Final Report for the Project

Sediment DNA and diatom analysis in the Koondrook- Perricoota Forest

Prepared for

Forestry Corporation of NSW

By

Peter Gell
Professorial Research Fellow
Federation University Australia

with

Phuong Doan, Research Associate, Federation University Australia, Mt Helen
Keely Mills, British Geological Survey, Keyworth, UK,
Mukesh Raipuria, Biomedical Science, Federation University Australia, Mt Helen

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Introduction

The Koondrook-Perricoota Forest (KPF) lies on the floodplain of the Murray River downstream of Moama. It forms part of a larger Ramsar wetland site and is also an icon site within The Living Murray Initiative. As such the managing authorities are concerned with the natural ecological character of the wetlands in the Forest and, more broadly, the degree to which they have changed over time.

The River Murray system has been subject to a diverse array of pressures from catchment development and climate variability and change. The NSW Central Murray State Forests Ramsar site was declared in 2003. The NSW Government has invested in infrastructure to enable Murray River water to be diverted through the Forest. Recently the wetlands received environmental flows to manage the wetland and floodplain ecosystems.

It is recognised that wetland ecosystems change over time and that long term perspectives, including those sourced from palaeoecology, can be useful in circumscribing the evolution of systems to inform their management into the future (Davidson, 2016). Palaeoecological approaches can extract from accumulated sediments the fossil remains of biota that have lived in a wetland over time. The development of a chronology for a sediment core can then enable change in wetland ecosystems to be reconstructed and tied to known historical events in the past.

Recognising this, a project was commissioned to explore the potential to use palaeoecological techniques to provide long term context to the understanding of the wetlands of the KPF. In May 2017 cores were taken from, amongst others, Dead River Lagoon in the Perricoota Forest of NSW. The core extracted from Dead River Lagoon proved the most promising for the reconstruction of long term change and so the analyses of fossil diatom and DNA remains focussed on the record from this site.

Diatoms are single-celled algae with 'bivalves' made of silica that preserve well in sediment sequences. They are reliable indicators of water quality and they have specific substrate and habitat requirements enabling wetland condition to be inferred through time (Reid *et al*., 1995). Biota living in and near wetlands shed body fragments that can enter the sediments. The extraction and sequencing of the preserved DNA fragments can be used to identify the presence of these organisms at the time the sediments were deposited. While fossil diatoms are readily identified to species, and their condition of the water inferred from preserved assemblages, recovered DNA is often fragmented and is difficult to attribute to known species. That said, it remains an exciting prospective approach to reconstructing the presence of the larger organisms that inhabit wetlands through time (e.g. Giguet-Covex *et al*., 2014).

Methods

In May 2017 four wetlands in KPF were visited (e.g. Figure 1) and the depth of sediment tested using a d-section (or Russian) coring device (Jowsey, 1964). As flowing water precludes the accumulation of sediment, the depth of sediment usually reflects the period since the meander or distributary was cut off from the main channel.

Figure 1. Coring Dead River Lagoon. The density of dead red gum saplings precluded coring from water craft and so hand coring was undertaken from within the lagoon.

Most sites yielded 40-60 cm of sediment (Figure 2) suggesting (based on typical sedimentation rates) that they have accumulated sediment for several decades only and so have been mostly inundated in that time. Prior to then they were likely mostly dry and so there was no net sediment accumulation.

Figure 2. A d-section sediment core extracted from Swan Lagoon within the Perricoota Forest.

Figure 3. DNA was extracted from sediment samples in the laboratory at Federation University.

By contrast, a 160 cm sediment sequence was extracted from Dead River Lagoon in 50 cm long sections (0-50 cm; 50 – 100 cm; 100 – 150 cm; 110-160 cm). Multiple replicate cores of each depth band were taken and stored in split PVC tubes and retained under refrigeration. Three (0-150 cm) sections were taken to France for DNA analysis to be undertaken later by Dr. Charline Giguex-Covex of the *Centre Nationale de Recherché Scientifique*. Five samples were retained for DNA extraction in the Biomedical laboratory at Federation University, Ballarat. Here, extracellular DNA was extracted from these core samples by using a Nucleospin Soil extraction kit with a modified protocol (Figure 3) following Macherey-Nagel (2016) and Taberlet *et al*., (2012).

One sequence of sections (0-160 cm) were prepared for diatom analysis following the technique of Battarbee (1986). Twenty samples were selected, spaced up the core with increasing resolution, for the identification and enumeration of fossil diatoms. At least 100, and as many as 220 diatom valves, were counted from each sample. Interpretation of the wetland condition from the assemblages relied on regional floras (Sonneman *et al*., 2000) and interpretation from previous fossil diatom records (e.g. Gell *et al*., 2005, 2007; Reid *et al*., 2007; Kattel *et al*., 2014).

Results

Sediments

The corer met hard sediments at ~ 170 cm depth and so the 160 cm core (10 cm nose on corer) represents the entire history of the wetland available from preserved sediments. The sediments in the core were fine, grey clays typical of many sediment records from wetlands across the Murray River floodplain (Gell *et al*., 2009). There was no clear stratigraphic variation that may mark major limnological or hydrological events in the past, but again, this is typical of Murray River floodplain wetland sediment records. The core from Swan Lagoon also contained fine grey clays but the granular nature of the middle section (Figure 2) suggested periodic drying.

Diatoms

The diatom assemblages from all samples were dominated by species of *Aulacoseira*. These are the dominant plankton in the River Murray and are known to enter wetlands during connectivity events (Gell *et al*., 2002). So, while the Lagoon was no longer part of the channel, it has remained well connected and has always received high numbers of river plankton. While *A. ambigua* and *A. alpigena* (syn. *A. subborealis*) were well represented in the basal sediments (160 – 110 cm), the early part of the record was dominated by *A. granulata*. This is a relatively heavy diatom that requires active, turbulent river flow. Above 50 cm the relative abundance of the lighter *A. granulata* var. *angustissima* increases consistent with records up, and downstream (Reid *et al*., 2007; Grundell *et al*., 2012).

Most other taxa are relatively rare. Despite these low numbers it is clear that the basal sediments contain benthic and epiphytic taxa including *Cocconeis placentula*, *Cymbella* spp., *Epithemia* spp., *Eunotia serpentina*, *Gomphonema* spp. and *Ulnaria* spp. These decline above 25 cm to be replaced by taxa indicating increased nutrient loads e.g. *Cyclotella meneghiniana*, *Discostella pseudostelligera* (named 'small *Cyclotella* spp.' in Fig 4*)* and *Nitzschia* spp. This turnover of taxa is shown in Fig 4 and the latter taxa separate the upper samples into cluster 1 in the ordination (Fig 5). It parallels similar records of change from other wetlands along the River (Grundell *et al*., 2012; Kattel *et al*., 2014).

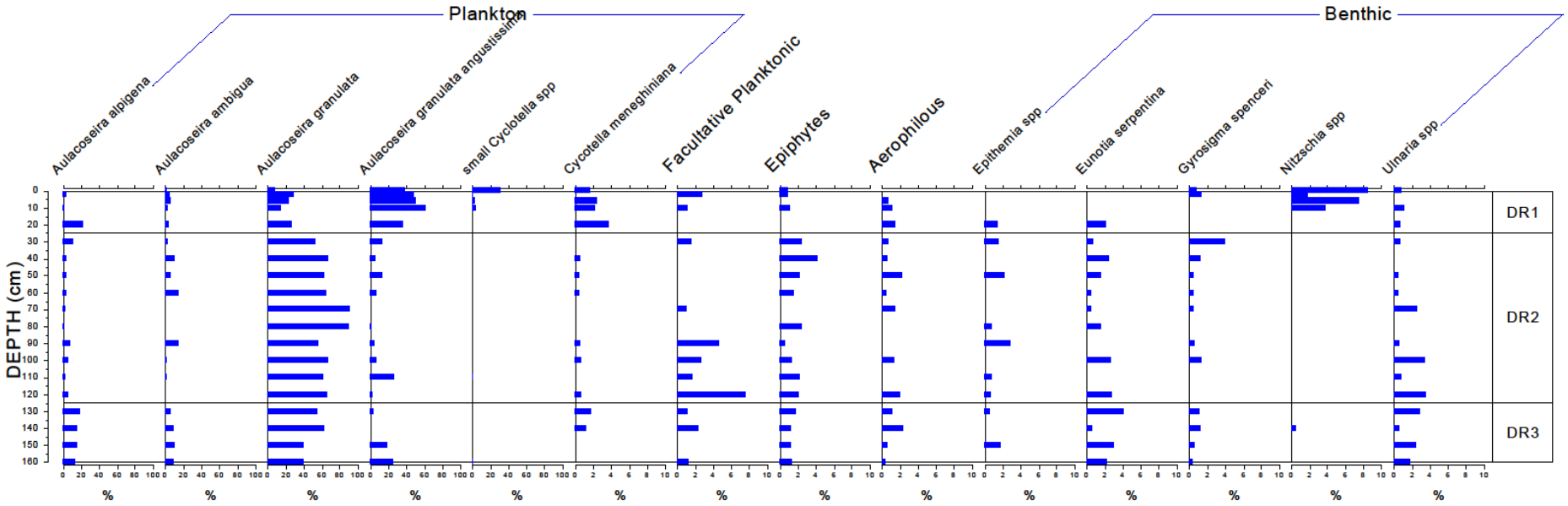

Figure 4. Stratigraphic record of selected diatom taxa from the Dead River Lagoon core (drawn using C2 (Juggins, 2003). Note the change in scale. The zone boundaries mark points of sustained change in the diatom assemblages.

The cores consistently record, albeit in very low abundance, diatom species that are reflective of acid waters, likely derived from organic acids from the floodplain. These include *Eunotia* spp., *Pinnularia* spp. and *Tabellaria flocculosa*. These are not in sufficient numbers in any sample to suggest a major acidification event (although this cannot be ruled out without contiguous sampling throughout the core). In several levels there are also taxa reflective of elevated salinity (*Gyrosigma* spp.; *Tryblionella* spp.), pH (*Cocconeis placentula, Epithemia* spp.) and turbidity (*Staurosira* spp.; *Staurosirella* spp.), again albeit in low numbers.

Detrended Correspondence Analysis (DCA) was applied to the square root transformed diatom data (with rare species down-weighted as outlined in Birks, 2010) from the sediment core samples. As the gradient length was < 2.5 both Correspondence Analysis (CA) and DCA were used further. The CA revealed an arch effect and so the detrended ordination was used to explore the relations between samples (Fig. 5).

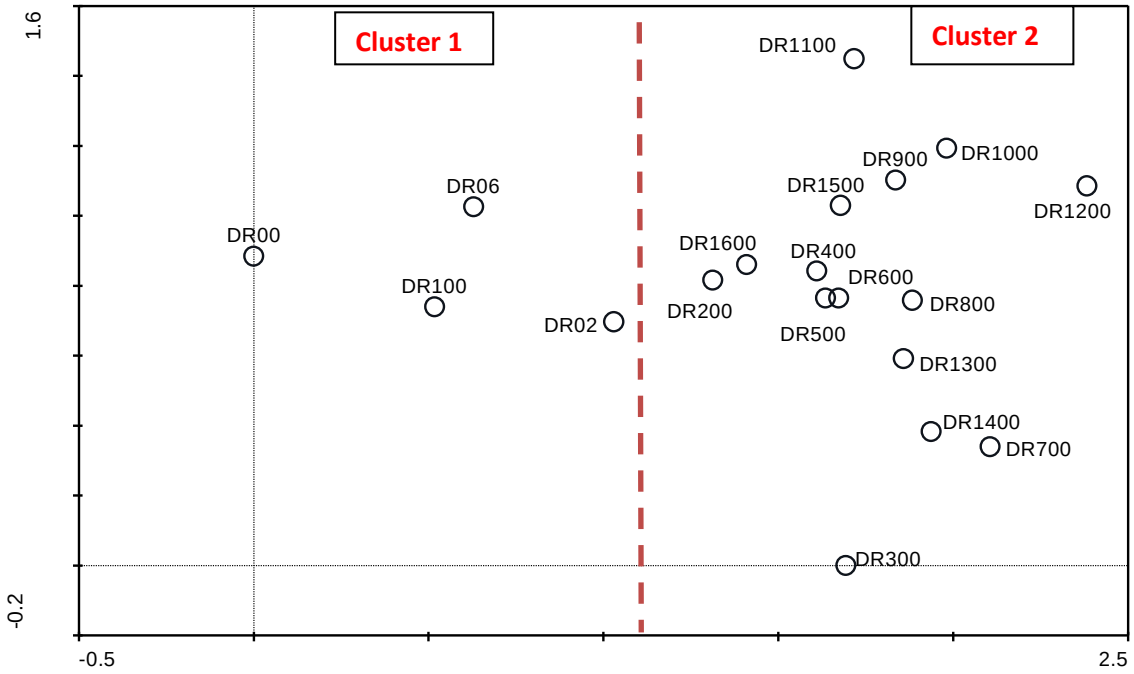

Figure 5. DCA of the Dead River Lagoon fossil diatom data. Labels refer to core depths in mm. The ordination separates all samples in the upper 10 cm of the core suggesting recent change in the diatom assemblage of the Lagoon.

While most of the deepest samples plotted to the top of cluster 2, the major change in the record as reflected by the ordination is in the upper 10-20 cm (cluster 1) where the sediments contained relatively high numbers of nutrient indicator taxa (Fig. 4) suggesting a recent deterioration in water quality.

Sedimentary DNA

The concentration of DNA extracted from the five samples is reported in Table 2. These are relatively high concentrations reflecting the potential of identifying evidence in the past of biota that are rarely detected from within sediment profiles.

Table 2. Concentrations of DNA extracted from Dead river lagoon sediment samples.

Sample CODE	Core Depth (cm)	DNA concentrations ng/ul	DNA identifications
DR1	81-83	165.50	
DR 2	91-93	151.60	
DR 3	101-103	119.00	
DR4	111-113	92.00	*Gambusia*
DR 5	158-160	668.00	

General primers were designed to identify the presence of five species of fish (Hardy *et al*., 2011) from these samples (*Galaxias*, *Gambusia*, *Cyprinus*, *Anguilla* and *Ambassis*). The qPCR was performed on a Viia™ 7 Real–Time PCR System (Life Technologies, USA) by using The SensiMix™ SYBR® Low-ROX Kit from Bioline Pvt Limited, Australia. The reactions were conducted under the conditions of 10 min at 95 °C, and 30 cycles of 30 s at 95 °C, 45 s at 54 °C and 30 s at 60 °C. Analysis of the data was performed with Viia™ 7 Software version 1.2.4. Samples were analysed in duplicate and for each primer, one negative template control (NTC) was used.

Of the primers used only *Gambusia* was detected (Figure 6). This is likely due to the challenge of matching the fragmented sequences and does not preclude the presence of other taxa in the wetland through time. This exotic species likely entered the Murray River after the 1940s (Merrick & Schmida, 1984) suggesting that the sediments at this depth were deposited some time over the last 70 years.

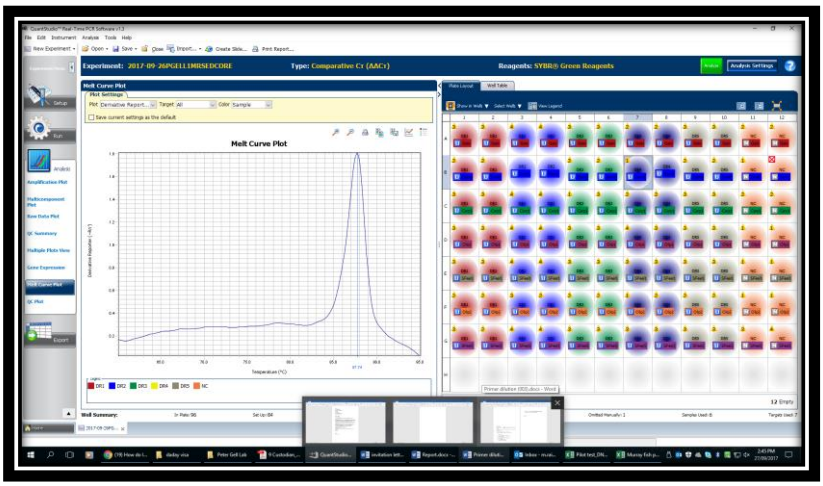

Figure 6. A melt curve typical of *Gambusia* from the sediment sample at 111-113 cm depth.

Discussion

Core Chronology

Without dating the chronology of the core remains uncertain. As the patterns in the changes of diatoms observed here can be seen also in many sites between Albury and Blanchetown it is possible to suggest a tentative chronology. Typically *Aulacoseira alpigena* increases greatly soon after regulation (Fluin *et al*., 2010), along with increases in species indicative of increased nutrients. This would place the onset of river regulation at 35 cm and suggest that the entire core may span ~ 200-300 years. Alternatively, the high *Aulacoseira alpigena* values between 160-130 cm may mark the post-regulation period suggesting the record only spans 90-100 years. As post regulation sediments have been found to accumulate at anything from 0.5 – 5 cm yr^{-1} either is plausible, however, the latter model would place the rise in nutrient indicators to the last 15 years which would be much later than recorded elsewhere.

The analysis of preserved DNA provides a new means of establishing a chronology for sediment sequences. The identification of *Gambusia* at 110 cm suggests that these sediments were deposited after the 1940s and possibly even more recently. On the basis of this evidence, it is likely that the 160 cm long sediment record spans the post regulation period. On average then, at least 2.0 cm of sediment was deposited annually since that time.

Wetland Change

The diatom assemblages preserved in 20 sediment samples from the Dead River Lagoon core reflect a clear water, mostly circum-neutral (pH 6.5-8) water body after regulation, with regular connection to the main river channel. Many disturbance-sensitive taxa declined to be replaced by those tolerant to elevated nutrient loads and water turbidity. The loss of benthic and epiphytic taxa suggests that the lagoon was once more rich in submerged aquatic plants in the past.

When wetlands are inundated they receive input of fluvial (and aeolian) sediments which is deposited. Also, there is an increase in organic productivity which adds to the sediment accumulation rate. While the wetland is inundated anoxia precludes decomposition. These sediments continue to accumulate and, as radiometric dating profiles from wetlands across the Murray Floodplain (Gell *et al*., 2009) suggest, appear not to be lost through scouring under flood. However, when the wetland has dried the organic matter may decompose and the sediments may deflate and be lost from the wetland. So, a sediment core records material from the time a wetland transitioned from a channel to a lagoon, or became inundated so regularly that the input and production of material exceeded the rate of loss during drying events.

The lack of preserved sediments beyond 160 cm suggests that there was no net sediment deposition before regulation. As the wetland is situated near to the main River channel this is unlikely to be due any geomorphic change physically isolating the Lagoon from river water. In this case then, while modelling suggests frequent overbank flows which may have inundated the Lagoon naturally in the past, it was not until sometime after regulation that water was sufficiently permanent to lead to a net accumulation of sediment. So, It is more likely that the natural state of the lagoon is one that was occasionally wet, when relatively low sediments loads could settle, with extended periods of drying whereupon the sediments were able to deflate. Since regulation, the high net accumulation

of sediments is likely attributable to sustained, elevated river levels that have changed the wetland into a permanently wet state.

Further Work

While the similarity of this record with others down the floodplain, and the presence of *Gambusia* at 111 cm, suggest that this record spans the period following river regulation (1922-36 and later) this interpretation would be reinforced by undertaking ^{210}Pb dating analysis. The inferred decline in water quality and plant cover would be supported by direct analysis of plant macrofossils and/or pollen. More detailed analysis of sediment DNA may provide insights into faunal changes associated with the inferred decline in water quality and support the chronology by identifying point of the arrival of carp.

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