2. MATERIALS and METHODS

2.1 Research Areas

The two Perth metropolitan wetlands chosen for this research project have similar extant vegetation, but very different histories of use. Herdsman Lake Regional Park (HL) has a complex history of agricultural and other use and the Eric Singleton Bird Sanctuary (ESBS) was previously a rubbish dump. Figure 2.1 shows the location of these wetlands relative to each other and the Perth CBD.

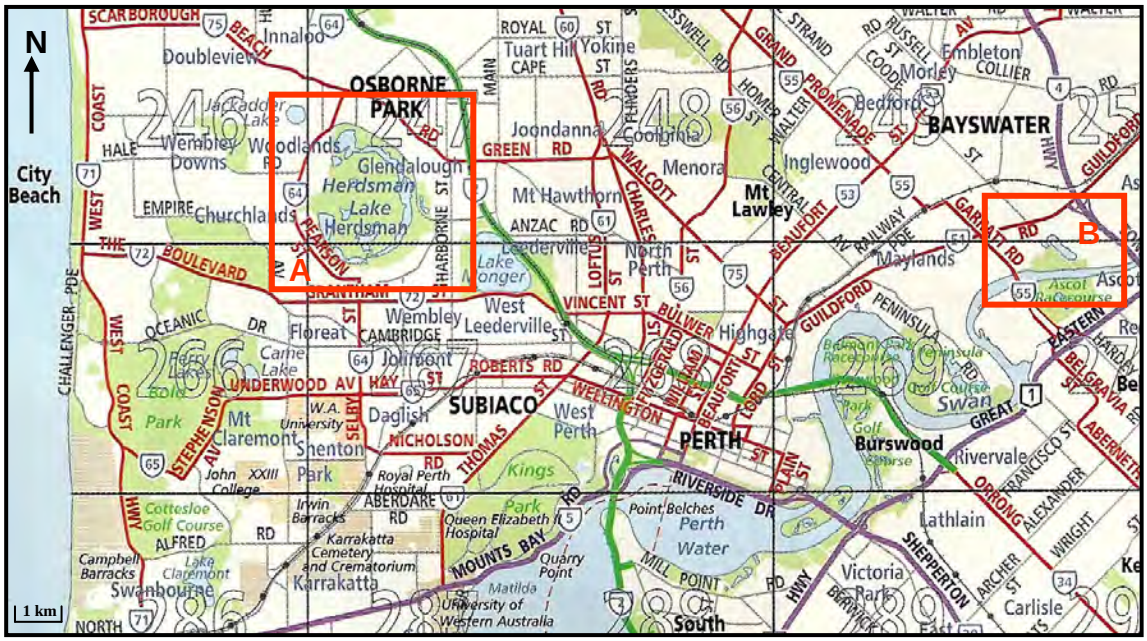


Figure 2.1 Location of Herdsman Lake (A) and Eric Singleton Bird Sanctuary (B). Map adapted from UBD Perth Street Directory, 2005, 47th edition.

Permission to take samples from these wetlands was granted by officers of the organisations that manage them: the Herdsman Lake Operations Officer of the Department of Conservation and Land Management (CALM) and the Environmental Co-ordinator of the City of Bayswater.

Terminology used throughout this work relating to sample sites is:

Research Area or Wetland = Herdsman Lake Regional Park (HL) or Eric Singleton Bird Sanctuary (ESBS).

Site = a place within a wetland with a distinct vegetation habitat e.g. HL Site One (Paperbark swamp).

Location = a place within a site e.g. lake edge or path edge, HL Site One

2.1.1 Herdsman Lake Regional Park

The following information is taken in part from 'Parks for People' Draft Interpretation Plan for Herdsman Lake Regional Park 2003 by the Department of Conservation and Land Management (CALM) and also in part from Jaensch (1992) in 'A Directory of Important Wetlands in Australia' by the Department of the Environment and Water Resources.

Located approximately 7 kilometres northwest of the Perth CBD, and surrounded by residential and industrial areas, is Herdsman Lake (HL); 'part of a chain of wetlands that extend north south within the Spearwood Dune System.' Herdsman Lake (WA080) is considered a wetland of 'national importance' and measures 2.5 km by 2 km (250 ha.) and together with surrounding areas measures almost 370 hectares (Fig. 2.2).

Figure 2.2 Location of Herdsman Lake Regional Park. Map adapted from www.whereis.com.

Herdsman Lake has undergone significant modification since indigenous Australian Aboriginal people, who call the area Ngurgenboro, utilised the area as a food and water source. Following European settlement the area has been used as a cattle grazing ground, as a market garden and a catchment for run off from surrounding market gardens. It has also been mined for the diatomaceous earth which lies below the lake bed.

The creation of two drains (Balgay Branch Drain and the Herdsman Main Drain) which cross the lake helps prevent it from becoming contaminated by the water within them. Many smaller drains flow directly into the lake from a catchment area that

contains urban housing, light industry and market gardens. The innermost area of the wetland is dry in the summer months but a moat has been dredged around the fringes of the lake to create some deep permanent water bodies.

Within the Herdsman Lake Regional Park are recreational areas, bush walking tracks and a Wildlife Centre which caters for education on conservation and environmental matters. The dominant vegetation of HL is Bulrush (*Typha orientalis*), which invaded the area as a consequence of the former use of the lake for agriculture, but there is also Swamp Paperbark (*Melaleuca rhaphiophylla*) and Flooded Gum (*Eucalyptus rudis*) woodlands and grassed areas. Many species of wildlife inhabit HL, particularly waterbirds.

2.1.2 Eric Singleton Bird Sanctuary

The following information is taken in part from an environmental management plan submitted to the City of Bayswater in 1990 by Brian J. O'Brien and Associates Pty. Ltd. and from signage at the site.

Located approximately 6 kilometres northeast of the Perth CBD, in a residential area, the Eric Singleton Bird Sanctuary (ESBS) is a 4 hectare lake (Fig. 2.3). The sanctuary is situated within 40 hectares of parkland known as Riverside Gardens. The lake and gardens lie on the floodplain of the Swan River which runs along the southern edge of the complex. The western edge of the ESBS is bordered by the Bayswater Main Drain (a very large stormwater drain).

Figure 2.3 Location of Eric Singleton Bird Sanctuary. Map adapted from www.whereis.com

The ESBS is a former natural paddock/occasional wetland. Early European settlers probably used the paddock as a market garden in the drier months and abandoned it in winter when it became a swamp. The lake was artificially created by piling rubbish on three sides of the occasional wetland but not in it. The rubbish dump operated from 1972 until 1981. It is unclear as to what soils were deposited in which location during the creation of the ESBS, but local sand and clay is known to have been used to protect the banks from erosion and the lake from pollution. The water level of the lake is maintained by rainfall, runoff and bore top-up.

The original Swamp Paperbarks (*Melaleuca rhaphiophylla*) and Flooded Gums (*Eucalyptus rudis*) have largely not survived but there has been successful reintroduction of these and other native vegetation. The weeds present are thought to have been introduced with the rubbish. The surrounding gardens are mainly grassed areas for recreational use. Aptly named, the ESBS is a haven for many species of birds.

2.2 Vegetation Survey

A vegetation survey of both research areas (HL and ESBS) was conducted with the kind assistance of Ms. Cate Tauss, a botanist, formerly with the Western Australian Herbarium and currently with Ecologia Environmental Consultants.

Knowledge of the vegetation present at each of the sites was deemed necessary in order to obtain pertinent reference pollen, and to establish which pollen may be expected in a sample. Four sites displaying distinct vegetation characteristics within each research area were chosen, surveyed and vegetation at each site was documented. The survey was conducted during the winter months of 2004 and full lists of vegetation noted, along with site descriptions, are provided in Chapter 3. Information detailing plant habitats and flowering periods is taken from FloraBase – the Western Australian Flora.

2.3 Sample Collection and Storage

2.3.1 Reference Samples

Using the methodology described by Jarzen and Jarzen (2006), two reference pollen samples each from 46 of the 76 plant species identified in the vegetation survey were collected from corresponding dried plant specimens held at the Western Australian Herbarium. A further five pollen samples were taken directly from living plants in the research areas. Reference pollen slides of a further 17 plant species from the University of Western Australia's Balme collection and from the private collection of Dr. Lynne

Milne were consulted. Reference pollen for eight plant species was unable to be accessed either because there was no plant specimen at the Herbarium or the pollen from the species does not survive the palynological preparation process. Various websites and books portraying pollen photomicrographs were consulted but no images of the eight plant species required were found.

2.3.2 Soil and Sediment Samples

- **Surface Samples**

  Surface soil samples were collected from several locations within each of four sites in each wetland at different times of the year. Samples were collected using the methodology described by Adam and Mehringer (1975); i.e. by taking 10 - 15 'pinches' from only the top 1 cm of soil in a grid-like fashion. These 'pinches' were then amalgamated in a clean container dedicated to each individual sample. This homogenous mix of soil from numerous spaced pinches ensures the pollen assemblage is more representative of the circumscribed area than if the sample was taken from one locale within the site. Clean gloves were used for each sample in order to avoid cross-contamination. Wet samples were treated with a few drops of Ethanol, to retard fungal growth, before storage in a cold room at 4^{o}C. Moist soil samples were dried in an oven before storage at room temperature.

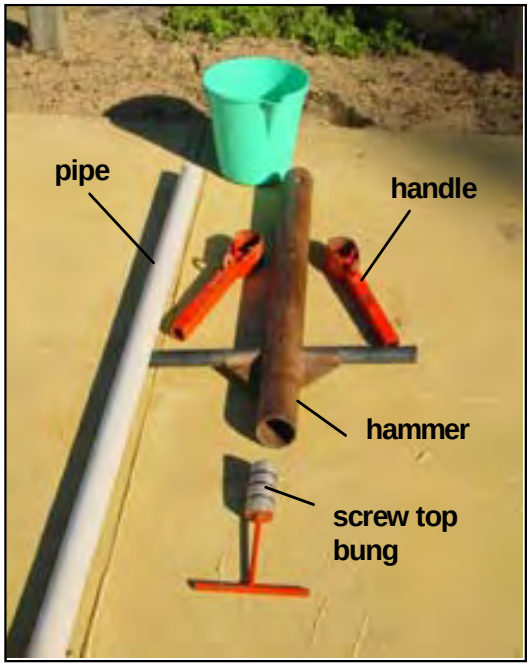


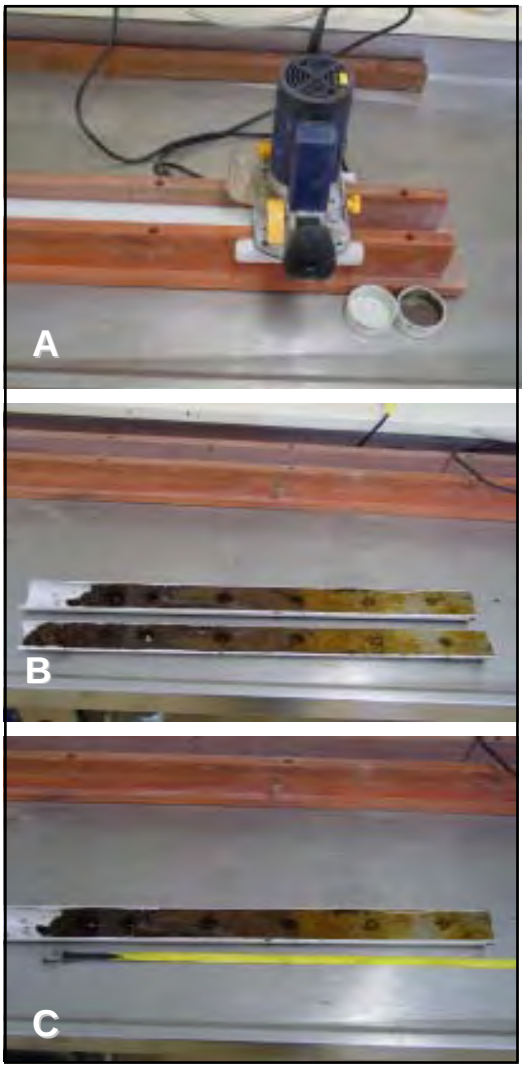
Figure 2.4 Equipment used to collect cores.

- **Core Samples**

One 50 cm core was taken at each of three sites in each wetland. No cores were taken at Site Two of either research area as the ground was considered too hard for the coring equipment available. The locations of the core samples were chosen as would be burial sites of a body in an imaginary crime. Each core was obtained by hammering vertically into the ground a 1 m length of polythene pipe (Fig. 2.4), measuring 10 cm in diameter, to a depth

of 50 cm. Water was then poured into the pipe to fill the void. A special screw top bung was inserted into the top of the polythene pipe and tightened, to form a seal, before the pipe containing the core was lifted out of the ground using the lifting handles. Once above ground the bung was removed and the water was then carefully decanted, the excess pipe sawn off and both ends of the pipe were then capped. The pipes were marked to show which end was surface level. The cores were then stored, inside the pipes, in a cold room (4^{o}C) at the School of Earth and Geographical Sciences, UWA.

Cutting of each core was achieved by removing the end caps and securing the pipe in a specially constructed open ended box (Fig. 2.5A). A router was applied along the full length of the pipe; the pipe was then rotated through 180^{0} and the router was applied again. A sharp blade was used to cut through the pipe at the indentations made by the router, and a fine toothed saw was then used to divide each core into two halves (Fig. 2.5B).



Approximately 2 cm^2 of sediment present in each core was taken at surface level and then at depths of 5 cm, 10 cm, 20 cm, 30 cm, 40 cm and 50 cm (Fig. 2.5C). To avoid possible contamination, as the saw had passed through the entire length of the core, the top 2 mm of sediment from each cut edge was discarded.

Figure 2.5A - C. Core cutting equipment. **A**. Router atop polythene pipe which is held in a specially constructed box. **B**. Two halves of core that has been cut. **C.** Tape measure alongside a cut core shows depths sampled.

2.4 Sample Preparation

The aim of the palynological preparation process is to destroy or remove almost the entire non-pollen fraction in a sample and to concentrate the pollen fraction that remains. As the palynological process destroys all but the pollen, if possible, at least a half of the sample is retained for repeat processing, if necessary, or for different types of analyses (e.g. mineralogy) by other scientists. Combining scientific disciplines has proved beneficial in a number of cases (e.g. Brown, Smith and Elmhurst, 2002; Mildenhall, 2003). The palynological process removes all of the internal protoplasm of the pollen grains and leaves only the empty 'shell' whose surface ornamentation is, along with other features detailed later, examined in an attempt to identify the plant that produced the pollen to the lowest taxonomic level possible.

The techniques used to prepare the samples for light microscopy are largely based on those described by Phipps and Playford (1984). During the preparation process each sample was monitored to determine if some of the steps described needed to be repeated or omitted altogether. Soil and sediment samples required all seven steps of the modified preparation process outlined below. Pollen taken directly from reference collections or fresh anthers required only steps 4 – 7. Eyring (1996) stated that the techniques employed need to "free it", "clean it", and "mount it". 'It' being the pollen present in the sample.

Possible contamination of all samples was avoided by the use of clean equipment dedicated to one sample only, and by the use of beaker covers and disposable pipettes where possible. Safety measures required in the preparation of samples were employed including the use of fume cupboards and protective clothing.

- **Reference Samples**

A minimum of five mature pollen bearing anthers was collected for processing from reference or field collections. In total, 92 pollen reference samples were processed.

- **Soil and Sediment Samples**

  The amount of soil from the surface samples considered adequate for processing was judged to be the amount required to cover the bottom of a 250 ml beaker. Twice as much was required for samples containing a lot of sand. A 2 cm^2 plug of sediment from each of the seven depths of the cores was processed. In total, 42 surface soil and 42 sediment samples were processed.

2.4.1 Palynological Processing Steps

STEP 1: Pre-HF Treatment
STEP 2: Silicate Removal
STEP 3: Fluoride Removal
STEP 4: Acetolysis
STEP 5: Alkali Treatment
STEP 6: Removal of Extraneous Material from Residue
STEP 7: Slide Making

Following completion of each step, samples were washed in hot or cold distilled water (DW) followed by three minutes centrifugation at 3000 rpm (except where noted). Supernatant fluid was discarded after each spin. Soil and sediment samples were prepared in beakers and 50 ml centrifuge tubes and reference samples were prepared in 10 ml tubes.

STEP 1: Pre-HF Treatment

Large organic matter such as twigs and leaves in surface soil samples was removed manually prior to treatment. The sediment in the cores required disaggregation, by crushing with a glass rod, prior to processing. Calcium and magnesium carbonates were then removed from the samples by heating in 50% hydrochloric acid (HCl). Time on the hotplate was determined by the cessation of a positive 'frothing' reaction.

STEP 2: Silicate Removal

A minimum of two days soaking in cold 50% hydrofluoric acid (HF) was required to 'digest' the clay, quartz, and other silicates in most of the samples. Some samples required further soaking (up to six days) with the addition of fresh HF to maximise the removal of silicates.

STEP 3: Fluoride Removal

Fluorosilicate compounds, which are a by-product of the reaction of HF with silica, were removed by heating the samples to boiling point in 50% HCl. The samples were then washed three times with hot DW to ensure the complete removal of the fluorosilicates.

STEP 4: Acetolysis

Acetolysis dissolves some of the cellulose present in a sample and clears pollen of its protoplasm and darkens pollen grains, which allows for better visualisation of apertures and surface morphology. Moist or wet samples required dehydrating with a minimum of two washes in glacial acetic acid (CH_3COOH) before coming in contact with the acetolysis mixture. This mixture is made up of 1 part concentrated sulphuric acid (H_2SO_4): 9 parts acetic anhydride [$(CH_3CO)_2O$]. Samples were placed in 50 ml or 10 ml centrifuge tubes with 20 ml or 5 ml of acetolysis mixture and placed in a hot water bath for 4 minutes, agitated, then heated for a further 4 minutes. Glacial acetic acid was then added to the mixture to stop the acetolysis reaction. The samples were then centrifuged, the supernatant fluid discarded and a further two washes in CH_3COOH with centrifugation in between was performed. The resultant residue was then washed with DW and centrifuged three times.

STEP 5: Alkali Treatment

Soil and sediment samples required removal of organic fines (fine humic material) from the residue. This was achieved by heating the sample in a hot water bath, in a 10% solution of sodium or potassium hydroxide (NaOH or KOH), for three minutes. The residue was then rinsed with DW until the supernatant appeared clear. A few drops of concentrated HCl were added to the residue and heated for a minute to ensure the pollen grains were kept in an acidic environment.

Reference samples did not require this step, however large or fresh anthers were given an alkali treatment as described above to soften them before the acetolysis treatment.

STEP 6: Removal of Extraneous Material from Residue

To concentrate the pollen fraction in the residue further the samples were passed through a nylon sieve with an aperture diameter of 150 microns (µm). Some samples required another hot HCl treatment followed by DW washes to free the residue of any clumped particles.

STEP 7: Slide Making

Three slides were made for each sample. The mounting medium used was Eukitt®, which has the advantage of preserving and permanently fixing the pollen grains in position, unlike glycerine jelly and silicone oil favoured by some palynologists. No staining of pollen grains was undertaken.

2.5 Microscopy and analysis

Microscopic examination of all of the slides was conducted in the University of Western Australia's School of Earth and Geographic Sciences Microscopy Laboratory. Of the reference pollen slides examined a minimum of 10 grains, on each of the two slides of each species made, were measured, recorded and their size averaged. Other basic morphological features were also noted (Appendix 4.).

Slides made from the soil and sediment samples were examined to characterise the pollen assemblage in each sample. This was arrived at by counting and identifying 200 pollen grains, and further scanning the slides to identify additional pollen types present not encountered during the count. A total of 200 grains is considered representative of a complete pollen grain assemblage (Bryant, 2000). Most slides produced a count of 200 grains or more, but some produced less, and some no count at all. The resulting data from individual surface and core samples (grave profile) was compared within and across sites and wetlands. The data from all samples within each core was combined to form composite burial assemblages for comparison.

The same Olympus BX60 transmitted light microscope (TLM) was used for all of the slides examined and pollen grains were then photographed at 1000x actual size (except where noted) using an Olympus DP11 digital camera attached to the TLM. Photomicrographs made were formatted using the Adobe®Photoshop®7.0 programme and selected ones are illustrated in Plates 1 - 5. Reference pollen sample photomicrographs made were kept as a permanent record to assist in the identification process and to add to a future database of locally sourced pollen grains. Photomicrographs of unidentified pollen were also made and compared to photomicrographs of the reference pollen and in most cases identification was made at least to family level.

Traditional statistical analysis has not been carried out on the data due to the vast number of variables (see Ch. 1, p. 15).