rugosity and rocky substrate.

This study consisted of two stages, firstly focusing on community structure then on a dietary analysis, to investigate the possible effects of *O. mossambicus* on the native fish communities in the lake (Table 11). The first stage was to determine the abundance and size structure of *O. mossambicus*, *A. caudavittata* and *C. pauciradiatus* in order to establish if there is overlap in the habitat distribution of these species and the potential of competition between the species. The selected ponds contained all three habitat types. These ponds were: Pete's Pond, Whistler's Pond, Harjie's Pond, Jack's Vent, Dave's Pond, Neil's Pond and Jana's Vent. A fyke net was used to capture fish from the pneumatophore habitat, while stereo remote underwater videography (RUV) was used to determine the abundance of fish in the nearshore and open vent habitat. Both of these sampling techniques allows for identification and abundance observations, as well as determining body-size structure of the populations.

**Table 11: Summary table of the sampling technique employed for the two stages of this study.**

Stage	Habitat type	Sampling method
Community structure	Pneumatophore bed	Fyke-seine net
	Nearshore	Stereo RUV 5 min
	Open vent	Stereo RUV 15 min
Dietary analysis	Pneumatophore bed	Fyke-seine net
	Nearshore	Seine net, angling, trap
	Open vent	Angling, trap, nets

The second stage of the study aimed to investigate possible overlap of diets between *O. mossambicus* and the native fish species, and possible predation of native species. *A. caudavittata* can be omnivorous, and therefore, is likely to exhibit dietary overlap with *O. mossambicus*. It is also likely that *A. caudavittata* inhabits similar habitats to *O. mossambicus*. To investigate the diets of the two fish species, a combination of stomach content analysis (SCA) and stable isotope analysis (SIA) was used. Both of these techniques are commonly used to investigate diets of animals and the trophic structures of ecosystems (Beaudoin et al. 1999, Davis et al. 2012). SCA gives useful information on which resources are being consumed and in what proportions they are being consumed (Hyslop 1980). Alternatively, stable isotope analysis (SIA) is utilised because it allows for temporal and spatial variation in diets to be distinguished, because the results are based on the items that have been assimilated into the consumer's tissue over a longer time periods (weeks to months). Such an analysis provides information on the trophic structure and relationships within a system (Gearing 1991).

While SCA allows for the identification of specific taxa and direct estimation of proportions or amounts of prey items consumed, this technique often requires large sample sizes, is limited to a very short time frame from ingestion (often only a few hours) and is highly dependent on the digestion rates of each species (Gearing 1991). Digestion rates between consumer species can vary, which can affect species comparisons. In this case, *O. mossambicus* is known to digest animal material rapidly, even showing evidence of rapid digestion of hard parts such as fish bones and crustacean exoskeletons (Doupé and Knott 2010). Stomach contents may be highly digested, especially soft-bodied animal matter, and different dietary items may be digested at various rates, rendering some items unrecognisable and often under-represented in the measurement of gut content proportions (Hyslop 1980).

SIA used for dietary studies generally look at the stable isotopes of carbon ( $^{13}\text{C}/^{12}\text{C} = \delta^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N} = \delta^{15}\text{N}$ ). The consistent enrichment of these isotopes as they are assimilated up the food web allows for this analysis to trace the major food sources from primary producers to consumers, and provides useful information on the trophic relationships of an ecosystem (Peterson and Fry 1987). As a general rule of thumb, the stable isotope ratios of animals reflect their diets up to 1.0‰ for  $\delta^{13}\text{C}$  and approximately 3-3.5‰ enrichment for  $\delta^{15}\text{N}$ , based on both field and laboratory studies (Peterson and Fry 1987).

## Results

### Community and size structure

Four species of fish were captured during this study at Lake MacLeod (Table 12). They included the Mozambique tilapia (*Oreochromis mossambicus*), yellowtail grunters (*Amniataba caudavittata*) few-ray hardyheads (*Craterocephalus pauciradiatus*) and the giant herring (*Elops hawaiiensis*). The yellowtail grunters and few-ray hardyheads were the only two species captured in every sampled pneumatophore habitat (although tilapia were frequently observed amongst the pneumatophores but never captured). In all the ponds, except Jana's Vent, the hardyheads were more abundant than the grunters. In the vent habitat, the grunters were also observed in every pond, with the greatest abundance.

Tilapia were also found at four of the vent locations, hardyheads in two ponds and the herring only found at Jack's Vent. Although sampling effort was limited in the nearshore habitat (due to the turbidity of the water), the grunners were observed at each of the three sites and the hardyheads observed at a single site. Tilapia were observed whilst snorkelling around the nearshore habitat, but no observations were made using the RUV.

**Table 12: Summary of the abundances (MaxN), mean body total length (mm) and range of total length (mm) for each species observed in each habitat in July 2012.**

Technique	Habitat	Pond	Species	MaxN	Mean TL	TL range
Netting	Pneumatophores	Pete's Pond	<i>A. caudavittata</i>	32	66	42-104
			<i>C. pauciradiatus</i>	188	25	20-41
		Dave's Pond	<i>A. caudavittata</i>	94	81	39-126
			<i>C. pauciradiatus</i>	131	27	20-53
		Harjie's Pond	<i>A. caudavittata</i>	182	72	39-97
			<i>C. pauciradiatus</i>	230	34	23-45
		Jack's Vent	<i>A. caudavittata</i>	3	45	43-46
			<i>C. pauciradiatus</i>	514	30	22-45
		Jana's Vent	<i>A. caudavittata</i>	236	95	76-135
			<i>C. pauciradiatus</i>	118	21	17-25
		Neil's Pond	<i>A. caudavittata</i>	19	87	54-158
			<i>C. pauciradiatus</i>	290	36	26-58
		Whistler's Pond	<i>A. caudavittata</i>	84	69	32-185
			<i>C. pauciradiatus</i>	311	39	25-60
Videography	Vents	Pete's Pond	<i>A. caudavittata</i>	34	98	59-130
			<i>O. mossambicus</i>	10	127	85-174
			<i>A. caudavittata</i>	20	56	27-89
		Dave's Pond	<i>O. mossambicus</i>	15	57	48-69
			<i>C. pauciradiatus</i>	10	22	20-25
			<i>A. caudavittata</i>	28	60	45-77
		Harjie's Pond	<i>A. caudavittata</i>	44	106	98-121
			<i>O. mossambicus</i>	101	368	341-385
			<i>E. hawaiiensis</i>	1	553	-
		Jana's Vent	<i>A. caudavittata</i>	46	75	54-95
			<i>O. mossambicus</i>	7	139	129-145
			<i>A. caudavittata</i>	14	107	99-121
		Neil's Pond	<i>O. mossambicus</i>	4	72	61-78
			<i>C. pauciradiatus</i>	6	-	-
		Whistler's Pond	<i>A. caudavittata</i>	11	113	105-122
	Nearshore	Pete's Pond	<i>A. caudavittata</i>	5	120	118-124
			<i>C. pauciradiatus</i>	27	21	19-24
		Neil's Pond	<i>A. caudavittata</i>	114	112	87-141
		Whistler's Pond	<i>A. caudavittata</i>	22	81	54-110

The giant herring were the largest fish observed in Lake MacLeod found in Jack's Vent, and other vents connected to it. Tilapia was relatively common throughout the lake and was the largest of the three main fish species. These fish fitted into three size cohorts: Jack's Vent where they were relatively large (up to 385mm), Jana's Vent with a max size of 145mm and Neil's Pond where they were less than 80mm in total length. The grunTERS were generally smaller in length in the pneumatophore habitats than the other habitats and had less distinct size cohorts. The hardyheads, which were mainly found in the pneumatophores, was the smallest of the three fish species and there were no size cohorts nor variation between habitats.

### Dietary analysis

Video analysis revealed that tilapia select food from the benthic substrate whereas the grunTERS consume food directly from the water column. Further evidence of the benthic feeding behaviour of tilapia was recorded by the large amounts of sand, sediment and diatoms in the stomachs of the tilapia (Table 13). In contrast, the stomachs of the grunTERS were dominated by filamentous algae, diatoms and amphipods. Nonetheless, tilapia and grunTERS seem to be consuming similar food items, but the quantities and proportions differed between species. For example, both species consumed moderate amounts of diatoms, but grunTERS consumed higher proportions of amphipods and seagrass and less foraminifera and detritus than tilapia.

Both species occasionally consumed bivalves, ostracods and gastropods. GrunTERS appeared to consume greater proportions of algae in 2013 compared to 2012. The hardyheads were observed selecting food from epiphytic algae on the pneumatophores and from disturbed sediment and macrophytes.

**Table 13: Summary of the percentage contribution of stomach contents (>5% volumetric contribution of dietary item) for tilapia and grunTERS.**

Dietary Item	<i>O. massambicus</i>				<i>A. caudavittata</i>				
	Pete's Pond	Jack's Vent	Dave's Pond 2012	Dave's Pond 2013	Pete's Pond	Neil's Pond	Jack's vent	Dave's Pond 2012	Dave's Pond 2013
Algae	6	17	10	10	38	34	13	33	51
Seagrass	2	0	0	0	6	3	0	9	5
Diatoms	0	19	29	24	7	14	14	22	17
Amphipods	5	14	5	7	20	14	25	12	13
Foraminifera	12	1	11	12	7	2	0	4	2
Detritus	9	5	6	4	0	2	0	1	0
Sand/Sediment	33	43	36	32	0	4	0	4	5

Mean  $\delta^{15}\text{N}$  was higher for the grunTERS than tilapia at each pond, while both species had the same  $\delta^{13}\text{C}$ . The ponds also had different  $\delta^{13}\text{C}$  signatures, with Jack's Vent having a different signature than the other ponds (Figure 12). The fish consistently had the highest  $\delta^{15}\text{N}$  than any other sources collected, except in Pete's Pond where the alga, *Acetabularia caliculus* had a  $\delta^{15}\text{N}$  signature almost identical to tilapia. At Pete's Pond and Dave's Pond, the mean  $\delta^{13}\text{C}$  values of the grunTERS and the tilapia were mid-way along the range of  $\delta^{13}\text{C}$  signature of potential source material, thus implying that the sources collected likely contribute to the diets of the fish. In comparison, the mean  $\delta^{13}\text{C}$  values of the two species at Neil's Pond and Jack's Vent were skewed to the right, with  $\delta^{13}\text{C}$  higher than most of all potential source material. *Cladophora* sp. were skewed in the same direction at these sites, while the seagrass *Halodule univervis* and amphipods were skewed in the same direction for Jack's Pond.

Mixing models revealed that amphipods contributed the most to the diets of tilapia and grunTERS at Dave's Pond and Jack's Vent, and contributed the least at Pete's Pond. Another important dietary source in all the ponds included the green alga, *Cladophora*. In Pete's Pond, dipteran larvae were found to be the most important component for grunTERS and the alga *Acetabularia caliculus* for tilapia. *Caulerpa chemnitzia*, a green alga only found in Neil's Pond, was found to be the most important component of the grunter's diet in Neil's Pond, although *cladophora*, *Halodule* and mangroves also contributed. The mangrove leaves contributed the greatest to the composition of detritus.

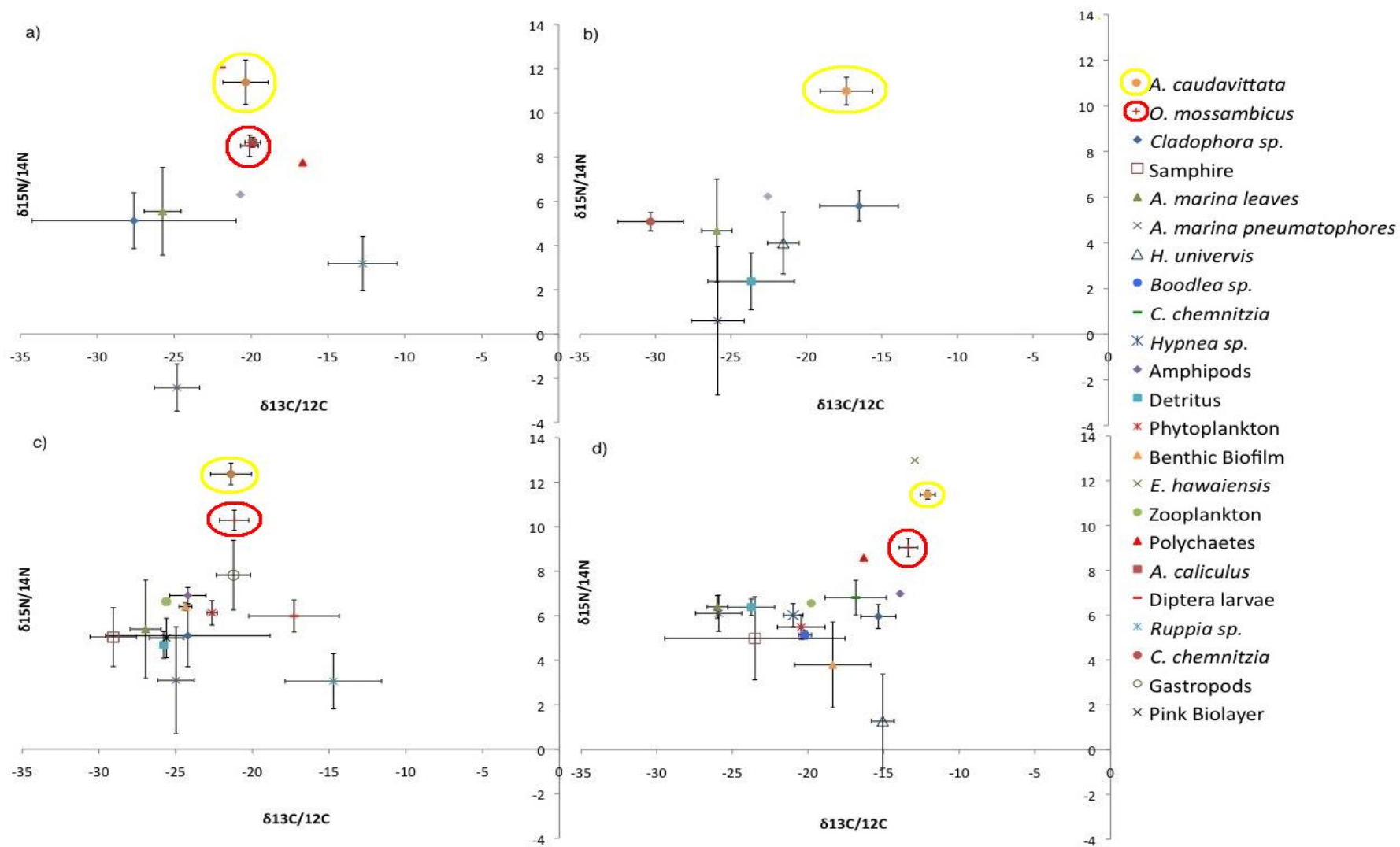


Figure 12: Scatterplots showing mean  $\delta^{15}\text{N}$  vs.  $\delta^{13}\text{C}$  mean with standard deviations for fish and source materials collected from a) Pete's Pond, b) Neil's Pond, c) Dave's Pond and d) Jack's Vent.

## Discussion

The low species richness of fish appears to be stable, based on the data collected in this study. Other than the four species reported here, no other species of fish were observed during the course of Lake MacLeod work (2011-2015), although two additional species are reported from the system anecdotally.

Common name	Scientific name	Comments
<b>Flag-tailed (yellowtail) grunters</b>	<i>Amniataba caudavittata</i>	Abundant and ubiquitous
<b>Few-Ray Hardyheads</b>	<i>Craterocephalus pauciradiatus</i>	Abundant and ubiquitous
<b>Tilapia</b>	<i>Oreochromis mossambicus</i>	Ubiquitous
<b>Giant herring</b>	<i>Elops hawaiiensis</i>	Northeastern vents of Cygnet pond only
<b>Sea mullet</b>	<i>Mugil cephalus</i>	Reported in the literature; unconfirmed.
<b>Spangled perch</b>	<i>Leiopotherapon unicolor</i>	Reported in the literature; unconfirmed.

Low richness of fish communities can be caused by a variety of factors which are relevant to Lake MacLeod, including isolation, limited ability for recruitment, spatial variation and changing salinities and occasional fluxes of freshwater, high predation pressure, competition, limited habitat diversity and complexity, fluctuating water temperature and flow regime (Rahel 1986, Oberdorff et al. 1995). The wide occurrence of the grunters and hardyheads is likely due to both species being euryhaline and thus able to withstand changing salinity. Further, the euryhaline nature of tilapia is also a factor which has enabled the species to successfully invade this saline system from a fresh Gascoyne River.

Tilapia were quantified in five of the seven study ponds, suggesting that it has a wide distribution and high abundance throughout the lake. In addition, tilapia was often visually observed within almost all sites, often swimming within and between all habitats. It is likely that they are widespread throughout many, if not all, of the ponds in the system and utilise all habitats. It is well established that this species can withstand a variety of environments, including conditions that are too harsh for other species (Doupé et al. 2010, Martin et al. 2010), which is likely a contributing factor to their success at Lake MacLeod. Although tilapia were not recorded in Neil's and Harjie's Pond, the vent habitats used in these ponds were small and located at the end of long channels, and may represent undesirable habitats for them., although they were directly observed in these ponds, and evidence of their presence was given by the leks that they build. The apparent preference for vent locations by tilapia may be due to a number of factors including cooler water temperatures, deeper and larger spaces for school formation, protection from predators due to increased depth and rugosity and the possible abundance of prey items in the substrate. There is no evidence that tilapia are excluding native species from available habitats. Although the vent habitats were the only locations where grunters and tilapia were captured together, there did not appear to be any association between the abundances and body sizes of the two species. Based on the size and abundance data, there is no evidence to suggest that tilapia presence negatively affects the abundance or size of the grunter population.

There were high abundances of the three fish species recorded at Jack's Vent. The population structure of tilapia was particularly different to the other systems, where they were significantly larger in size (341-385mm at Jack's Vent compared to 45-174mm at all other ponds). These differences in the fish community structure in Jack's Vent could be due to a variety of factors, including water chemistry, physical characteristics, high connectivity with Cygnet Pond and other vent regions, susceptibility to flooding from the Gascoyne River and increased predation from water birds. Jack's Vent is much deeper, wider and colder than any of the other ponds in this study and these conditions may provide a more favourable and spacious habitat for larger sized fish. The distinctive size distribution of tilapia at Jack's Vent suggest that the individuals may have originated from a single invasion event, and have not successfully reproduced since. Although leks have been observed within Jack's Vent, no active mating or juveniles were observed. Predation levels surrounding is likely to be greater than at other ponds because of the higher abundance of piscivorous water birds (Phillips et al. 2005). These birds may be selecting the smaller sized tilapia (and giant herring) because they are easily consumed, leaving behind the larger sized fish. The tilapia could also be stunted, which is a phenomenon which occurs when sexually mature fish have reduced maximum size. This has been recorded before in tilapia assemblages (Lorenzen 2000), although the causes are unclear. Stunting is mostly likely to occur in small water bodies with isolated populations and low species diversity (Lorenzen 2000), conditions which are found at Lake MacLeod. Further work is needed to determine the mechanisms behind the unique community found at Jack's Vent.

Specific food availability is not likely a major controlling factor for tilapia populations because of their opportunistic nature and highly plastic diets (Maitipe and De Silva 1985). In this study, the tilapia and grunter populations of Lake MacLeod were observed to consume a variety of items but mainly invertebrates and plant matter. The diet of the tilapia from the nearby Chapman and Gascoyne Rivers were different though (Maddern et al. 2007), and both species were not observed to be consuming gastropods and amphipods. Even though there was overlap in the diets of the tilapia and grunter populations, the items they consumed were at vastly different ratios. Interestingly, it seems that the grunters are less selective in their choice of diet than the tilapia, as grunters were found to consume a greater variety of food sources, albeit at lower proportions than tilapia. Based on the video footage, the two species exhibit different feeding behaviours, with tilapia constantly seen selecting food items from the benthos whereas the grunters appeared to be selecting food directly from the water column. The pelagic feeding behaviour of the grunters is in contrast to observations made by other studies, where they were mainly found to be benthic feeders (Wise et al. 1994) but similar observations in the eastern states (Davis et al. 2011). The invasive tilapia and the native grunters therefore appear to co-exist because of their separate feeding strategies.

There were shifts in the diets of both grunters and tilapia in Jack's Vent as both species were found to become increasingly carnivorous because of the higher amount of amphipod material found in their guts. The fact that both species seem to alter their diets at Jack's Vent suggests that diets between ponds may be dependent on the availability of specific food resources. Alternatively, these differences may be caused by shifts in the size structure of the populations, which has been shown to affect many aspects of fish ecology, including diet (Werner and Gilliam 1984).



Tilapia within Lake MacLeod is widespread and abundant. This study aimed to quantify and describe the distribution and habitat use of the invasive tilapia and native fish species within and across the ponds of Lake MacLeod, and determine if the invasive species is likely to be competing for the same food resources and/or predating on native fish species. However, these impacts appear unlikely. The complexity of this system demands further study to fully understand the effects of tilapia, not only on the native fish, but on all aspects of the unique and important Lake MacLeod ecosystem.



Plate: Post-flood (2011) tilapia at the southern end of Lake MacLeod on Blowholes road. Photo: P.Horwitz

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## SHOREBIRD FORAGING AND FEEDING ECOLOGY

Source document:

Estrella, S. M., R. A Davis, and P. Horwitz. 2016. *Shorebird foraging ecology in northwestern Australian salt works*. Edith Cowan University

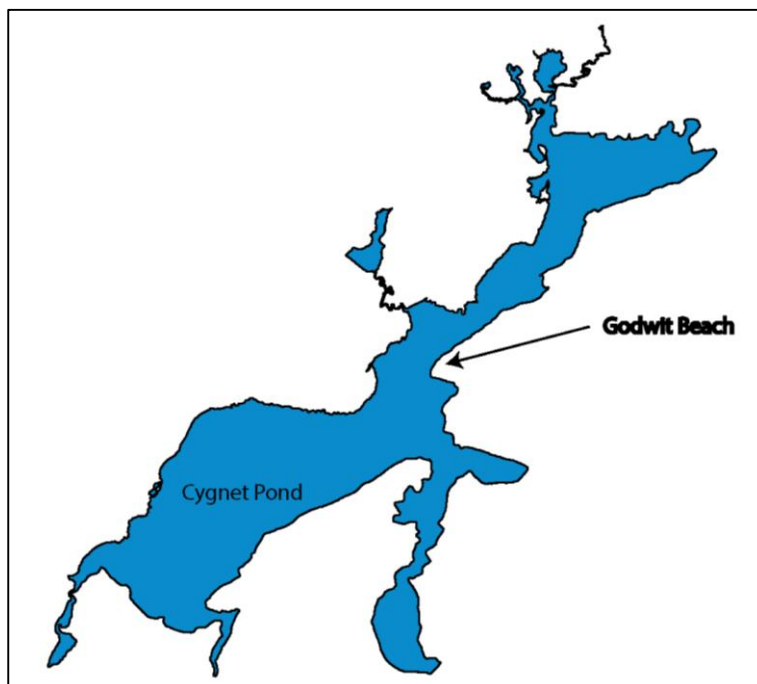
There are 37 species of shorebirds which have been recorded at Lake MacLeod, many of which are migrants that use the East Asian-Australasian Flyway to spend the austral summer season in Australia (Bertzeletos et al. 2012). The remaining species are bound to the Australian continent, but may visit the lake for varying periods of time in response to rainfall. Lake MacLeod often contains more than 1% of the flyway populations of *Calidris canutus* (Red Knot), *Calidris ruficollis* (Red-Necked Stint) and *Calidris ferruginea* (Curlew Sandpiper). There have also been records of more than 1% of the national population of the endemic *Recurvirostra novaehollandiae* (Red-Necked Avocet), *Cladorynchus leucocephalus* (Banded Stilt) and *Charadrius ruficapillus* (Red-capped Plover). In 2003, the Curlew Sandpiper and Banded Stilt were present at Lake MacLeod at abundances up to 16 and 26% of the flyway population, respectively. For some of the other species, the importance of Lake MacLeod increases when regional weather conditions make the lake one of the few water sources available inland from the coast. Consequently, Lake MacLeod is included in *A Directory of Important Wetlands in Australia* (Environment Australia 2001).

The extremely long distance migrations undertaken by many of the shorebirds inhabiting Lake MacLeod demand high amounts of energy. These species therefore rely on a limited number of intertidal habitats that occur across the Flyway to provide prey (energy) that allow them to meet these energy demands required for such long distanced flight. The loss and degradation of these habitats pose a serious threat to the species as it limits the availability of feeding habitats and thus increases the risk of the birds depleting their energy reserves. In the last 20 years, some species have shown declines of up to 80% in the East Australian Flyway. This includes the Curlew Sandpiper, Great Knot (*Calidris tenuirostris*), the Red Knot, the Bar-tailed Godwit (*Limosa lapponica*) and Eastern Curlew (*Numenius madagascariensis*). Salinas, such as Lake MacLeod, provide important feeding grounds, roosting sites and breeding areas for these birds, and they are often preferred by the birds as feeding and roosting habitats over the nearby intertidal mudflats.

Although there have been many bird surveys conducted at Lake MacLeod, and the species found there and the locations they prefer are reasonably well understood, there is little understanding on the foraging ecology within the system. The report of Estrella et al. (2016) investigates the shorebird foraging ecology at three Dampier Salt Limited salt work operation sites in north-western Australia. These sites include the intake ponds at Port Headland and Dampier, as well as the Northern Ponds at Lake MacLeod. This project identifies the primary biotic factors influencing shorebird habitat use, the abiotic conditions that affect habitat use by shorebirds and an evaluation into the quality of these habitats as a shorebird feeding habitat. Information relevant to Lake MacLeod has been extracted from the report of Estrella et al. (2016) and will be summarised here.

## Methods

Two locations in the Northern Ponds were sampled during post-migration and pre-migration periods in order to understand the shift in invertebrate communities in the feeding habitats before and after the shorebirds arrived. The post-migration period starts in October and finishes at the end of November, and represents the time when the shorebirds are arriving at the feeding habitats after their southward migration. The pre-migration period commences in February and finishes at the end of March and represents the time just prior to the commencement of the northward migration. The two feeding habitats sampled were Godwit Beach, which is found on the eastern side of Cygnet Pond (Figure 13), and the marginal sheet environment found between Harjie's and Whistler's Ponds in the Chirrida system.



**Figure 13: Location of Godwit Beach in relation to Cygnet Pond.**

### **Biotic determinants of shorebird distributions**

Video footage was used to understand the biotic factors that explain habitat characteristics used by the shorebirds. This component of the study was completed in three sections. Firstly, direct feeding observations of the birds were made in order to understand the amount and type of prey consumed, as well as changes in feeding behaviour and strategies between species and habitats. The second component involved an investigation into the spatial and temporal variation in prey abundances in the different habitats and migratory periods. Thirdly, an investigation into the energy value of the different prey items was calculated.

Direct feeding observations were made using video recordings for 30 seconds following the method of Estrella and Masero (2007). Between 16 and 82 individuals were filmed during each migratory period for each habitat.

The video footage enables for observations to be made on the feeding mode utilised by the bird, as some birds are known to rely on visual cues to locate prey whilst other birds rely on tactile cues by inserting their beaks into the substratum. The capture strategy employed by the birds can also be determined using the video footage. The birds either capture prey by *pecking* prey from the water column or sediment surface or *probe* prey by inserting their beak into the sediment column to retrieve prey. Other useful data which the video footage allows to be collected include: the proportion of successful feeding attempts per minute, the rate of prey collection, the type (polychaete, amphipod or bivalve) and size of prey consumed, depth of water column where prey is collected.



Plate: Shorebird feeding, Godwit Beach, Lake MacLeod 2012. Photo: P.Horwitz

Temporal and spatial variation of prey availability was obtained for the two migratory periods at each location. These data was collected using cores to sample the benthic invertebrates greater than 0.5mm in size. Prey suspended in the water column with body sizes greater than 0.25mm in length was also collected. All individuals were identified to the family level, with their abundance and body size recorded, allowing the density of prey to be calculated. Temporal variation was investigated by recording the changes in invertebrate communities over a 24 hour period. Vertical distribution patterns of the invertebrates was also measured during the 24 hour period by observing shifts in the preferred depths (<5cm or >5cm deep) for the different families.

In order to understand the energy content of each type of prey consumed by the shorebirds, ash free dry mass (AFDM) for polychaetes, bivalves and chironomids was measured. Dry mass (DM) was measured after the samples were dried for 24 hours at 60°C. Samples were then combusted at 500°C for 2 hours and AFDM was calculated as the difference between non-combusted mass and DM. Allometric equations were used to provide an equation between prey size and biomass. By collecting shorebird faeces, and determining equations to relate

chironomid head size and polychaete jaws with length, the amount of biomass, type of prey and prey size selection can be determined.

### **Abiotic determinants of shorebird habitat use**

Tides in adjacent coastal areas may be an important factor in affecting the use of the Northern Pond's feeding habitats as intertidal coastal habitats may be available at different times during the tidal cycle (Masero 2003, Dias 2009). Similarly, wind strength and direction can influence the distribution of surface waters at the Northern Ponds (Shepherd 1990), and thus render potential feeding habitats unusable, at least for some species (Ntiamoa-Baidu et al. 1998, Davis and Smith 2001, Takekawa et al. 2009). Wind can also have a significant effect on invertebrate distributions (Sánchez et al. 2006) and therefore on prey availability for shorebirds (Verkuil et al. 2003). Therefore time to low tide, water depth, wind direction and wind speed were measured for each sampling occasion. Invertebrate communities in salt systems have been shown to be affected by water chemistry characteristics such as pH, temperature and conductivity (Sánchez et al. 2006), which were also measured using a portable water quality meter.

Time of day also has a profound effect on the feeding behaviour of shorebirds (Kuwaie 2007, Santiago-Quesada et al. 2014), and is also a factor that needs considering when determining daily energy balances. Nocturnal feeding was found to contribute about a third of the daily energy consumption in Godwits, with the authors suggesting that nocturnal feeding may be important when daily energy requirements are not met during the daylight hours (Santiago-Quesada et al. 2014). Nocturnal observations were therefore made of bird feeding behaviour in order to assess if the birds are feeding at night and if they modify their behaviour. Feeding behaviour was assessed as the number of steps taken per minute (Beauchamp 2012) and the number of successful and unsuccessful attempts per minute to capture prey.

### **Habitat quality as a shorebird feeding ground**

The quality of the different habitats could be measured by comparing the energy consumption by the birds for each of the locations, and comparing this with the total daily energy requirement. The biomass, or energy, ingested per unit of feeding time was used to calculate intake rate. Video recordings of feeding birds were used to calculate the biomass intake rate by multiplying the number of prey size class taken per unit time by the AFDM of the invertebrate species. These biomass intake rates can be converted to energy intake rates by using the conversion factors (Brey et al. 1988, Dos Santos et al. 2012).

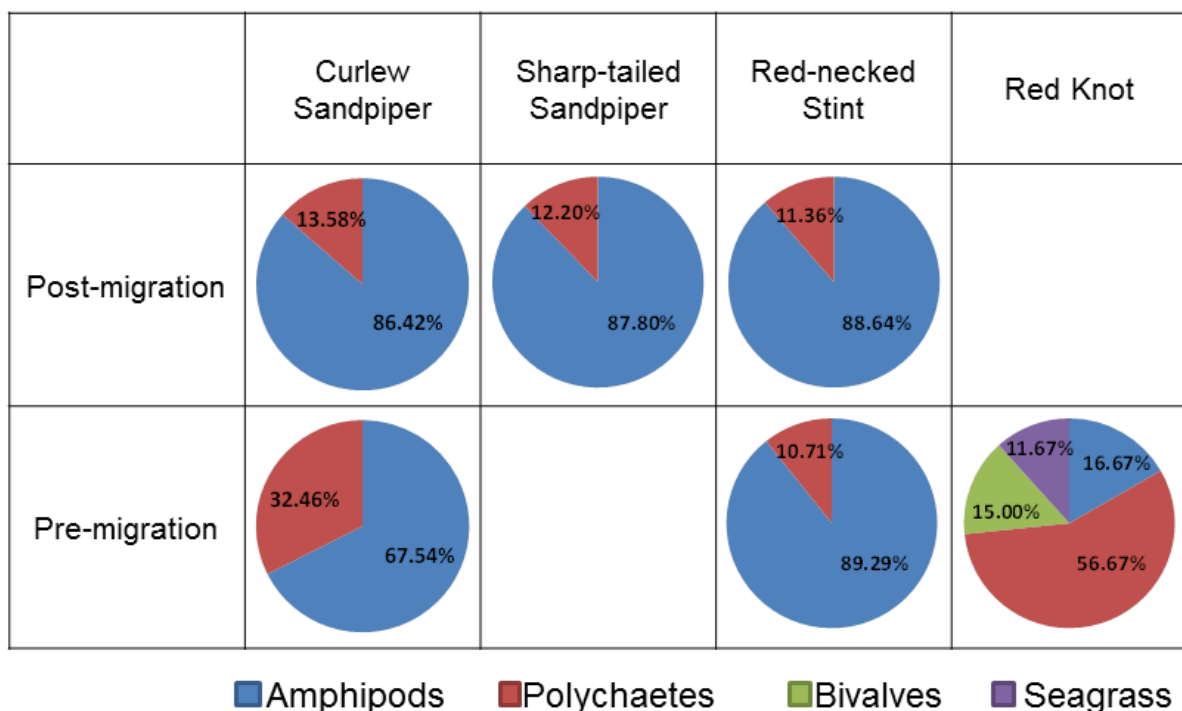
In order to assess whether the birds are meeting their daily energy requirements, energy acquisition and expenditure needs to be compared. Energy acquisition (EA) obtained per species per hour per site per period was compared with the theoretical daily energy expenditure (DEE) per period. DEE for each shorebird can be calculated as a function of their body mass in each migratory period (Nagy 1987). The length of time required by a bird to feed and accomplish their DEE was calculated. When the time required to achieve DEE was less than 24 hours, the feeding habitat was considered to be a high quality feeding ground that is an alternative to the

nearby intertidal regions. However, if the time required to reach DEE exceeded 24 hours, the habitat is assumed to be a complementary feeding ground.

## Results and discussion

Whistler Pond was the site with the highest abundance and biomass of macroinvertebrates. Whistler Pond is an expansive, inundated spill sheet habitat close to the vent, receiving a constant supply of water of, or close to the salinity of seawater, which would preclude the site from experiencing extreme conditions (high water salinity). There is also support for the idea that distance from the water source, or vent, is an important influence on prey abundance and biomass at saltworks, as sites closer to the water source experience less extreme fluctuations in salinity conditions and are more reliably inundated. Therefore, it could be expected that the Whistler Pond site would have more prey in terms of increased abundance, biomass or diversity.

The highest prey densities were found in the top 5 cm of the sediment during the day. The hypothesis that there would be daily changes in macroinvertebrate availability was therefore rejected, since macroinvertebrates were continuously available for shorebirds. The lack of a daily vertical movement of prey could be related to the small size of the macroinvertebrates at Lake MacLeod which limits their burrowing capacity (Esselink and Zwarts 1989). The apparent shallow depth of the reduced sediment layer (where anoxic conditions begin) can also prevent invertebrates from moving deeper into the sediment (Nilsson and Rosenberg 2000).



**Figure 14: Composition of prey items which different species were observed feeding on in Godwit Beach.**

Amphipods were the main prey for shorebirds at Lake MacLeod, except for both species of Knots, for whom bivalves, polychaetes and seagrass were the main food items (Figure 14). Amphipods were selectively eaten by

the majority of shorebird species despite the presence of other highly available prey such as polychaetes. There are 2 complementary explanations for this finding. The available polychaetes at Lake MacLeod were relatively small and offered on average a smaller amount of biomass per unit of handling time than amphipods. Additionally, recent studies have shown that Semipalmated Sandpipers (*Calidris pusilla*) target the amphipod *Corophium vulgatum* which is rich in highly unsaturated fatty acids (HUFAs; Maillet and Weber 2006). HUFAs can enhance shorebirds aerobic performance and thereby help shorebird migrations (Maillet and Weber 2006, 2007). Bar-tailed Godwits and Great Knots were the only shorebirds to not favour amphipods in our study, probably because in the case of the godwits their large beaks made small amphipods difficult to handle (Zwarts and Wanink 1993) and Great Knots are known to be a highly specialized molluscivorous species (Tulp and de Goeij 1994).

Small to medium-size shorebirds (Stints and Sandpipers) were particularly limited in their habitat use by water depth, always feeding in shallow waters from 0 to 3 cm. Areas that had a high abundance of macroinvertebrates but were not in shallow water were not used. At Godwit Beach Flats, strong northerly winds pushed the water out of the mudflats and through Linda's Creek. Conversely, strong southerly winds pushed the water into the flats from the creek. This wind-driven movement of water determined the availability of foraging habitat for shorebirds in that part of the Northern Ponds. This sort of wind-driven system has been described previously for the Black-fronted Plover (*Elseyornis melanops*) feeding in saline lagoons in Australia (Taylor 2004). Since Lake MacLeod is a complex system with extensive, patchily distributed mudflats, it is possible that strong winds blowing from a particular direction makes some areas unavailable for shorebirds while making other areas suitable by exposing alternative feeding habitats. This process is repeated in other parts of the lake with every change of wind direction. This progression of sequential availability of different foraging habitats has been previously identified as an important aspect of managing habitats in saltworks (Estrella and Masero 2010).

Feeding location and period (post or pre-migration) had a significant effect on feeding and intake rates of shorebirds at all operations. In general, birds achieved higher feeding and intake rates at sites with high prey abundance, like Whistler Pond at Lake MacLeod. When prey availability decreased due to predation or seasonally related changes in abundance, the feeding rates and intake rates also decreased, as was recorded at Godwit Beach Flats, Lake MacLeod, from post to pre-migration. Long-distance migratory shorebird species have high energy requirements both post and pre-migration, in order to recover from the demanding migration or to build up the reserves that will allow them to migrate (Velasquez and Hockey 1992). To achieve this energy gain, birds tend to increase their intake rates (Zwarts et al. 1990, Zharikov and Skilleter 2002) or the time they spend foraging (Zwarts et al. 1990, Lourenço et al. 2008, Santiago-Quesada et al. 2014). Of the migratory species studied, most of them, except the Bar-tailed Godwit, had lower or similar intake rates pre-migration than post-migration, suggesting that the birds would need to feed for longer. In most cases, migratory shorebirds had higher intake rates than resident shorebirds both post and pre-migration. This difference could be related to the higher energy requirements for migration, something that is not a concern for resident species.



**Table 14: Summary of daily energy intake rates for different species at Godwit beach post-migration and pre-migration. \* indicate non-resident species that migrate to the northern hemisphere. All values are mean  $\pm$  SE.**

Migratory period	Species	kJ.min <sup>-1</sup>	Average hours to meet DEE
Post-migration	Red-necked avocet	0.72 $\pm$ 0.02	13.5 $\pm$ 0.1
	Curlew Sandpiper*	0.33 $\pm$ 0.01	7.4 $\pm$ 0.1
	Red-necked Stint*	0.30 $\pm$ 0.01	6.7 $\pm$ 0.1
	Sharp-tailed Sandpiper*	0.24 $\pm$ 0.01	14.9 $\pm$ 0.2
Pre-migration	Banded Stilt	0.23 $\pm$ 0.01	29.1 $\pm$ 0.1
	Curlew Sandpiper*	0.15 $\pm$ 0.01	23.9 $\pm$ 0.1
	Red Knot*	0.53 $\pm$ 0.01	10.0 $\pm$ 0.1
	Red-necked Stint*	0.09 $\pm$ 0.01	28.4 $\pm$ 0.1

Several species of shorebirds were observed feeding at night, including Red-necked Stints and Red-capped Plovers. Several theories have been hypothesised to explain nocturnal foraging in shorebirds. The supplementary hypothesis (McNeil et al. 1992) states that when birds are not able to achieve their energetic requirements during daylight, they feed at night (Lourenço et al. 2008, Santiago-Quesada et al. 2014). During the study period, there were nearly 13 hours of daylight, and the number of feeding hours that plovers and stints required to achieve their energy requirements was 12 and 11 hours, respectively. Consequently, birds should not need to feed at night. Alternatively, the preference hypothesis (McNeil et al. 1992) proposes that shorebirds prefer to feed at night because it is more profitable due to higher prey availability (Pienkowski 1983, Mouritsen 1994, Gillings and Sutherland 2007), activity (Mouritsen 1994, Kuwae 2007) or decreased predation/disturbance pressure at night (Sitters et al. 2001).

Plovers' foraging speed (steps/min) decreased from day to night, while their prey capture attempts per minute increased. A decrease in night foraging speeds has been observed in several plover species (Turpie and Hockey 1993, Lourenço et al. 2008) and has been related to a lower capacity for visual prey detection at night (Turpie and Hockey 1993). However, higher availability and activity of the one Corophiidae amphipod species (*Corophium vulgator*) recorded on the intertidal sediment surface, has been observed at night (Mouritsen 1994) and this could explain the increase in Red-capped Plover feeding attempts without an increase in the foraging area. It is well known that birds tend to move slower on highly profitable patches (Nolet and Mooij 2002, Lourenço et al. 2008). In addition, plovers are well-adapted to nocturnal visual searching for prey because of their large eyes and retinal visual receptors (Rojas de Azuaje et al. 1993, Rojas de Azuaje et al. 1999, Thomas et al. 2006).

Contrary to other studies on sandpiper nocturnal foraging behaviour, Red-necked Stints continued feeding visually at night, increasing their feeding speed while marginally lowering their capture attempts. A study of Dunlins (*Calidris alpina*) tactile feeding at night on highly available surface *Corophium* also recorded a higher foraging speed than that in daylight, but this was accompanied by a higher number of capture attempts (Mouritsen 1994). Assuming that Whistler Pond also had a high availability of amphipods on the sediment

surface at night, it is reasonable to suggest that the stints kept trying to detect their prey visually. However, with a lower night-vision capacity than the plovers (Thomas et al. 2006), they likely increased their foraging speed while maintaining their prey capture attempts at similar rates to those recorded in daylight. Our results therefore appear to support the preference hypothesis, at least for Red-capped Plovers, which reduced their feeding area at night but increased their number of feeding attempts. However it remains unclear why Red-necked Stints feed at night.

Across all three DSL salt production areas, Whistler Pond was the best feeding ground for shorebirds, with all the species able to meet their theoretical energy requirements. The importance of this site as a feeding ground for shorebirds, especially small to medium-size shorebirds, appears to be related to the abundance of amphipods, especially the family Corophiidae, and bivalves. However, the small area of feeding habitat prevents large flocks from forming there. Godwit Beach Flats was also an exceptional feeding ground for small shorebirds post-migration, although its quality as a feeding ground decreased towards the pre-nuptial migration due to a decrease in prey availability. Although monthly data on prey availability were not collected, it is possible that the decrease in prey abundance at Godwit Beach Flats was related to prey depletion by shorebirds during the non-breeding period. Previous surveys and the current study have found high numbers of shorebirds feeding on Godwit Beach Flats (Bertzeletos et al. 2012), which can significantly impact prey abundance (Székely and Bamberger 1992, Zharikov and Skilleter 2002, Sánchez et al. 2006).

The East Asian–Australasian flyway has, globally, the highest number of shorebirds (Milton 2003), including more globally threatened or near-threatened species than any other. The region of the flyway also contains over 45% of the world's human population and consequently extensive areas of intertidal flats have been lost to urban, industrial, and agricultural land reclamations (Yang et al. 2011, Murray et al. 2014). At the same time, serious population declines of migratory shorebird species that use sites in Southeast Asia as a stopover have been observed (Amano et al. 2010, Wilson et al. 2011, Szabo et al. 2012). In fact, of the 7 species of migratory shorebirds included here, the Curlew Sandpiper is listed as critically endangered, the Bar-tailed Godwit, Red Knot and Great Knot are listed as vulnerable and the Great Knot is listed as vulnerable globally. This study reveals the remarkable value that Lake MacLeod has as a feeding ground for non-breeding migratory shorebirds. Although the study focused on two particular sites, it is likely that other similar or better feeding grounds may occur at Lake MacLeod, due to the complex heterogeneity of feeding habitat and extensive mudflats, especially on the south-eastern side of Cygnet Pond. This valuable area supports high numbers of shorebirds (Davis and Smith 2001, Bertzeletos et al. 2012) and is also an important non-breeding foraging site for migratory and non-migratory species, as it is strategically situated between the southern part of the continent and the northwest and its future protection is of high importance for shorebirds.

# ROLE OF HYDROLOGY AND ENVIRONMENTAL CONDITIONS ON MANGROVE MORPHOLOGY AND PHYSIOLOGY

Source Document:

Dunham, N. R. 2014. *Influence of hydrological and environmental conditions on mangrove vegetation at coastal and inland semi-arid areas of the Gascoyne region*. Edith Cowan University.

## Introduction

Mangroves are marine plants with a growth form that ranges from trees to shrubs and are typically found on the fringes of the ocean (Nybakken and Bertness 2005). A mangrove forest usually consists of single to multi-species communities and they provide important ecosystem functions and services, including erosion buffering (Lovelock et al. 2004, Duke and Larkum 2008), filtering nutrients and pollution (Connolly and Lee 2007) and provide spawning and feeding habitat for a variety of organisms (Saenger 1982, Boorman 1999, Johnstone et al. 2000). Mangrove dominated habitats usually establish in tropical and sub-tropical locations, but can persist in the semi-arid conditions found along the Gascoyne Coast.

The interface of marine and terrestrial environments is a challenging environment for most plant species, due to tidal inundation and exposure to variable salinities. Mangroves are, however, well adapted to these environments (Hogarth 1999) as their roots allow for gas exchange to continue while being inundated or subject to anaerobic conditions (Nybakken and Bertness 2005). Salt tolerance strategies are key to their survival and the plants use mechanisms such as salt secretion, exclusion and tolerance of their internal fluids to manage the fluctuation external conditions (Hogarth 1999).

Existing theories highlight that when faced with elevated environmental stress, physical components of the environment, rather than biological interactions, are important in structuring the plant communities (Grime 1977, Menge and Sutherland 1987). The key environmental factors that influence mangrove presence are salinity, temperature, soil type and freshwater supply (Connolly and Lee 2007). Within specific sites, and at local scales, topography and ocean interactions are important factors (Matthijs et al. 1999). Soil salinity appears to be important as it has been shown to reduce mangrove plant height, growth and productivity (Dawes et al. 1999, Ajmal Khan and Aziz 2001, Ellison and Simmonds 2003, Connolly and Lee 2007). Reduced or absent tidal flushing lowers nutrient availability and has been observed to cause mangroves to be stunted in size (McKee et al. 2002, Lovelock et al. 2004).

Inland mangrove stands are rare, with only seven known inland mangrove systems known globally (Ellison 1997). These systems have all had a historical connection to the ocean (Ellison and Simmonds 2003), and have a restricted hydrological link to the marine environment. Because of these marine connections, these habitats are able to maintain similar hydrological conditions as those found within coast mangrove habitats (Nybakken and Bertness 2005). Some examples of inland mangrove systems include geological embayment relics in Bermuda

(Thomas et al. 1992), Bahamas (Lugo 1981) 80 Mile Beach at Western Australia (Beard 1967, Storey et al. 2011) and Lake MacLeod (Ellison 1997). All these systems are either located in tropical or subtropical climates with the exception of Lake MacLeod, which endures semi-arid condition (Ellison 1997). In contrast to coastal and riverine mangrove communities, inland mangrove systems are not exposed to tidal fluctuations, experience greater evapotranspiration stress and have high variability in salinity. These factors are likely to result in restricted habitat distributions and cause greater physiological stress to the mangroves, as the frequency and magnitude of either seawater or river water flushing is reduced (Ellison and Simmonds 2003, Naidoo 2010).

Mangroves possess morphological, anatomical, physiological and reproductive features which allow for survival under the high and variable salinity levels (Stewart and Popp 1987). Morphological features of mangroves that aid survival in the intertidal zone generally function to minimise water loss and improve water-use efficiency (WUE; Naidoo 2010). Aerial root morphological adaptations such as pneumatophores, allows for gas exchange to occur in anoxic sediments (Hogarth 1999) and to cope well with temporary inundation, although prolonged inundation may cause mortality (Ellison 2001). In highly saline conditions, leaves on stressed plants have a reduced specific leaf area and thicker cuticles and specific weight (Lugo 1981, Naidoo 2010, Naidoo et al. 2011). Furthermore, regulation of ionic concentrations of cell fluids occurs as salt is expelled via salt glands on the leaves of *Avicenia* species.

Water-use efficiency has been shown to increase at high salinities while photosynthesis decreases (Medina and Francisco 1997). Photosynthesis is regulated depending on environmental conditions. Daily increases in light intensity reduce the efficiency of the plant's photosystems over and above any changes to water availability or salinity levels (Sobrado 1999). However, down regulation of the photosystems occurs when trees are exposed to hypersaline conditions and other environmental stresses (Larcher et al. 1990). Therefore, habitats with higher salinity are likely to have smaller plants, which use less water physiologically to maintain productivity.

Temperature, salinity and aridity are key environmental factors influencing growth and survival of individual mangrove species (Clough et al. 1997). At a regional scale, temperature and aridity are the prominent factors influencing mangroves, whereas at the local scale, salinity is the most influential factor. Tropical and subtropical mangrove trees are larger, have higher production rates and larger above-ground biomass than the same species in temperate or semi-arid climates (Naidoo 2010). The local hydrological regime and salinity levels are the principal abiotic factor affecting mangrove tree growth and productivity (Wei et al. 2008, Naidoo 2010). It is common to see reductions in above-ground biomass with increased elevation and distance from water bodies. There is a distinct gradient in sediment salinities that can become hypersaline due to increased variability and harsher environmental conditions (Dawes et al. 1999).

A unique opportunity exists to assess the largest inland mangrove system in the southern hemisphere at Lake MacLeod, and to compare it to nearby coastal and riverine mangrove systems growing in a semi-arid climate. Coastal mangroves stands within a semi-arid climate are rare and research is needed on relating the environmental and mangrove vegetation characteristics of this habitat to coastal and riverine habitats. This study investigated the distribution and structure of *A. marina* in relation to hydrological and sediment conditions

existing at the semi-arid, inland mangrove system at Lake MacLeod. In addition, the productivity, water-use efficiency and morphological traits of the mangroves growing in three different hydrological regimes (inland, coastal and riverine) of the Gascoyne were compared.

## **Methodology**

At Lake MacLeod, Goat Bay, Whistler's Pond, Pete's Pond and Neil's Pond were chosen study sites. Together, these sites span the seepage face from the north (Pete's Pond) to the south (Goat Bay) in Cygnet Pond. Coastal sites were selected at the nearby township of Carnarvon, where there are fringing coastal mangrove stands. Two other sites were selected which are associated with the Gascoyne River mouth and represent the riverine habitat.

### **Part 1: Identification of environmental parameters**

The aim of this section is to identify the environmental parameters associated with the variability in the distribution and structure of the mangroves-dominated vegetation at Lake MacLeod. To address this objective, a survey of mangrove dominated vegetation and the associated variables was undertaken in February and July 2012 at Goat Bay, Whistler's Pond, Pete's Pond and Neil's Pond. These four sites represent a range of hydrological conditions that are found at Lake MacLeod. Hydrological conditions were measured by assessing water quality and quantity using a gradsect design (Austin and Heyligers 1991). This design determines sampling locations based on changes in elevation which influences the hydrological conditions experienced by the vegetation. Up to 7, 10x10 m plots were established along each gradsect, and were at least 100 m long and extended up slope to 20 m beyond the last mangrove tree.

Within each plot, samphire cover (percentage coverage), mangrove density (number per plot), canopy cover, basal area (trunk diameter at 30 cm above the ground), maximum and average tree height, number of dead branches, pneumatophore density and height, and leaf litter cover. Sediment samples were taken from three locations in each plot in order to measure sediment moisture content, electrical conductivity, pH, clay content and organic matter.

### **Part 2: Ecophysiological condition and morphological features**

The four sites at Lake MacLeod, which constituted the inland region, and four sites along the Gascoyne coast and river, which constituted the coastal region, were used in this part of the study to 1) compare productivity, water-use efficiency, morphological features and physiological attributes of inland and coastal mangroves and 2) examine the relationship between environmental and hydrological conditions and ecophysiological features of the mangroves. The gradsect system, as described in part 1, was used to select four trees per plot for ecophysiological traits and environmental conditions in July 2012 at both inland and coastal sites.

Four mature plants were tagged in each plot in February 2012, and each plant was revisited in July 2012 to assess productivity and sediment samples collected for soil moisture content, sediment salinity (electrical conductivity),

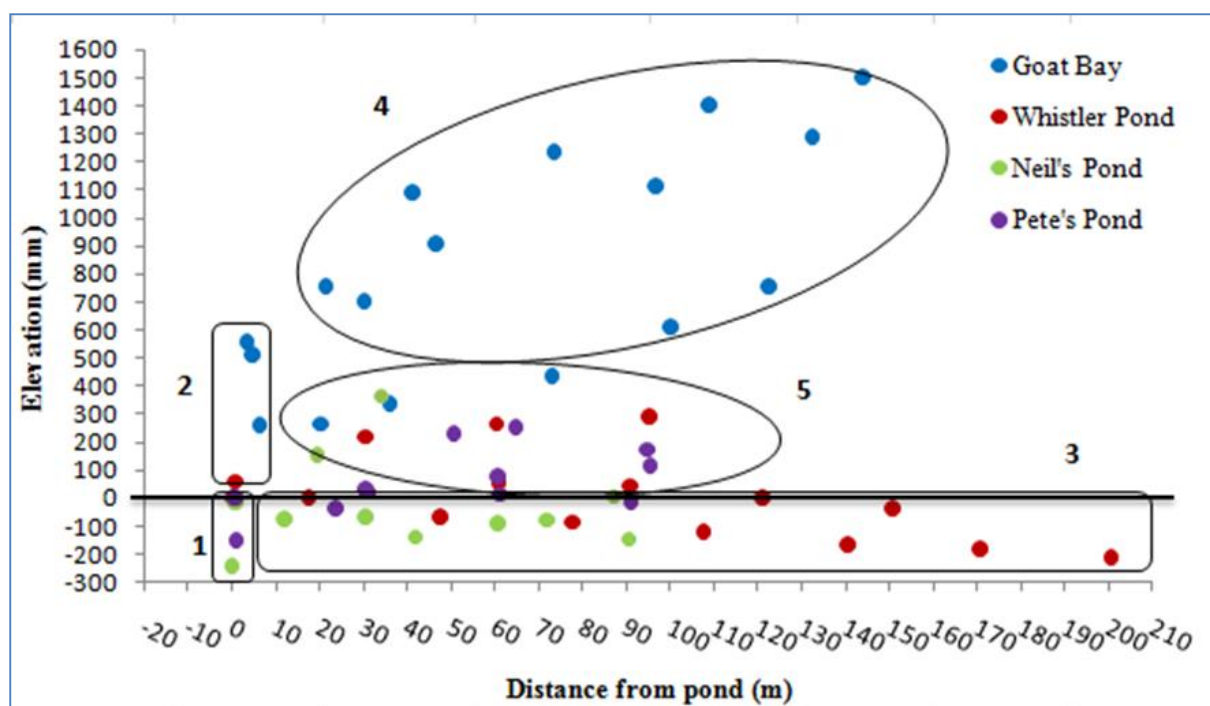
pH and organic matter. Eight ecophysiological and morphological measures were taken for each tree. Photosynthesis rate (relative electron transfer rate) and photosynthetic health (potential quantum yields of photosystem II) were measured using fluorometry. Productivity was estimated by accounting for new growth of four north facing apical shoots of each tree during the 5 month period. Wire was used to mark the apical node in February 2012, and new growth beyond this point after 5 months was removed and dry weight of biomass per shoot per day was calculated. Above ground biomass was used to estimate long-term tree productivity following (Clough et al. 1997). Tree height was recorded and three leaf anatomical features were measured using collected foliage used for measuring productivity. These variables included leaf lamina thickness, leaf area and specific leaf weight.

Carbon isotope readings were used as a proxy for water-use efficiency as leaf carbon isotopes ( $^{13}\text{C}$ ) provides a measure of the volume of water used to fix a unit of carbon (Farquhar 1989). Changes in the ratio of  $^{12}\text{C}$  and  $^{13}\text{C}$  can be related to the environmental conditions, such as water quality and salinity, in which the plants are experiencing (Medina and Francisco 1997, Macfarlane et al. 1999). Leaf ion analysis was also performed to measure chloride concentration.

## **Results and discussion - Identification of Environmental Parameters**

Significant association between hydrological and sediment characteristics were revealed for the vegetation at Lake MacLeod. The inland mangrove system experience highly variable environmental conditions, both spatially and temporally. This variability contributes to the variability observed in the hydrological conditions, sediment properties and vegetation characteristics. The dominant factors associated with mangrove presence and structures were relative elevation and distance from a permanent water source (Figure 15 & Figure 16). The interaction between these factors represents subtle hydrological and sediment difference that influence mangrove vegetation.

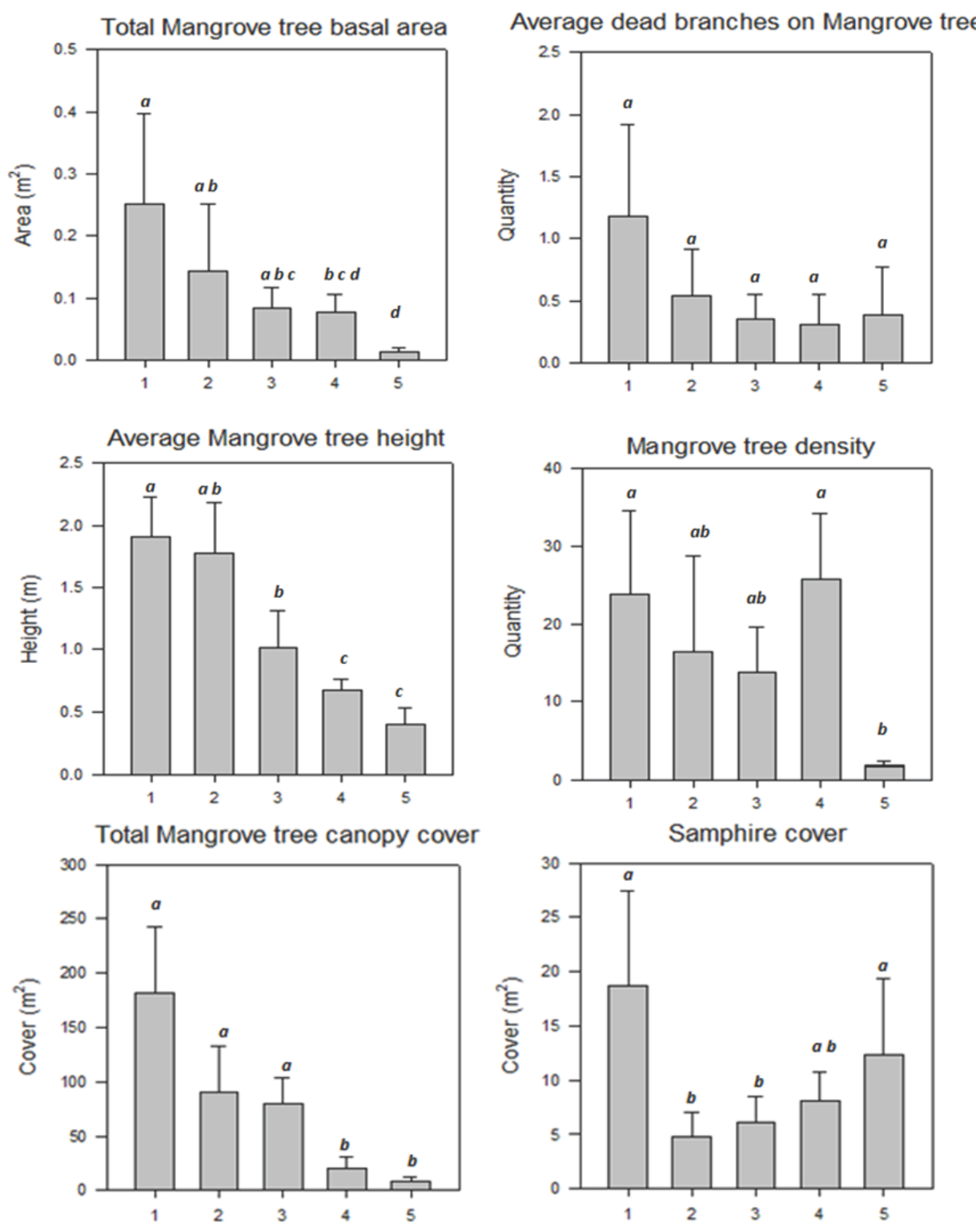
Environmental variables which were important in influencing the vegetation characteristics were sediment properties soil moisture content, organic content and electrical conductivity, as well as the distance from nearest pond shore. Distance from a permanent water source and elevation are two well documented factors which contribute to the hydrological condition of mangrove dominated habitats (Paliyavuth et al. 2004, Naidoo 2010). Generally, relative elevation increased slightly with increasing distance from the ponds, although there was not a consistent pattern.



**Figure 15: Plot showing the characteristics of hydrological categories identified at Lake MacLeod based on distance from pond edge and elevation.**

Usually, hydrological conditions within mangrove stands vary along an environmental gradient, where changes in vegetation structure depends primarily on elevation. In coastal habitats, soil moisture content and electrical conductivity both decrease as elevation from the water body increases. At Lake MacLeod, this study revealed that soil moisture content was highest at low elevations, despite proximity to the permanent water bodies. At low elevations, high soil moisture content, and occasionally surface water, can persist due to the existence of smaller vents and seepages distributed throughout the lakebed. Similar to coastal habitats, electrical conductivity was greatest near the ponds, where consistent marine water discharge and evapo-concentration occurs and was lowest at distant, low elevation sites where influences from seepage, and episodic freshwater inflow may reduce the concentration of the salts. Organic matter in the sediment was also greatest at high elevation, and was not influenced with distance from pond. This relationship may reflect the presence of vegetation and litter accumulation and entrapment which raise sediment elevation over time (Saintilan and Williams 1999).

Elevation and distance from permanent water supplies are known to reduce mangrove biomass in other inland (Lugo 1981, Logan 1987, Vilarrubia 2000) and coastal mangrove systems (Dawes et al. 1999, Paliyavuth et al. 2004, Lara and Cohen 2006). At Lake MacLeod, mangrove cover, basal area and height decreased as distance from the pond increased and the influence of drying and episodic freshwater inputs increased. In some habitats, such as Goat Bay, mangrove density remained high although cover, basal area and height decreased with distance from pond. This habitat may be representative of other inland mangrove stands, such as Inagua (Lugo 1981) and the Mandora Marshes (Beard 1967, Storey et al. 2011) and Barbuda (Stoddart et al. 1973).



**Figure 16: Mangrove and samphire vegetation characteristics for each hydrological categories.**

Salinity gradients are a key environmental determinant of mangrove habitat (Menge and Sutherland 1987, Hogarth 1999, Matthijs et al. 1999, Ajmal Khan and Aziz 2001, Lara and Cohen 2006, Feller et al. 2010). At Lake MacLeod, biomass attributes did increase as sediment electrical conductivity increased and both were associated with proximity to pond edge. Interestingly, there was an association between salinity and the number of dead branches on a tree, with canopy dieback greatest at plots close to the pond edge where salinity was



greatest. These sites also had the lowest elevations, and are therefore subject to the greatest frequency and duration of inundation. Because of significant freshwater inputs and prolonged flooding at Lake MacLeod (Ellison 2001), it is likely that the dieback is a result of prolonged submergence of pneumatophores (Ellison 2009), as well as elevated salinity.

Consistency of marine water supply appears to be a key environmental variable responsible for the presence and persistence of mangrove vegetation at Lake MacLeod. Ellison and Simmonds (2003) suggested that frequency, quantity and quality of water supply influence the Lake MacLeod vegetation condition. The environmental gradients identified in this study support this suggestion and represent the interaction between discharge of marine water, evapo-concentration and episodic catchment freshwater inflows which suppress marine inflows. The absence of tidal fluctuation limits the extent of hydrological and sediment characteristics that are optimal for mangrove growth, as represented by structural attributes. Consistency of marine discharge therefore appears to be critical for maintaining a narrow hydrological envelope that supports optimal mangrove productivity in this inland mangrove system.



Plate: Mangroves growing in a spillsheet west of Dave's Vent. Photo: P.Horwitz

## Results and Discussion - Ecophysiological Condition and Morphological Features

Previous research suggests that as sediment salinity increases, mangrove trees have higher water-use efficiency and are less productive (Medina and Francisco 1997, Naidoo 2010). However, these studies were limited in the range of salinities investigated and by focusing on an environment at the high end of salt tolerance for *A. marina*. The inland locations within the Lake MacLeod basin were more saline than the nearby coastal environments, and also had higher soil moisture content.

Long-term productivity was significantly lower at the inland sites, especially in plots distant from the ponds and at high elevations, where, although soil salinity was variable, the moisture content was low. Similarly, trees at the coastal habitats growing at close proximity to the water edge were the most productive, despite the salinity ranging from fresh to seawater. These results suggest that water supply, irrespective of salinity is the most important driver of long-term productivity, and that *A. marina* is tolerant of a broad range of salinities.

Water use efficiency and photosynthetic rates were not consistently different between coastal and inland sites (Figure 17). Unexpectedly, water-use efficiency was low at two coastal habitats fringing the water edge in the riverine habitat and greatest for coastal mangroves on the landward edge. Similarly, photosynthetic rates were lowest at the coastal riverine habitats and these patterns could not be related to changes in soil salinity or soil moisture content. For the inland mangroves, plots distant to the ponds edge, but at similar elevation to the water level had the lowest quantum yield and lowest electron transfer rate. These plants also had the lowest above ground biomass but were not found in the driest nor most saline sediments. It remains unclear which environmental conditions are driving the low photosynthetic capacity and shoot production for these inland areas.

Vegetation traits, such as leaf size and tree height, were consistently greater at coastal sites compared to the inland sites. Specific leaf weight and leaf chloride concentration were greater at the inland sites. With the exception of leaf chloride concentration, all the vegetation traits for inland mangroves were similar to those growing on the landward edge of the coastal sites. It is likely that a lack of water quantity rather than quality, because of the reduced tidal flushing of landward trees, is responsible for the similarity of vegetation traits between these two communities.

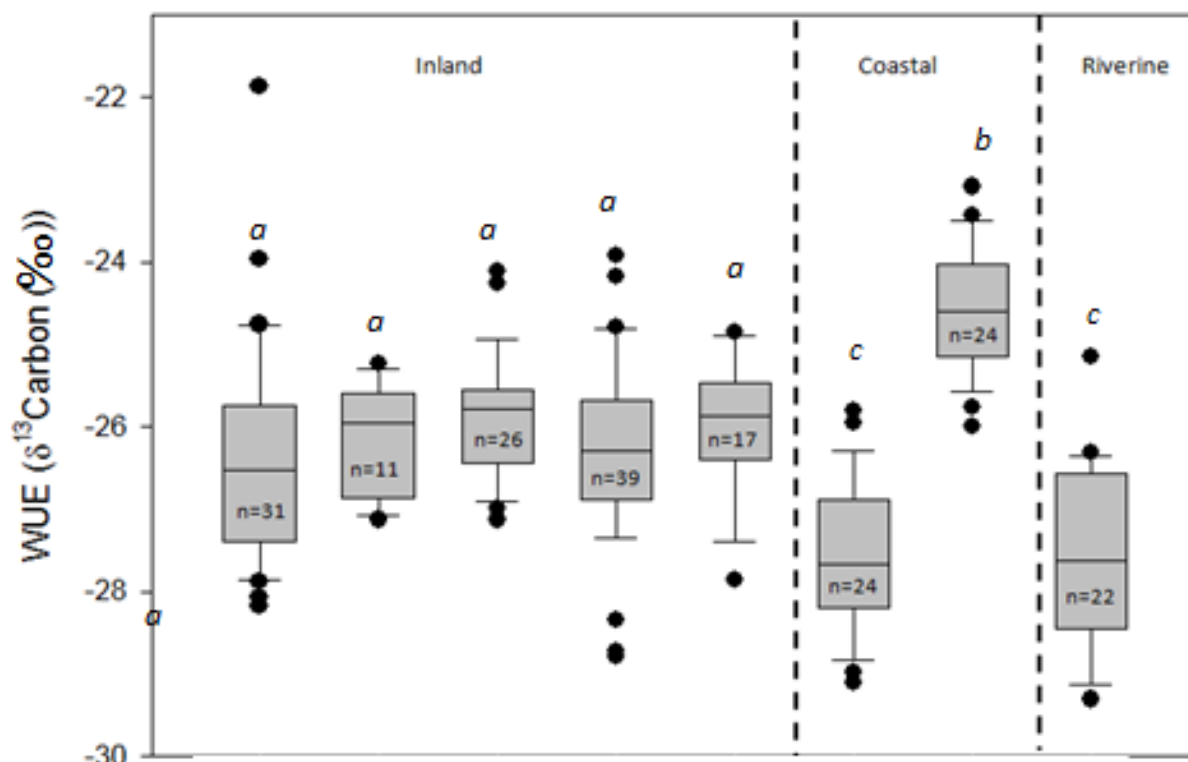


Figure 17: Water-use efficiency values for plants from each hydrological category at Lake MacLeod compared to plants from coastal and riverine sites. Different letters represent significantly different means.

## Conclusion

The relationship between environmental conditions in the sediment and the presence and structure of the vegetation dominated by the mangrove *Avicenia marina* at Lake MacLeod was examined in this study. Sediment salinity at Lake MacLeod is high, with a maximum of 105 mS/cm, and possibly one of the highest salinities recorded for sediments where *A. marina* stands are found worldwide (Beard 1967, Lugo 1981, Van Steenis 1984). Sediment moisture was also higher than those of coastal sites. Most of the mangroves were found growing in proximity to the edge of the ponds, and extend up to 20 m away from the ponds. The mangroves were also commonly found growing with samphire saltmarsh vegetation. *Avicenia marina* is found inhabiting a variety of environmental conditions globally, and has been recorded in many different climatic zones (Sobrado 1999). This study is unique in that it examines the relationship between vegetation structure and environmental conditions at an extreme and unique environment of low rainfall, high evaporation and constant marine water supply.

The environment at Lake MacLeod is complex, and five hydrological categories which relate distance from the pond and elevation relative to pond water level were identified. Typically, typically, high salinity and moisture were found close to the ponds at high and low elevations, and moisture content decreased further from the pond. However, points of underground seepage, identified by low elevations, were identified because moisture content was high, and salinity was slightly higher than sea water. These smaller, and probably permanent seepage points, are responsible for the complexity in sediment conditions found at Lake MacLeod. Nonetheless,

salinity and moisture are strongly associated with the presence of mangroves at Lake MacLeod, and the plants seem able to persist because of a constant water supply, irrespective of the salinity conditions.

The trees growing at Lake MacLeod had intermediate levels of water-use efficiency when compared to the coastal sites. Even though the main water supply at Lake MacLeod is saline, it is the constant supply of water that is influencing the water-use efficiency of these inland trees. Trees with highest productivity levels at Lake MacLeod were those growing close to permanent bodies of water, such as the ponds. These results suggest that distance from a permanent water source, and not salinity, is the key factor driving short term and long-term production of mangroves at Lake MacLeod and coastal locations. There are a number of processes which influence water supply to plants in coastal, riverine and inland habitats. Coastal habitats, and to a lesser degree the riverine habitat, are delivered regular inputs of seawater via tidal movement, whilst the inland habitat at Lake MacLeod is delivered via seepage. However, the key difference between inland and coastal systems is the constant supply of seawater at the inland system, compared to the irregular but greater supply of freshwater in coastal habitats.



Plate: Flowering mangrove, December 2013, Annie's Vent. Photo: P.Horwitz

# RECENT SEDIMENTS REVEAL PERIODS OF MARINE AND TERRESTRIAL INFLUENCE

Source document:

Oscar Serrano-Gras, Christopher Kavazos and Pierre Horwitz, Unpublished material

## Introduction

The MacLeod basin is endorheic, and therefore acts as a sink for materials deposited from flooding in the inflowing catchments. European settlement in the area could lead to a disruption of the catchments through agriculture and deforestation as reported elsewhere and in Australia (Lotze et al. 2006). Reconstructing nutrient, metal contamination, sediment accumulation rates, grain size, carbon stocks and isotopic signatures using seagrass sedimentary archives in coastal environments has provided new insights into the ecological dynamics and processes over millennial scales in coastal ecosystem since European arrival in Australia (Serrano et al. 2016a, Serrano et al. 2016b).

During the late Holocene the Lake MacLeod basin experienced changes, primarily because of rising sea levels. During the late Holocene (6500 years BP), sea level transgression was at its highest, and the Lake MacLeod basin was inundated. Connection to the Indian Ocean was maintained through the Texada Sill. This sill was at least 5m deep, and encroachment of surrounding dunes eventually led to the sill being closed, and loss of the surface ocean-basin connection at about 5800 year BP (Logan 1987). By 5100 years BP, the evaporate system which dominates today was established. Sedimentation and accretion has acted to raise the basin floor, eventually resulting in the environment present today. During this period, seepage has been continual but has slowly declined due to regressing sea levels, which has resulted in the extinction of some seepage faces and the 'choking' of vents.

By analysing seagrass sedimentary archives collected at two sites in Cygnet Pond, it is hoped that longer term fluctuations in the environment can be detected. These results will enhance our understanding of the systems evolution during the mid-late Holocene, as well as provide a tool to better understand natural and anthropologically induced changes in Lake MacLeod.

## Methods

Cygnet Pond, being one of the largest and deepest water bodies in the system, and the sediments in it is probably provides a better representation of the sequence of sedimentary depositional layering than other non-permanent parts of the basin. Therefore two sediment cores were sampled from dense *Ruppia* spp. meadows at 0.4 m water depth at Cygnet Pond in December 2013. Coring was carried out by manual percussion and rotation. The corers consisted of 2 m-long PVC pipes with an inner diameter of 60 mm. The bottom-ends of the corers were sharpened to penetrate and cut fibrous material and thus minimise compression of the sediment within the core. The maximum core penetration into the sediment was recorded by measuring the total length



of pipe used, whilst the sediment recovered was also recorded. Compression of loose sediments during coring is inevitable and was corrected by distributing the spatial discordance proportionally between the expected and observed sediment column layers (Glew et al. 2001). The cores were sealed at both ends, transported vertically and stored at 4 °C before processing. All results reported in this study refer to the decompressed (corrected) depths.



Plate: Coring, Cygnet Pond. Photo: P.Horwitz

The cores were cut longitudinally into half and sliced at regular intervals (i.e. 1 cm-thick slices). Each slice/sample was weighed before and after oven drying to constant weight at 70°C (DW), grounded in an agate mortar and subsequently sub-divided for analysis. The  $C_{org}$ , N, elemental and isotopic composition of the organic matter was measured in milled subsamples from every second slice. These sub-samples were acidified with 1 M HCl, centrifuged (3500 RPM; 5 minutes) and the supernatant with acid residues was removed using a pipette, then washed in deionized water, centrifuged again and the supernatant removed. The residual samples were re-dried (70°C) before carbon elemental and isotopic analyses using a Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at University California Davis Facilities. The relative contents of  $C_{org}$  were calculated for the bulk (pre-acidified) samples. Carbon and nitrogen isotope ratios are expressed as  $\delta$  values in parts per thousand (‰) relative to VPDB (Vienna Pee Dee Belemnite).

Calcium carbonate ( $CaCO_3$ ) content was determined with a Calcimeter (Pressure Gauge Model 432 (Fann ®); ASTM D 4373-84 Standard) by reacting the  $CaCO_3$  present in the sample with 10% HCl in a sealed reaction cell.

The pressure build up due to the CO<sub>2</sub> was measured with a bourdon tube pressure gauge that was pre-calibrated with reagent grade CaCO<sub>3</sub>.

Concentrations of chemical elements (Al, Si, P, S, K, Ti, Fe, Cr, Mn, Co, Ni, Cu, Zn, As, Rb, Zr, Cd, Hg and Pb) were measured using inductively coupled plasma (ICP-MS) (Agilent, model 7500ce) at Universitat Autònoma de Barcelona facilities. 0.25 g of dried and homogenized bulk sample of every slice of the cores was digested at 240°C using a mixture of concentrated HNO<sub>3</sub>, HF and HCl. After digestion, the samples were diluted with HCl 1% prior to injection. Reagent blanks and standard reference material NIST 2702 (Inorganics in Marine Sediment) were run in parallel to the analyses of the samples to ensure the accuracy of digestion and analytical procedures. In addition, reference material and selected sediment samples were digested at 260°C to improve the accuracy of chemical element determinations in cases where recoveries were not satisfactory with the chosen methodology. The concentrations reported in this study are indicative of total chemical element concentrations.

Good analytical precision was obtained for chemical element concentrations with an average relative standard deviation (RSD%) of 2%; average recovery was 86%, being the majority of them above 90%. At 240°C, recoveries for Al, K and Rb were low (9%, 39% and 3%, respectively) while those at 260°C increased (15%, 102% and 89%). The accuracy of Si and Zr concentrations could not be tested since certified values were not available.

For sediment grain-size analysis, a Coulter LS230 laser-diffraction particle analyzer was used following digestion of bulk samples with 10% hydrogen peroxide. Sediments were classified as coarse sand (<1 mm and >0.5 mm) medium sand (<0.5 mm and >0.25 mm), fine sand (<0.25 mm and >0.125 mm), very fine sand (<0.125 mm and >0.063 mm), and mud (<0.063 mm).

The age of the soil along the cores was determined by means of radiocarbon. Five samples of bulk sediments radiocarbon dated at AMS Direct Laboratory after an acid-base-acid treatment, following standard procedures (ISO 17025 and ISO 9001). The raw radiocarbon dates reported by the Laboratory were calibrated (Marine13 curve; Reimer et al. 2013). The raw <sup>14</sup>C ages were used to produce an age-depth model using a Bayesian approach with the Bacon 2.2 and the Marine13 calibration curve (Blaauw and Christen 2011).

Principal component analysis (PCA) of chemical element concentrations (Al, Fe, Mn, Pb, Zn, Cr, Cd, Co, As, Cu, Ni and S) and organic matter content were carried out to resolve the structure of the variance and detect fundamental factors accounting for their distribution. All multivariate analyses were conducted using vegan software for R (Oksanen et al. 2013). The Sequential Regime Shift Detection software (significance level = 0.1; Rodinov 2004, Andersen et al. 2009) was used to detect discontinuities in the properties measured signaling at the presence of ecological thresholds and regime shifts over the period reconstructed.

## Results

The two sediment cores, CP6 and GB2, had similar patterns in shifts in sediment characteristics and elemental composition. Both cores show a region of high carbonate content which during contemporary times has decreased in concentration. The opposite trends were seen for fine sediment content. Organic matter and

elemental composition also tend to increase in both cores. The sediments beneath the *Ruppia* sp. meadows are mainly composed of biogenic and geogenic carbonates (~75%), with low levels of organic matter (<1%; Table 15 and

Dry bulk density values ranged from 0.2 to 1.5 g cm<sup>-3</sup>, while organic matter and carbonate contents ranged from 0.2 to 6.0% and 18 to 91%, respectively. Age-depth models using Bayesian models (Blaauw and Christen 2011) and <sup>14</sup>C ages suggest that the bottom of the cores were 6373 and 5457 years BP for CP6 and GB2, respectively. Accumulation rates averaged 0.014 cm year<sup>-1</sup> for both cores over the last 2700 years. From 2900 years BP to the end of each core, accumulation rates were relatively higher (0.025 and 0.022 cm year<sup>-1</sup> for cores CP6 and GB2, respectively).

The deposits, however, were not homogeneous in density, organic matter and carbonate contents, with high levels of variation with depth. Si was the major element in both cores, ranging in concentration from 500 to 57,000 mg/kg. Other elements, such as S and Fe had mean concentrations greater than 5,000 mg/kg in both cores. Variability in Al concentration during the last ~6000 years in Cygnet Pond ranged between 118 and 158% in the GB2 and CP6 cores, respectively. As showed the lowest levels of variation in each core. The concentration records of all the major elements follow similar patterns, rising in concentration at the top of the core (Figure 18 and Figure 19). As was an exception to this rule, and did not show the clear increase in concentration which the other elements showed.

The highest concentration of major elements were found in the top 5cm of the sediment corresponding to the last 15 and 760 years. Concentrations of the major elements remained consistently low in the cores for the period between ~1000 and 3500 years BP, with the exceptions for As, P and S. S displayed a near linear decrease in concentration with depth throughout the core during this period, while As had higher concentrations than present day levels. The bottom sections of the cores were highly variable in major element concentrations. Sediment density and carbonate content showed similar trends in both cores, with declines in both variables within the top 5cm. On the other hand, organic matter and nitrogen levels both increased during the same period. Both cores also displayed similar trends in recent increases in fine sediment (<125µm) content.



**Table 15: Summary statistics for the different components of the sediment cores.**

	Variable	Mean	S.E.M.	Minimum	Maximum	CV (%)
<b>Core CP6</b>	Density (g/cm <sup>3</sup> )	0.88	0.04	0.19	1.49	33
	Carbonate (%)	75.17	2.37	18.32	90.91	24
	Organic (%)	0.92	0.15	0.25	5.98	125
	Nitrogen (%)	0.11	0.02	0.03	0.69	129
	δ <sup>13</sup> C	-16.48	0.15	-19.74	-15.1	7
	δ <sup>15</sup> N	4.64	0.14	2.83	8.22	24
	Fine sediment (%)	38.32	3.4	5.7	94.3	67
<b>Core GB2</b>	Density (g/cm <sup>3</sup> )	1.02	0.05	0.4	1.53	31
	Carbonate (%)	75.53	1.81	47.36	89	14
	Organic (%)	0.97	0.14	0.24	5.33	88
	Nitrogen (%)	0.1	0.01	0.03	0.54	90
	δ <sup>13</sup> C	-16.99	0.31	-22.37	-10.88	11
	δ <sup>15</sup> N	4.11	0.09	2.82	5.56	13
	Fine sediment (%)	29.16	3.54	5.02	73.13	73

**Table 16: Summary statistics of element concentrations in the two cores. All units are mg/kg. Coefficient of variation (CV) expressed as %.**

Variable	Core CP6					Core GB2				
	Mean	S.E.M.	Min	Max	CV	Mean	S.E.M.	Min	Max	CV
<b>Al</b>	1082	226	49	11427	158	636	122	30	3427	118
<b>Si</b>	7951	1150	500	44548	109	15732	1991	2411	46901	78
<b>P</b>	120	15	46	600	95	147	22	51	602	90
<b>S</b>	5527	463	2804	22978	63	7735	419	4550	17788	33
<b>K</b>	2370	273	933	10099	87	3366	406	997	10217	74
<b>Ti</b>	445	57	152	2492	96	576	73	137	1984	79
<b>Fe</b>	5156	660	1788	33287	97	6400	614	2545	19985	59
<b>Cr</b>	8	1	3	43	75	8.9	0.7	4.3	23.4	51
<b>Mn</b>	97	10	32	402	78	127.8	16.6	26.3	415.6	80
<b>Co</b>	1.7	0.2	0.6	9.6	89	2.2	0.2	0.7	7.4	63
<b>Ni</b>	5.5	0.5	1.9	22.5	70	7	0.7	2.3	21.1	64
<b>Cu</b>	3.6	0.5	0.9	21.7	102	4.2	0.6	0.9	15.4	84
<b>As</b>	4.9	0.3	1	12.3	51	7.6	0.4	2.7	12.3	30
<b>Rb</b>	9.3	0.8	3.8	31.3	61	11.8	1.2	3.7	31	63
<b>Pb</b>	2.7	0.3	1.2	15.3	86	3.5	0.3	1.6	10	48

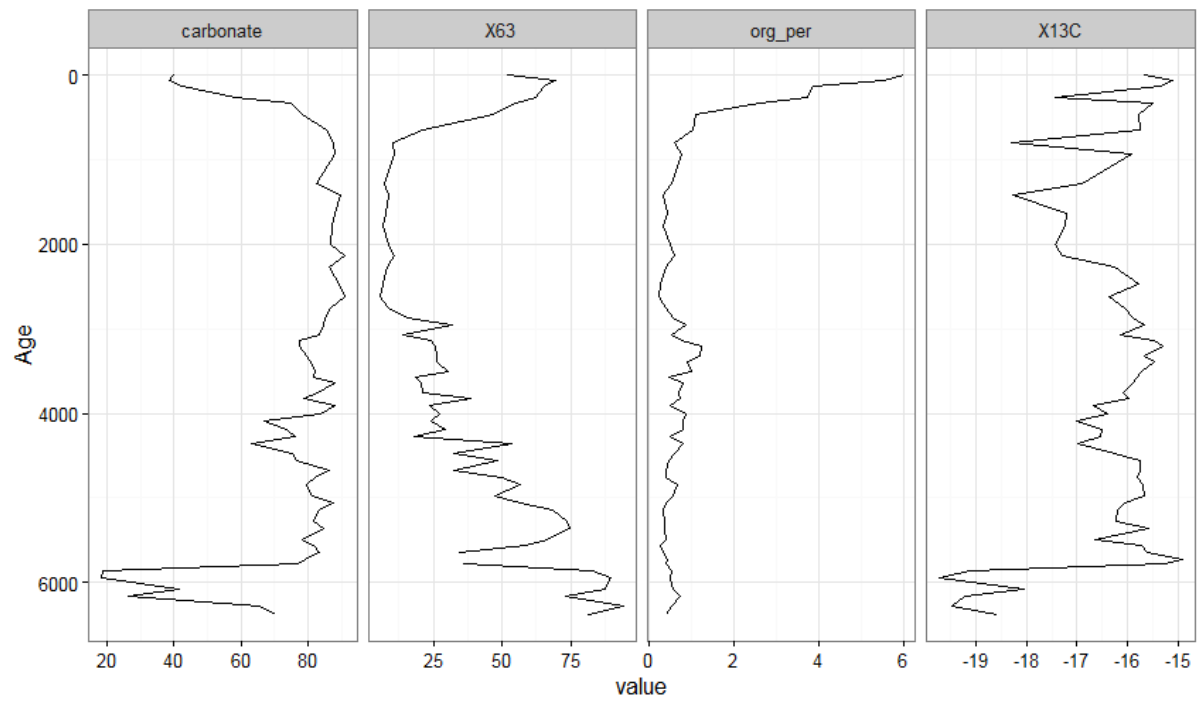


Figure 18: Archival history of carbonate content (%), fine sediments (%), organic matter (%) and  $\delta^{13}C$  for CP6 core.

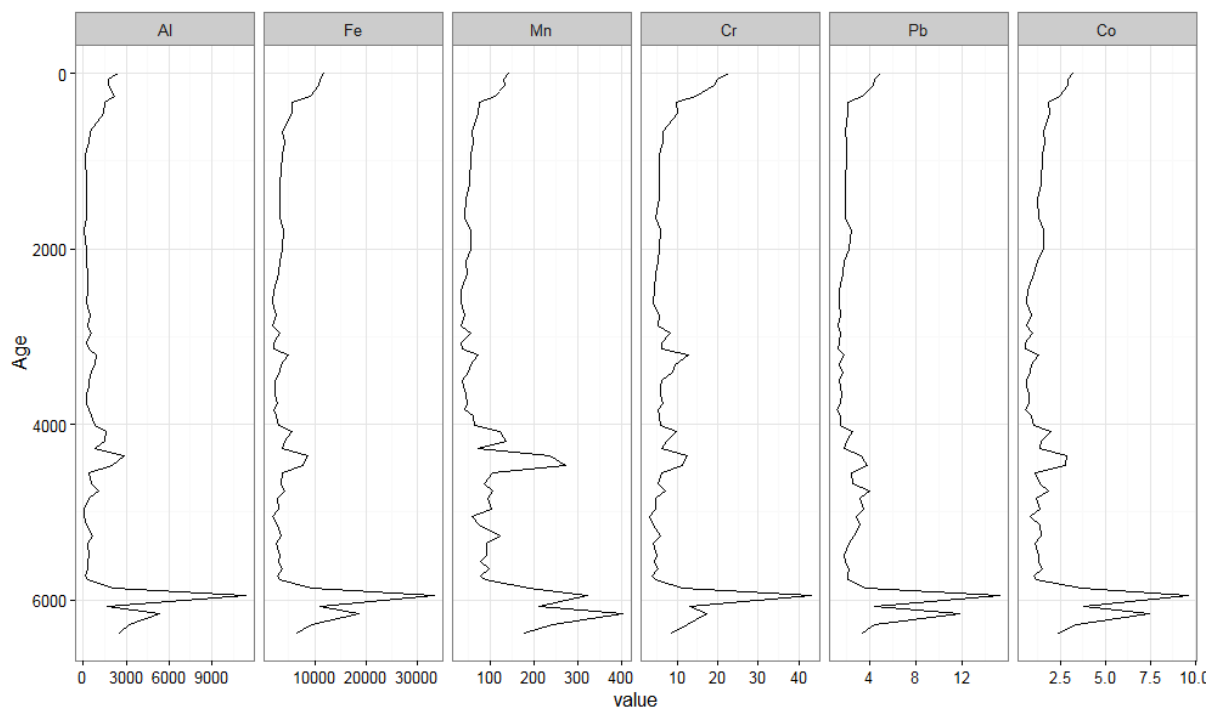


Figure 19: Archival history of selected elements in the CP6 core.

## Discussion

The analysis of the two seagrass cores analysed here provide a record of the concentration and fluxes of chemical elements and biogeochemical dynamics throughout the last ~6000 years in the Cygnet Pond. The records show similar trends, with an abrupt increase in elemental concentration and decrease in carbonate concentration since ~200-250 years BP. These contemporary changes may be indicative of anthropogenic disturbances after European settlement (Serrano et al. 2016a), and is consistent with the arrival of agriculture in the catchment areas. Such activities can cause degradation of land surfaces, deforestation and land clearance, which in turn lead to increases in erosion and run-off of fine sediments in rivers. The rapid decrease in carbonate content and the increase in fine sediments during this period are consistent with higher levels of terrestrial inputs. Nonetheless, regime shift analysis revealed four distinct shifts in the composition of the cores. The first period, only observed in CP6 represents the basin before the basin was disconnected from the Indian Ocean around 5800 years BP. The second and third periods both represent changes from 5800 years BP to approximately 1000 years BP, whilst the fourth period represents contemporary changes from 1000 years BP to present day.

Approximately 8000 years BP, surface connection between the MacLeod Basin and the Indian Ocean was established as rising sea levels inundated the area. A permanent connection with the ocean was maintained until 5800 years BP, when closure of the Texada Sill (where the surface connection was established) due to dune encroachment commenced. By 5100 years BP, complete closure of the sill had occurred and there was no longer any surface connection of the basin with the Indian Ocean, despite the rising sea levels. This period is clearly visible in the sedimentary history of CP6. During this period (before 5800 year BP), the sediments were characterised by low carbonate content and high fraction of fine sediments. The sediments were also rich in chemical elements, and  $\delta^{13}\text{C}$  levels were approximately -19, indicating marine processes were occurring. The maximum duration for the system to become an evaporite (and endorheic) environment was possibly 200 years, but it could have been much quicker (Logan 1987). The decrease in carbonate material in this core could be an indication of the system rapidly 'drying out' as it switched from a marine dominated system to one dominated by terrestrial processes.

From 5100 years BP to approximately 1500 years BP corresponds to the second and third periods determined using regime shift analysis. During these periods, discharge from the vents declined from about 1.3 billion  $\text{m}^3/\text{year}$  to 380 million  $\text{m}^3/\text{year}$ . This gradual decrease in seepage volume may be reflected by the gradual decrease in carbonate content and the retention of fine sediments. The decrease in carbonate material could be an indication of the system rapidly 'drying out' as it switched from a marine dominated system to one dominated by terrestrial processes. The terrestrial influence during this time was also detected by the shift in  $\delta^{13}\text{C}$  signature. Variation in sediment characteristics was found for the two cores during the second period, which may be a reflection of the cores being collected in different parts of the basin.

The fourth period determined using regime shift analysis, which covers the period from 650 and 1000 years BP to present for, CP6 and GB2, respectively, is characterised by further drops in discharged brine. Logan (1987) estimates that during the last 1500 years, free discharge of brine into the basin through the Cygnet seepage face

has declined from about 380 million m<sup>3</sup>/year to the present day levels of 270 million m<sup>3</sup>/year. This decline has partly be attributed to the reduced hydrostatic head because of regressing sea levels, but also because of the choking of the vents by sediments. However, the two cores analysed here were collected from seagrass meadows in Cygnet Pond which have likely been inundated since seepage commenced 5000 years BP. The gradual dominance of terrestrial processes which would be expected to occur as the basin becomes drier are therefore probably not as evident inside the ponds than they are elsewhere in the basin.

The analysis of these two cores provides a record of changes in element concentrations and sediment characteristics over the last 6000 years BP in Lake MacLeod. Major shifts in ecosystem dynamics linked to changes in sea level and climate can be distinguished in the PCA analysis, and match the events hypothesised to have occurred during the evolution of the basin by Logan (1987) and Shepherd (1990). Recent increases in fine sediment content of the sediments, which may explain the increase in chemical elements of the sediments, indicates increased run-off from surrounding catchments which may be caused by climatic changes and/or agriculture. Increased pressures from sedimentation, eutrophication and contamination have been related to loss in seagrass meadows in Oyster Harbour (Serrano et al. 2016a). These results add to a growing body of literature demonstrating the role of sedimentary archives to reconstruct the trajectories of anthropogenic pressures and structural changes on aquatic ecosystems, which can improve management outcomes to better manage ecological change.



Plate: Seagrass communities near Goat Bay, Cygnet Pond. Photo: P.Horwitz

# **DERIVING A HABITAT CLASSIFICATION SCHEME FOR THE NORTHERN PONDS**

Source Document:

Dave Blake, Holly Winkle, and Pierre Horwitz Unpublished material

## **Background**

A habitat classification scheme can be defined as a structured system of habitat types (or classes), often arranged in a hierarchy, where the classification types are clearly defined and repeat in different geographical places. There are numerous classification schemes being utilised throughout the world. These incorporate both hierarchal and non-hierarchical structures. In a hierarchical system classification classes are defined at various levels of detail and are nested so that numerous detailed habitats lay within a smaller number of more broadly-defined habitat classes. Detailed habitats (at low levels in the hierarchy) that are grouped into the same upper-level class are more similar to each other than to those in another class (Connor et al, 2003).

## **Methodology**

### **Research Design**

Initially, Landsat 7 and high resolution ortho-rectified aerial imagery were investigated to determine their suitability to map the habitat of Lake MacLeod's Northern Ponds. Landsat 7 imagery provides a spatial resolution of 30 m whereas aerial imagery provides a spatial resolution of 20 cm. Landsat 8 imagery was used to develop band ratios used to identify or differentiate between vegetation, water and mineralogy in the region.

Supervised and unsupervised classification of imagery are commonly employed in mapping landuse and habitat across a landscape and are particularly useful in remote inaccessible regions such as those found at the Northern Ponds of Lake MacLeod.

Landsat sensors provide approximately 45 years of complete temporal coverage of the earth's surface. They were initially designed to capture high quality global data of the earth's land surfaces. As such, they provide a rich resource with which to map the different land surfaces that occur throughout the Lake MacLeod system.

The process of classifying the habitat of Lake MacLeod's northern ponds was divided into five components;

- Pre-processing
- Unsupervised classification
- Hand-digitized classification
- Accuracy assessment/Field ground-truthing
- Supervised classification

## **Landsat 7 ETM+ Image pre-processing**

Landsat 7 images were downloaded from the Landsat Archive via the USGS Earth Explorer site. Path 115 Row's 76 and 77 provide coverage of Lake MacLeod and surrounding areas. Images were selected for the most recent cloud free dates which corresponded to the 2<sup>nd</sup> September 2014. Level 1 products were downloaded and processed.

Due to a scanner malfunction, namely the scan line corrector, on the Landsat 7 MSS sensor, stripes often appear on images over the area of interest where the sensor has not recorded any information. These images are referred to as Landsat 7 ETM+ SLC-off data and have affected all Landsat 7 ETM+ imagery since 2003. This limits the integrity of the data for scientific purposes, including geostatistical analysis. A number of methods have been developed to overcome this flaw imagery. This study used the Focal Analysis method using ERDAS Imagine 2014 software (version 10). Focal analysis is an interpolation method that computes values for the missing pixels based on neighbouring pixels, this method is used to fill in missing data on single images (Pratt, W. 1991).

As two satellite paths are required to cover the area of interest, these were mosaicked together using ArcGIS software (ESRI, version 10.2.2), following SLC correction, to create a single, seamless image for classification. Due to the heterogeneity of the lake system it was felt that the 30 m pixel size of Landsat imagery may not capture the detail required, therefore, the mosaicked imagery was pan-sharpened using the panchromatic band (15 m pixel size) of the Landsat 7 ETM+ sensor to provide a final image with a pixel size of 15 m. Unsupervised and supervised classification techniques work better over smaller less heterogeneous landscapes therefore the initial classification was performed over the Neil's Pond region of Lake MacLeod. The mosaicked imagery was clipped to the Neil's Pond extent using ArcGIS (ESRI, version 10.2.2).

## **Unsupervised classification**

Unsupervised classification is useful when the number of land cover types is unknown or there is not a pre-defined habitat classification system in place. The unsupervised classification was used as a pathway habitat map to select spectrally homogeneous sites which assisted in the development of the hand-digitized map, as well as for selecting ground-truthing sites to be verified in the field. An unsupervised classification of Neil's Pond was done using Iso-cluster analysis (Ball and Hall, 1965) in ArcGIS software (ESRI, version 10.2.2). This is an iterative statistical method that groups pixels into groups or geographical clusters based upon their reflective attributes.

Due to concerns about capturing the appropriate amount of detail about the system it was decided to also perform an unsupervised classification on the geo-referenced aerial image of the region (pixel size of 20 centimetres) which would have produced too much spectral 'noise' and taken an extended period to process. The aerial image was resampled to provide a spatial resolution of 5 metres. Once again an Iso-cluster unsupervised classification was performed using ArcGIS software (ESRI, version 10.2.2). Following classification,

both images were reclassified to provide meaningful labels to the identified classification groups. During this process the number of classifications was reduced to 19.

Based upon the unsupervised classification of the Landsat 7 ETM+ imagery for Neil's Pond and using the resampled aerial image for verification, polygons were hand digitized for each of the 19 classification types using ArcGIS software (ESRI, version 10.2.2).

### **Accuracy assessment/Field ground-truthing**

Following the classification of the Neil's Pond area of Lake MacLeod an accuracy assessment was performed. Three stratified random sampling points per classification were identified (54 sites) and the coordinates recorded. Field staff then navigated to the identified points using hand-held GPS and recorded the characteristics of the land surface within a 15 m radius of the designated point (approximate positional accuracy of the GPS was +/- 5 m). Characteristics recorded included vegetation type and percentage cover, sediment type, presence of water and any other distinguishing features. Photographs were also taken at each sampling point. Based on the results of the ground-truthing exercise a base error matrix was calculated to determine the agreement accuracy, overall accuracy, commission error, omission error and the Kappa coefficient. This was then used to determine the final classification categories for the habitat classification of Lake MacLeod.

Following the Supervised Classification of Lake MacLeod (see below) another field ground-truthing event was undertaken. The boundary of the northern ponds was divided into three areas (Area 1: northernmost Ponds, Area 2: Neil's Pond and Area 3: northern part of Cygnet system). Again, three stratified random sites were selected per classification category.

### **Habitat Classification Map - Supervised Classification**

Based on the results of the Neil's Pond ground-truthing a supervised classification was performed on Landsat 7 ETM+ imagery for the whole of the Lake MacLeod system. Training sites were selected based on ground-truthing and knowledge gained by scientists working in the region. Ten sites were selected per classification category. Training polygons were digitized in each classification category and then merged to provide a single class training file. A Maximum Likelihood Classification algorithm (Lillesand and Kiefer, 2000) is then applied that assigns pixels based upon their statistical similarity with the variance and covariance of the training file. The supervised classification was performed using ArcGIS software (ESRI, version 10.2.2). This did not include Cygnet Pond.

Following the supervised classification, a habitat map was compiled by hand-digitizing the classification categories using the aerial imagery for verification. On compilation of the habitat map the Lake MacLeod was divided into three distinct areas and sites selected for ground-truthing (see above section on accuracy assessment). Based on the results of the ground-truthing the classifications were reclassified and the final map produced.

## Results

Unsupervised classification of the Landsat 7 ETM+ imagery resulted in the identification of 19 distinct classification types (see Figure 1 and Table 1). Unsupervised classification, using similar methods, of the resampled aerial image (see Figure 2) resulted in too much spectral noise to warrant further classification at this spatial scale. The resampled aerial image was used for verification purposes from this point forward.

Ground-truthing was carried out with 3 sites selected per classification category. An accuracy assessment of the unsupervised classification measured against the ground-truthing results performed poorly with an overall accuracy of 0.22 or 22% and a Kappa coefficient of 0.18 with a Kappa coefficient of 1 indicating perfect agreement between predicted and observed classification types.

Based on these results the classification categories were reduced from 19 to 12 categories: 6 sediment, 4 vegetation, 1 water and an unresolved category. A supervised classification was then performed on the Landsat 7 ETM+ imagery for the whole of the Northern Ponds region of Lake MacLeod.

Ground-truthing was carried out in 3 regions measuring the same criteria as the initial ground-truthing exercise for the initial classification at Neil's Pond. The number of ground-truthing sites visited during the field survey was dictated somewhat by available resources and accessibility issues. At least three sites per classification category were sampled for each of the transects. In Area 1, situated around the northern section of the Northern Ponds, there were 8 x W1 sample sites, 8 x V1 sample sites, 6 x V2 sample sites and 3 x sample sites for V3 – V4 and S1 – s6 sample sites, resulting in a total of 45 sample sites. In Area 2, situated around the Neil's Pond area, there were up to 3 sample sites per classification category resulting in 30 ground-truthing sample sites in this area. In Area 3, situated around Cygnet Pond, again up to 3 ground-truthing sample sites per classification category were selected, resulting in a total of 33 ground-truthing sample sites in this area.

Based on the results of the ground-truthing, the Northern Ponds of Lake MacLeod were reclassified into 15 broad categories and the whole region mapped (see Figure 9 and Table 2).



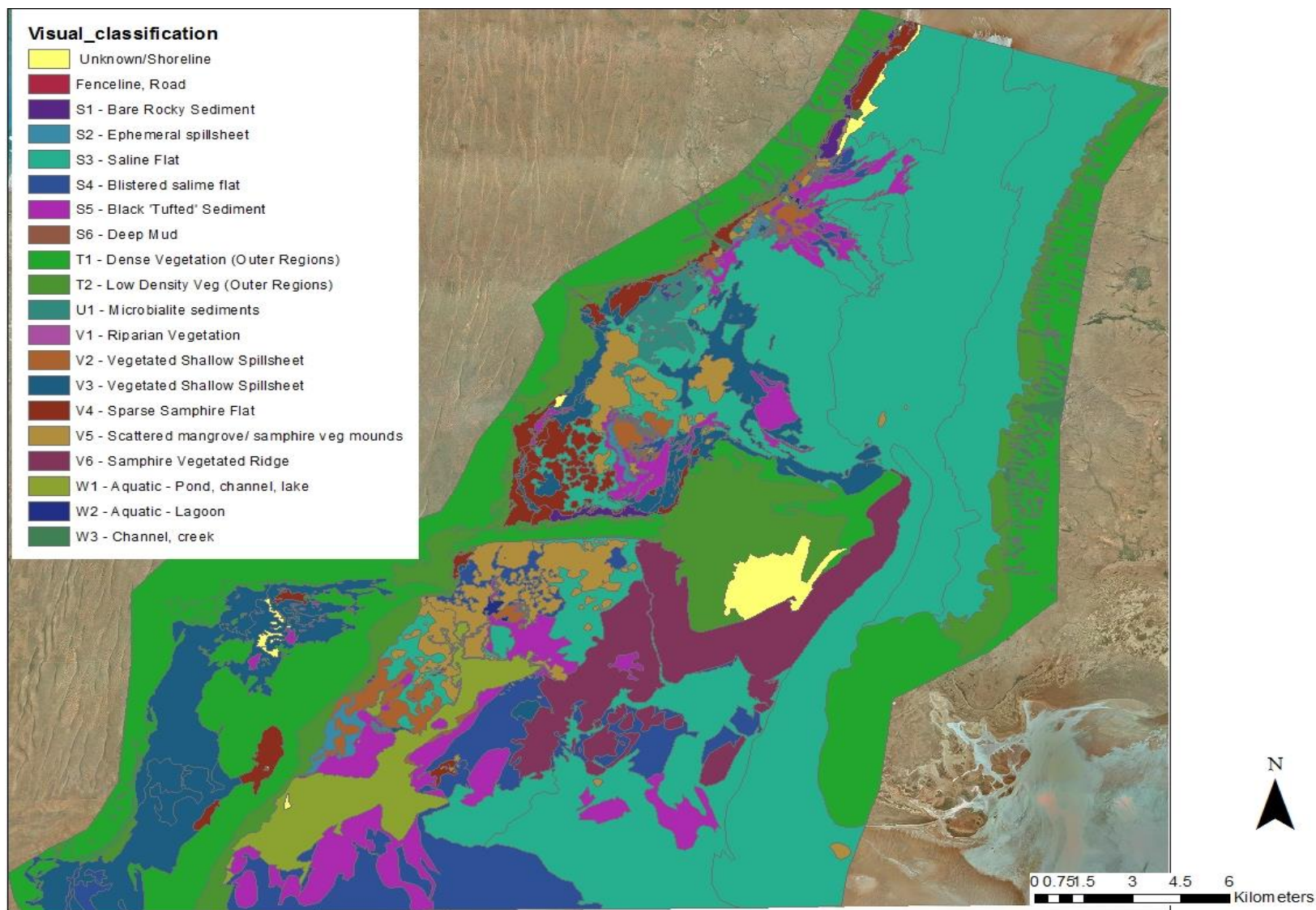


Figure 20: Habitat classification map of the Northern Ponds of Lake MacLeod.

**Table 17: Final habitat classification categories for the Northern Ponds of Lake Macleod.**

New Landform Type	Code	Descriptor
<b>Pond, channel, lake</b>	<b>W1</b>	Purely aquatic habitat (surface water). Well defined banks with V1 littoral vegetation. More than 20cm average depth. Water varies from green to clear. Water originates from a vent. Important habitat for fish and invertebrates
<b>Lagoon</b>	<b>W2</b>	Water body that receives water from a pond (no vent) Often shallow (<50 cm). Can be green to clear. Banks often undefined. With or without V1 vegetation.  Salinity greater than ponds? No macrophytes
<b>Riparian Vegetation</b>	<b>V1</b>	Dense riparian vegetation (50%-80% cover in total) adjacent to any W1. Mangroves (often tall 2m to 5m) with understorey of often dense leaf litter. Samphire usually inhabit zone opposite side of the mangroves as water. Where leaf litter is absent, sediment is white salt crust. When occurring around W1, mangroves form steep banks with pneumatophores. Important habitat for birds, insect, aquatic inverts and fish
<b>Vegetated shallow spillsheet</b>	<b>V2</b>	Dominated by expansive areas of very shallow (2-15cm), flowing water. Sediments are typically white, and muddy. Footprints sink 30 cm. Lines of pneumatophores, and beds of <i>Ruppia</i> (very occasional <i>Halodule</i> ). Habitat for gastropods and small fish. Pneumatophores usually surrounded by filamentous algae. Waterbirds often found here.  'Islands' of V1 habitat dispersed throughout on raised mounds.
<b>Samphire Flat</b>	<b>V3</b>	Samphire density of more than 40% cover, species diversity variable. Bushes of variable height and width. Light brown sands, gritty, few to no rock fragments, sometimes shells, often moist. No mangroves (if present, very small and stunted or dead)
<b>Sparse Samphire Flat</b>	<b>V4</b>	Samphire low density (<30%) and low height/width bushes (similar to V3). Can be of high and low diversity Sediments typically drier than V3 Sediments may vary from rocky fragments with sub-fossils to bare surface with blisters Sediments vary from brown/red clay to white sand . No mangroves
<b>Scattered mangrove/samphire vegetated mounds</b>	<b>V5</b>	Areas of 'vegetated islands' dispersed throughout bare sediments. Islands consist of mounds which are raised above surrounding sediments (S4)  Mounds can have tall mangrove, usually less than 5 individuals  Exposed areas of mounds covered with samphire and white salt crust. Cauliflower microbialites (U1) common in seepage spots

New Landform Type	Code	Descriptor
<b>Samphire vegetated ridge</b>	<b>V6</b>	<p>Elongated ridge (or mound?) with highly diverse samphire community (.15 species)</p> <p>Sediment dry (no seepage)</p> <p>Sub-fossil fragments and samphire detritus (wood) scattered on surface</p>
<b>Bare rocky sediment</b>	<b>S1</b>	<p>Non-vegetated areas (very occasional samphire) with rocky surface. Rocky surface can be gypsum sheets or rounds stromatolitic fossils. Surface clay, usually moist</p>
<b>Ephemeral spillsheet</b>	<b>S2</b>	<p>Probably an inundated example of S4. Expansive areas of shallow (2-15cm deep) flowing water on soft fine white sediment (ephemeral) occasionally with sparse <i>Ruppia</i> or biofilm occasional pneumatophore beds</p>
<b>Saline flat</b>	<b>S3</b>	<p>Non-vegetated light brown hard compacted sediments, often dry and featureless. Sediments vary from sand to clay.</p> <p>Sediments always moist.</p>
<b>Blistered saline flat</b>	<b>S4</b>	<p>Similar to S3, except with some degree of ‘blistering’ of surface sediments. Blisters vary from large blister &gt;1cm to small salt crusts. Non-vegetated. Varies from brown softer sediments to red-brown blister mats, often moist or with shallow surface water (windblown and probably ephemeral). Very expansive areas</p>
<b>Black ‘tufted’ sediment</b>	<b>S5</b>	<p>Often very expansive areas of Grey – Black ‘tufted’ cyanobacterial mats. Non-vegetated but can have isolated patches of dead mangroves. Occasionally submerged (windblown spill sheets). Probably turns red when inundated. Some mats are highly developed and very thick (&gt;10cm in depth). Some areas have been destroyed by cyclonic winds, leaving patches of bare sediments</p>
<b>Deep mud</b>	<b>S6</b>	<p>Deep soft sediment under shallow, non-flowing, exposed water.</p> <p>Non-vegetated and often with very dense, dead, mangrove forests</p> <p>Surrounded by samphire or dead mangroves. Surface smooth, Not ‘tufted’.</p>
<b>Microbialite sediments</b>	<b>U1</b>	<p>Dark crystalline cauliflower like hard wet sediment</p> <p>microbial with one species of samphire close to seepages (very small). Varied microbial structures, from cauliflower to ridges.</p> <p>Water is running over these structures (at a micro scale)</p> <p>Often very expansive areas, but can also be found dispersed throughout other habitats such as V5</p>

## SUMMARY AND CONCLUSIONS

Lake MacLeod is a dynamic environment. Since the late Holocene, the basin has been transformed from a marine basin into a marine evaporite system. These dramatic changes have resulted in the establishment of a rather unique, permanent wetland system in arid Australia of which the Northern Ponds are regarded as having the greatest biodiversity values. The work presented in this report provides information on the heterogeneity and structural characteristics of the habitats found in the Northern Ponds, as well as enhancing our understanding of the spatial distribution and structure of some of the biological communities found there. Taken together, these studies provide an insight into the how the ecosystem is functioning and what are the important components which contribute to the diversity of the system. These studies have addressed many of the knowledge gaps for the system identified in the report by Horwitz et al. (2010).

The Northern Ponds support extensive areas of terrestrial habitats which have been shown to be important for the mangrove forests, benthic invertebrates and shorebirds. The extensive habitat mapping shows the high amount of heterogeneity in the terrestrial habitats associated with the Northern Ponds. Some of these habitats are important for mangroves, particularly those near the ponds, whilst other areas are important habitats for a diverse samphire community. The work done by Dunham (2014) provides information on the abiotic determinants and structural characteristics of the mangrove stands, while the work done using satellite imagery and mapping of habitats (Blake et al, unpublished) provides an estimate for their spatial distribution.

At certain times, Lake MacLeod supports hundreds of thousands of birds, which makes the basin one of the most important wetlands in Australia for migratory shorebirds. Estrella et al. (2016) show that prey availability and quality at Lake MacLeod is probably superior to other inland feeding sites available to shorebirds in the region. Not only is prey abundance higher, it appears that the birds can select higher quality prey, such as copepods, because of their higher HUFA content. Nonetheless, the mudflats at Lake MacLeod allow the birds to feed and meet their daily energy demands as they recover from and prepare for migration. Again, the work done by Blake et al. will provide additional information on the spatial coverage of these habitats.

The aquatic habitats, which continuously feed seawater through the vents, are an unusual feature of the otherwise arid landscape. The sizes of the ponds are not only determined by topographic features, but also by the volume of seawater that is discharged by the vents. The larger the discharge volume either the larger the pond body is or the larger the loss via outflow channels is. Because the ponds are all shallow the waters are well mixed, and because of the high flushing times evapoconcentration of the brines is not significant. These features maintain a marine-like environment throughout the ponds, where the water does not differ greatly in ionic composition from marine waters, and post-mortem accumulation of marine organism remains has created extensive biogenic sediment habitats.

Analysis of the microbial communities provides useful information on the functioning of the pond ecosystems. Small scaled biogeographic patterns within and between the ponds revealed that environmental drivers are not

important, and that the ecological communities within each pond may be differentiating because of stochastic events. Analysis of the littoral invertebrate communities also provides evidence that ecological drift may be an important factor in determining the beta-diversity of the system. Analysis of the taxonomic composition of the bacterial and invertebrate communities reveal that both communities consist mostly of marine taxa, which further supports the idea that the ponds are dominated by marine-like processes rather than terrestrial processes. Interestingly, the bacterial communities contained taxa belonging to deep sea communities, which may provide evidence of where seawater is penetrating the karst system.

Although tilapia are recent invaders of the ponds, their effect on the native communities is hard to discern. The work done by Cameron-Caluori (2014) suggests that competitive pressure between tilapia and the native grunTERS is low because the two species consume prey items in different proportions and have different feeding behaviours. Benthic biofilm material was found to be at the bottom of the food chain although, and highlights the importance of microbial processes to the functioning of the systems.

Even though tilapia seem to be having a minimal effect on the trophic structure of the fish communities, increases in element composition of sediments being deposited in the last 200 years provide evidence of the impact of agriculture in the catchment areas since European settlement. Other potential threats to the system include increased grazing pressure from goats as well as human and goat traffic disturbing the sediments and microbial communities in the surrounding mudflats.

### **Update on knowledge gaps**

An updated table of knowledge gaps is provided in Table 18. This project has broadly enhanced our knowledge of the spatial coverage, temporal changes, habitat descriptions and ecosystem functioning for the aquatic invertebrates, shorebirds, fish, mangroves, littoral vegetation, microbial benthic biofilm and bacterioplankton components of the Lake MacLeod community. There are however, areas where our knowledge remains particularly poor, particularly in relation to boom-bust cycles, macro-algae, samphire and microbial mat communities. Although not directly quantified, ECU research at Lake MacLeod began after the flood event of 2010, and the subsequent boom and bust in productivity was noted by researchers. This cycle appears to be an important process in the functioning of the system. Other knowledge gaps exist in understanding the diversity and distributions of the extensive samphire communities, macro-algal communities and microbial mats found in the extensive mudflat areas. All of these communities are likely to contain novel taxa and contribute to the biodiversity value of Lake MacLeod.

**Table 18: An updated synthesis of research gaps and risks and significant biological assets, organised according to the biotic and abiotic characteristics of the Northern Ponds. Four overall dimensions of research (where red indicates high priority for research, orange a medium priority for research and green a low priority for research). Ideally any proposal for research should address high priority issues.**

Fields			Northern Ponds			
			Spatial Coverage	Temporal Change	Habitat Description	Ecosystem Function
Biotic	Invertebrate Fauna	Aquatic Invertebrates				
		Terrestrial Invertebrates				
	Vertebrate Fauna	Mammals				
		Terrestrial Birds				
		Waterbirds				
		Reptiles				
		Amphibians				
		Fish				
		Littoral Vegetation, Mangroves & Aquatic Habitats	Littoral vegetation			
	Mangroves					
	Riparian					
	Seagrass/macro algae					
	Diatoms/phytoplankton					
	Algae (mats & benthic)					
Cyanobacterial mats						
Microbial	Fungi					
	Stromatolites					
Social & Cultural		Cultural History				
		Significant sites				
Abiotic	Water Quality	Physiochemistry				
		Nutrients				
		Ions				
	Hydrology	Vent systems				
		Surface water				
		Aquifers				
		Sinks				
		Floods				
	Geology	Geology				
		Geomorphology				
		Sediments				
	Climate	Temperature				
		Rainfall				
		Cyclones				
Evaporation						

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## **APPENDIX**

This appendix contains the bathymetric and temperature profile maps for each of the eight study ponds. All maps (Figures 20-27) were produced following the kriging and simulation analysis detailed in the methodology section of paper. Latitude and longitude unites are Mercator meters, which correspond to distances in meters. A temperature profile maps could not be produced for Cygnet Pond because of large fluctuation in water temperature during the period when measurements were being taken.

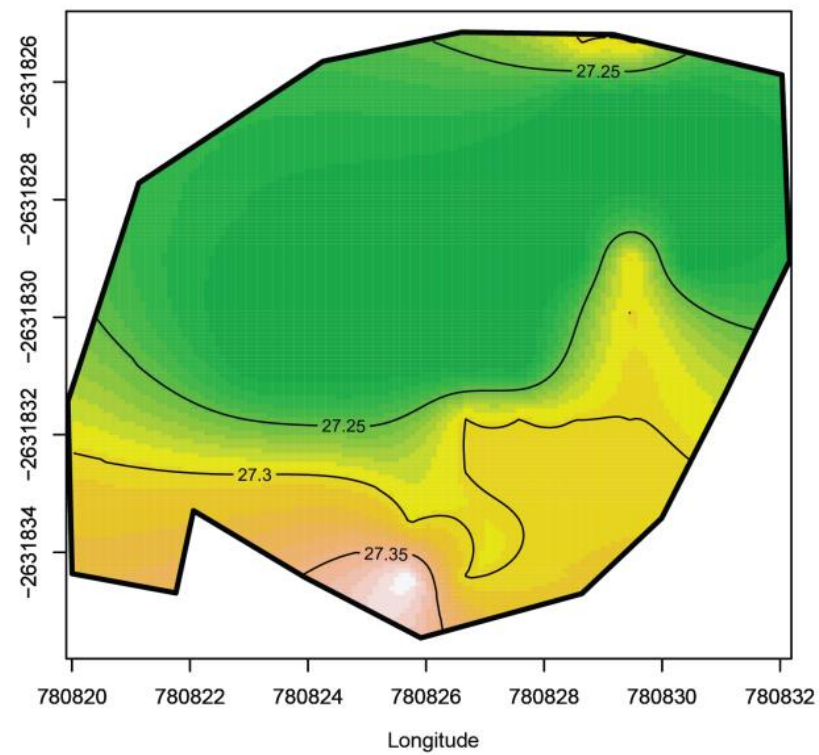
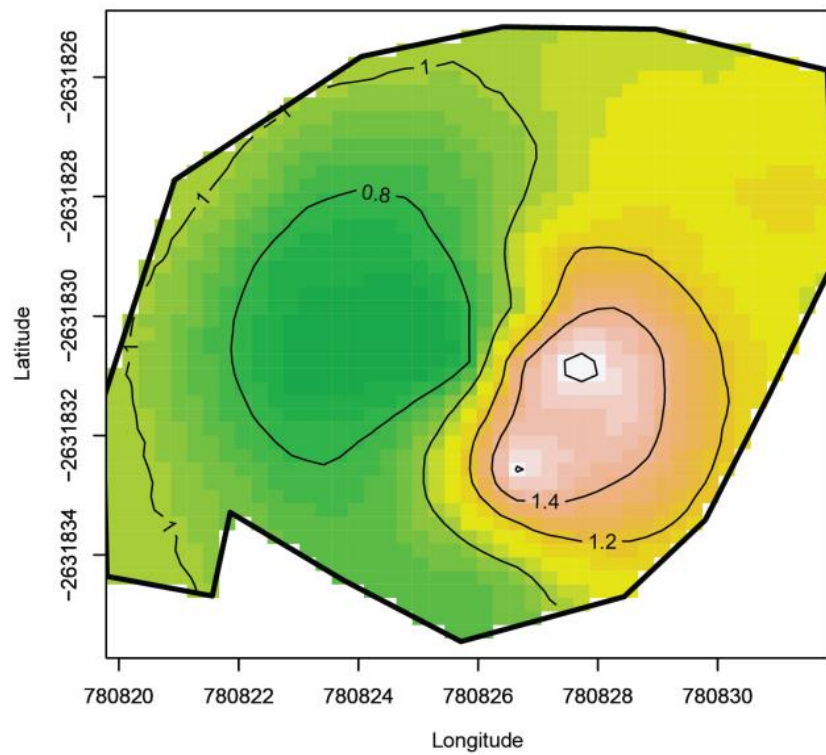


Figure 21: Bathymetric map of Pete's Vent with depth contours in meters (left) and water temperature ( $^{\circ}\text{C}$ ; right). All measurements were taken in July 2013.

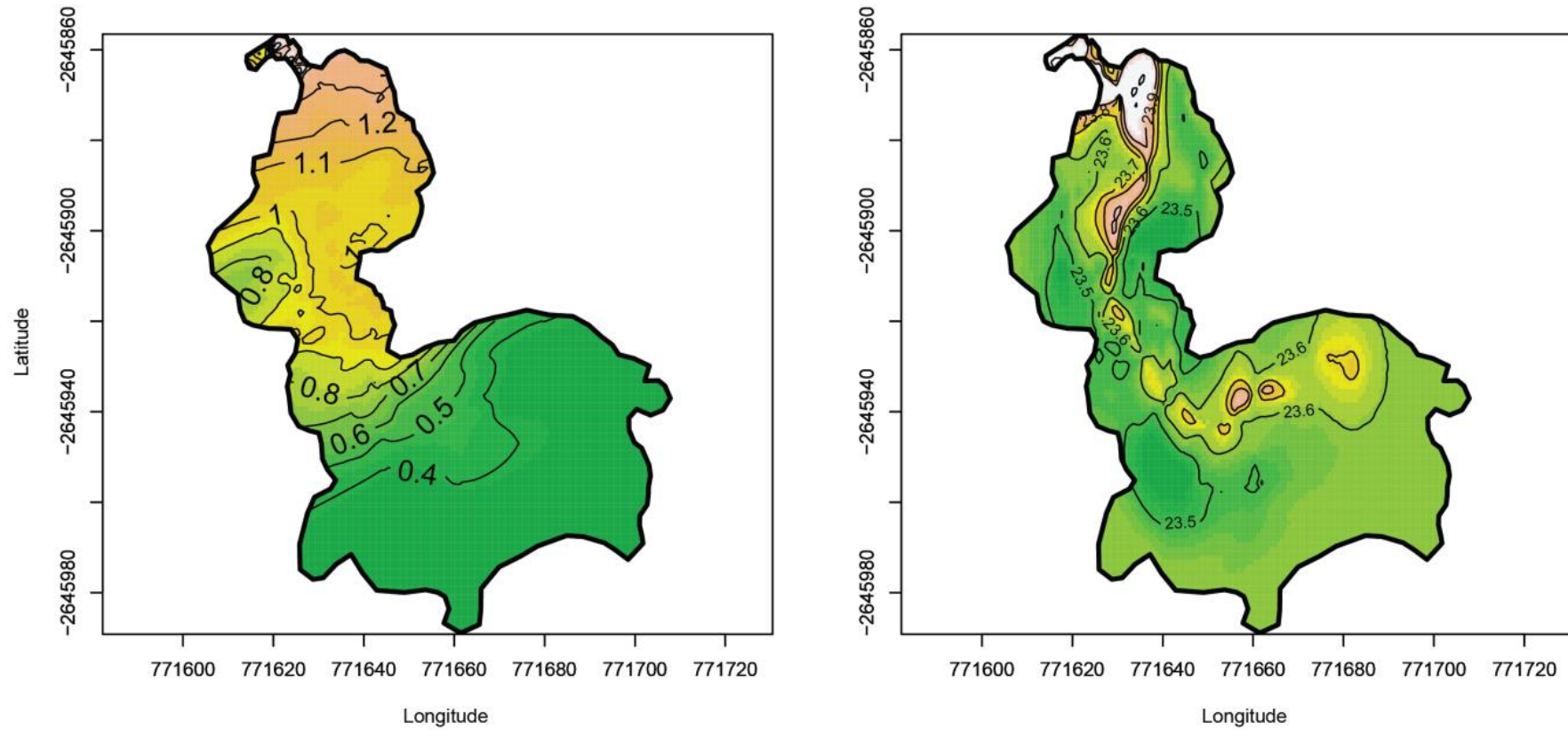


Figure 22: Bathymetric map of Donut Pond with depth contours in meters (left) and water temperature (°C; right). All measurements were taken in July 2013.

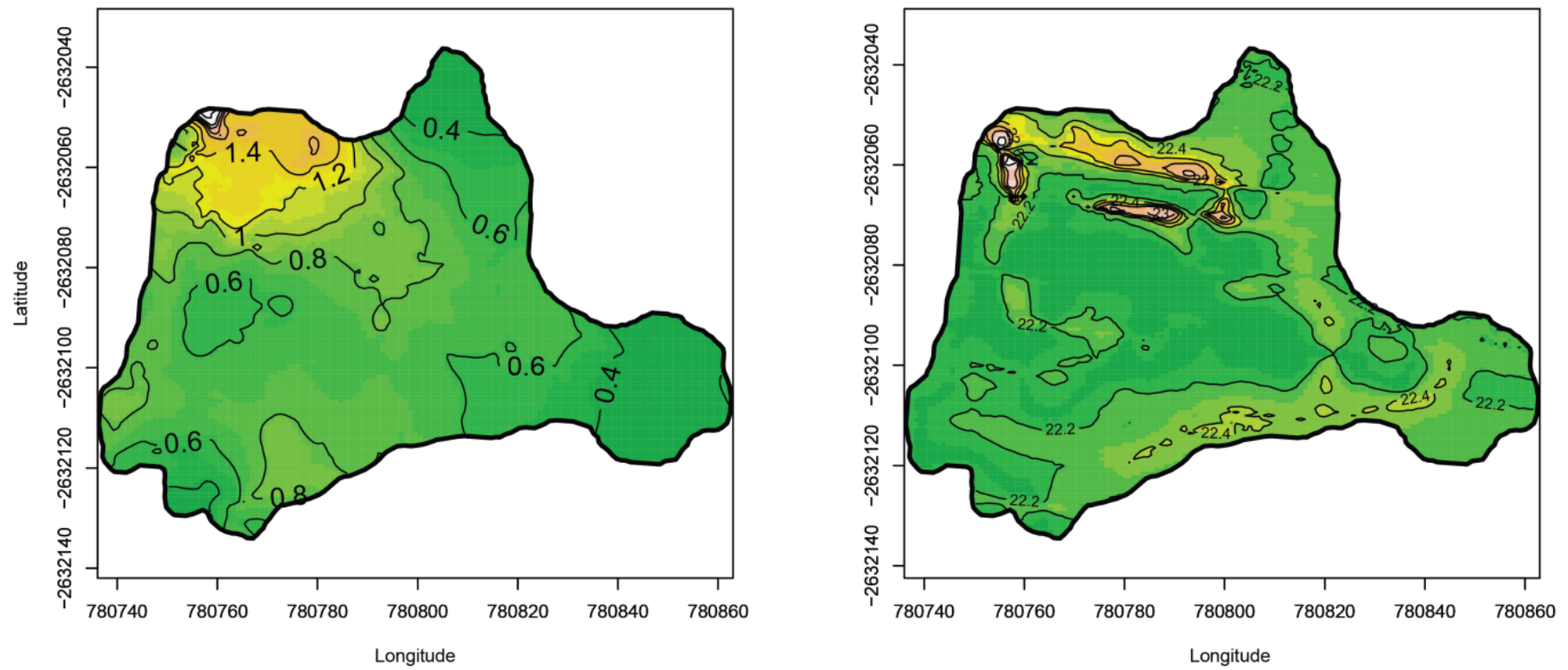


Figure 23: Bathymetric map of Annie's Pond with depth contours in meters (left) and water temperature (°C; right). All measurements were taken in July 2013.

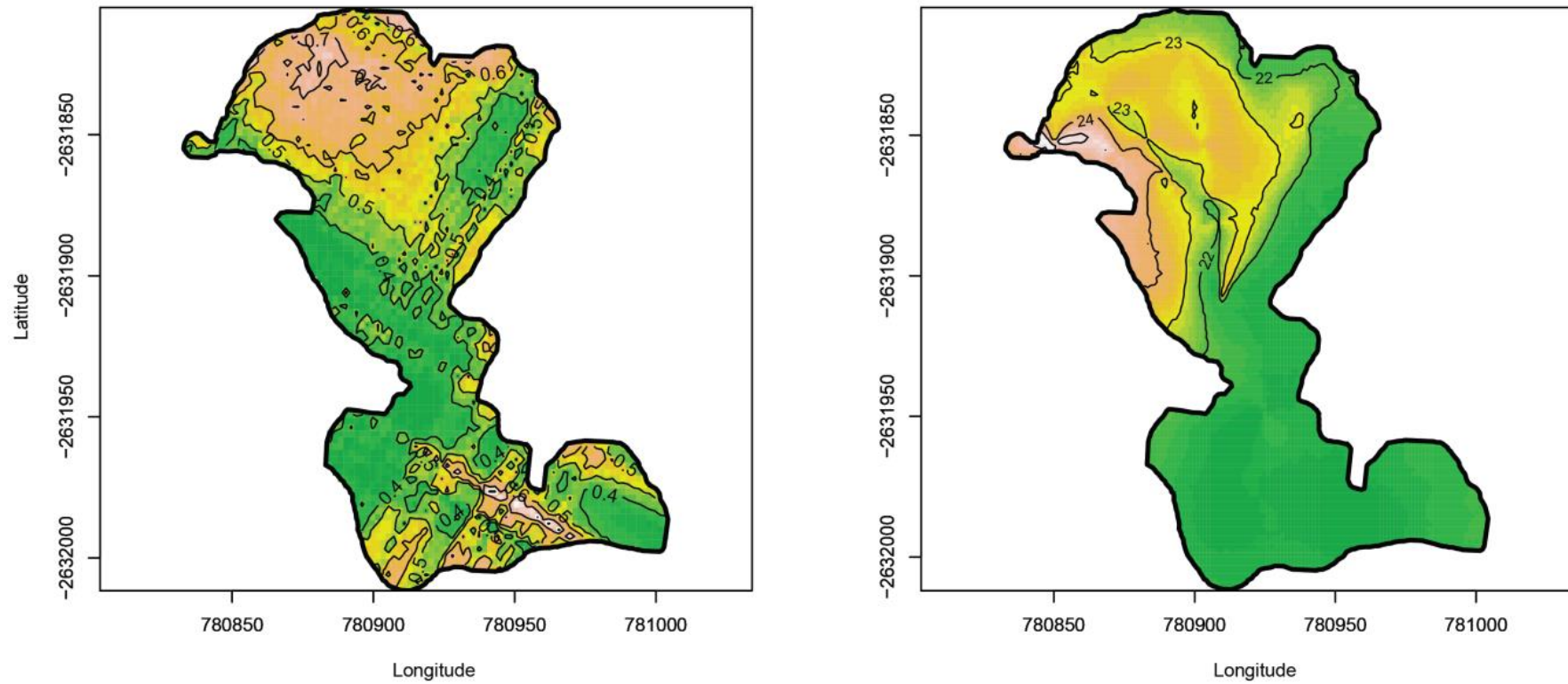


Figure 24: Bathymetric map of Pete's Pond with depth contours in meters (left) and water temperature ( $^{\circ}\text{C}$ ; right). All measurements were taken in July 2013.



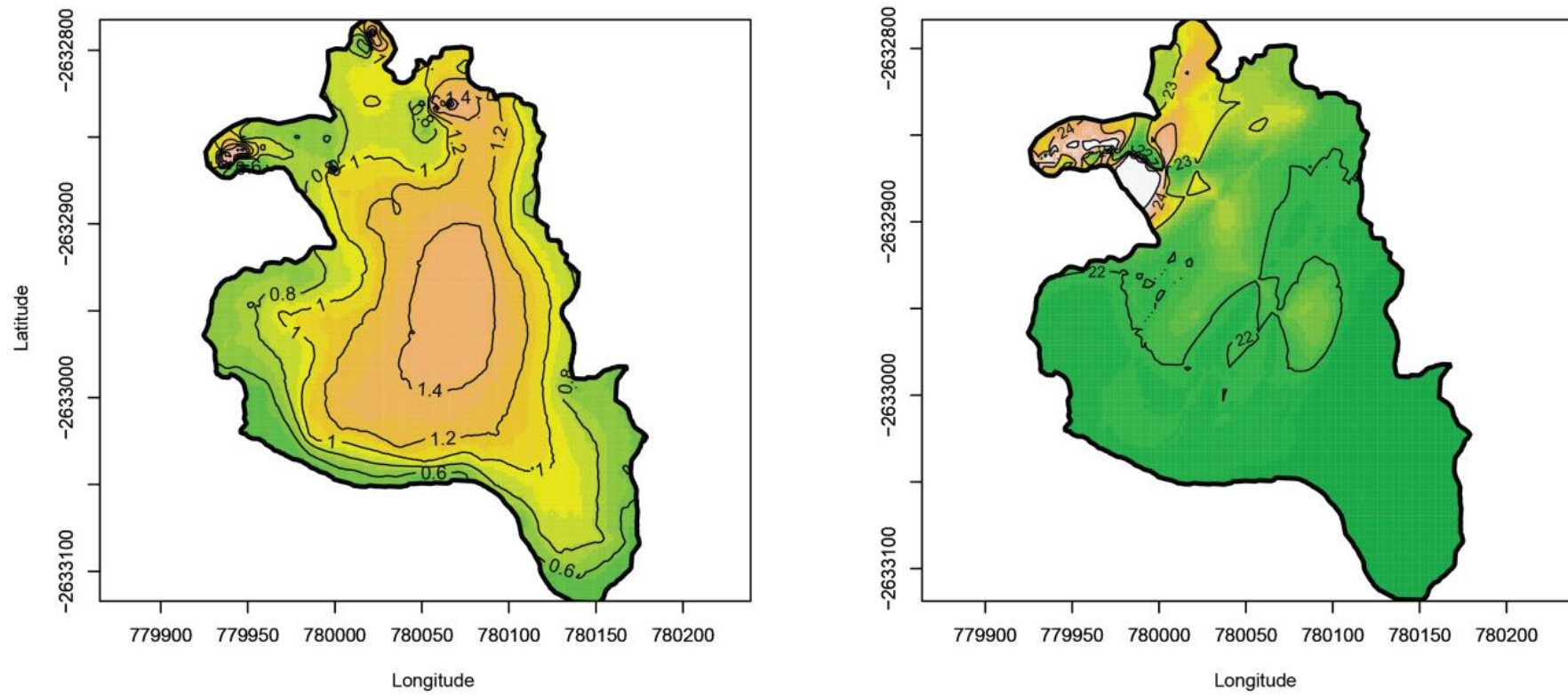
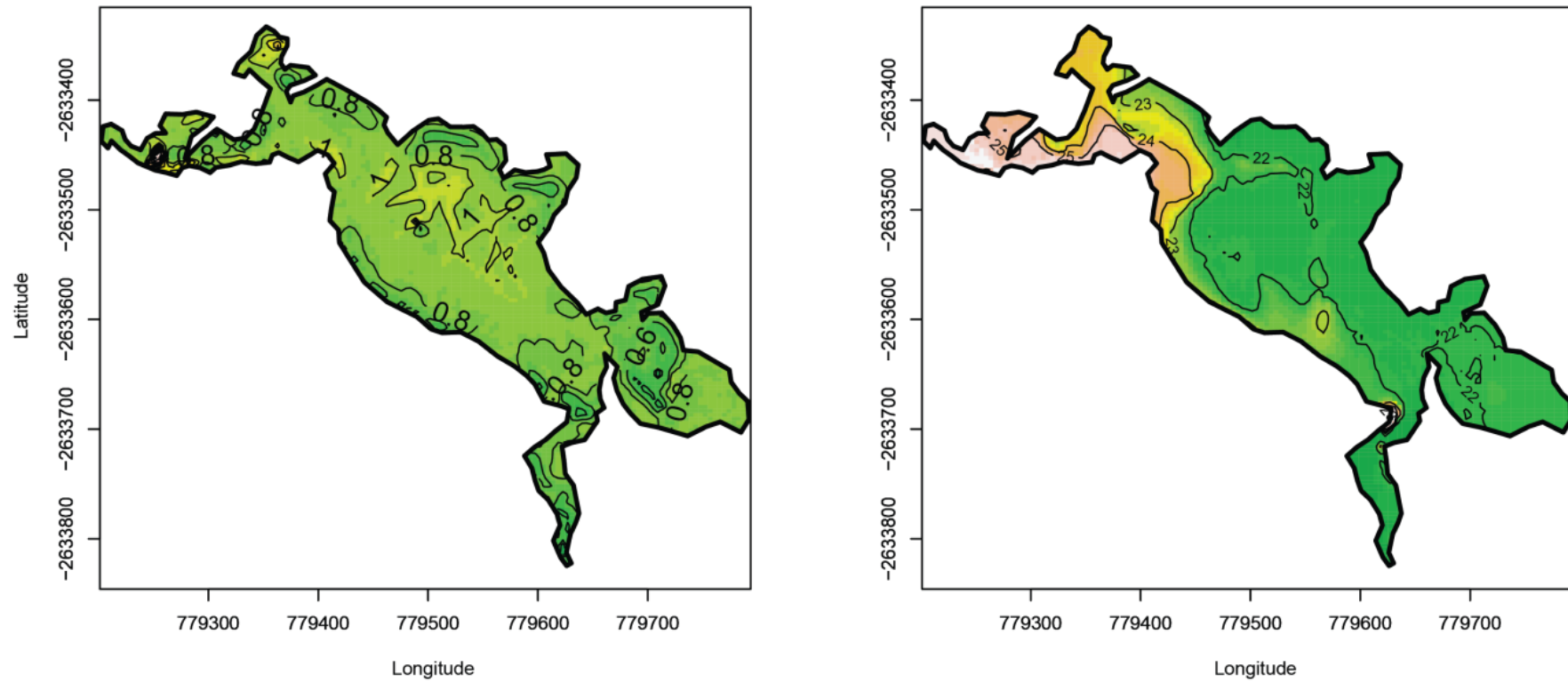


Figure 25: Bathymetric map of Whistler's Pond with depth contours in meters (left) and water temperature (°C; right). All measurements were taken in July 2013.



**Figure 26: Bathymetric map of Harjie's Pond with depth contours in meters (left) and water temperature (°C; right). All measurements were taken in July 2013.**

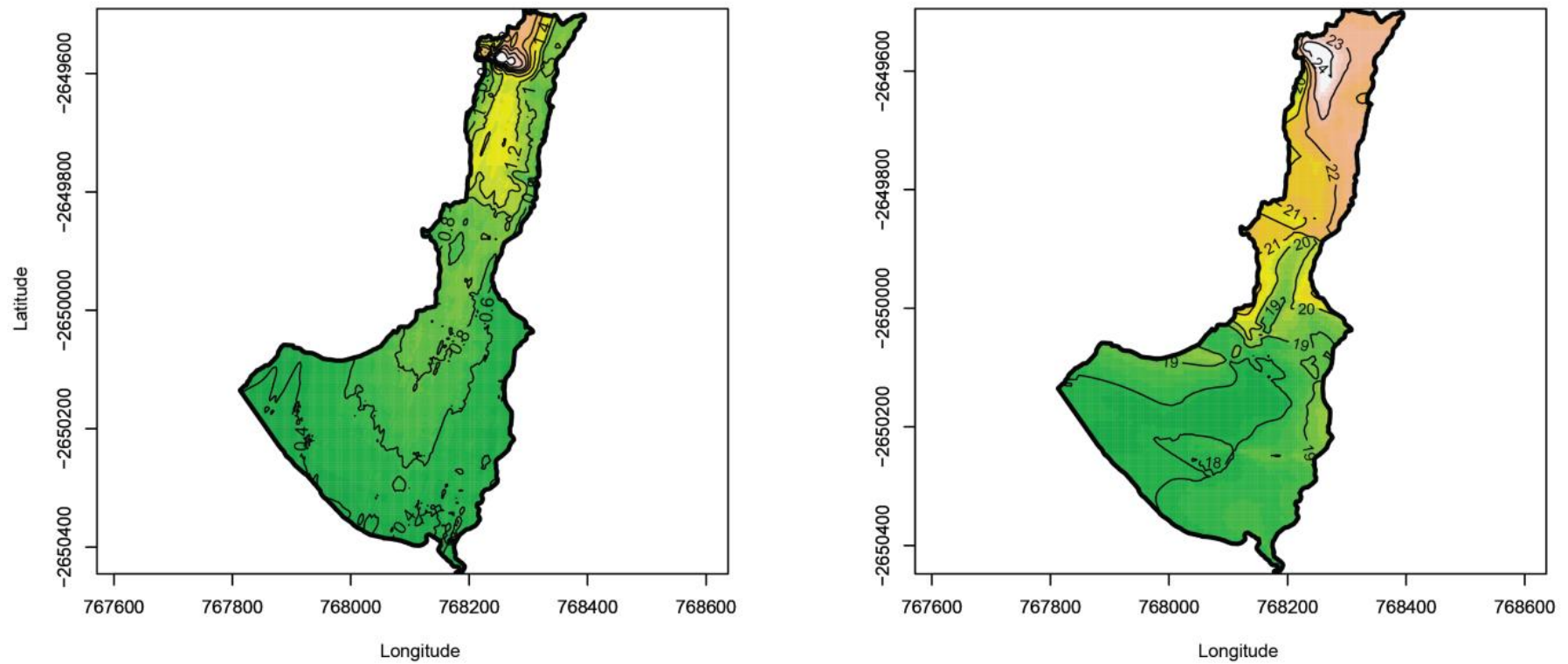


Figure 27: Bathymetric map of Jana's Vent with depth contours in meters (left) and water temperature (°C; right). All measurements were taken in July 2013.

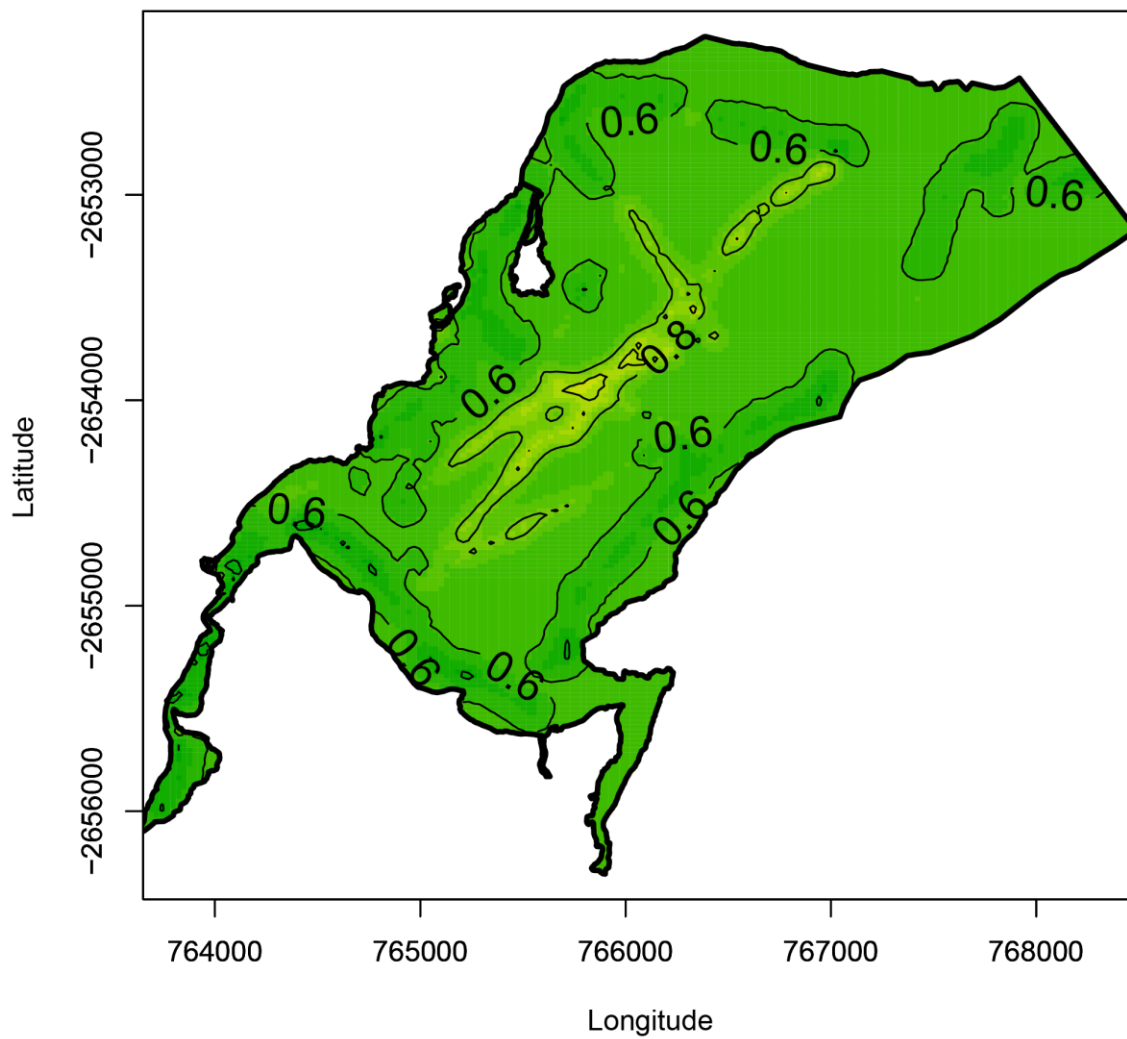


Figure 28: Bathymetric map of Cygnet Pond. Goat Island can be seen on near the western shore. Contours are in meters. All measurements were taken in July 2013.