Australian National University

Wildlife Biodiversity in Ginninderry Conservation Corridor:

DNA-based assessment from water samples

CEG Lab

Conservation and Environmental Genomics Labs

Fenner School of Society and Environment

+61 2 6125 5018

Linda.Neaves@anu.edu.au

The Australian National University

Canberra ACT 2600 Australia

www.anu.edu.au

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Aims/purpose

Assessment of biodiversity present within the Ginninderry Conservation Corridor via environmental DNA from water samples.

Background and methods

All organisms shed DNA into the surrounding environment via skin, hair, scales etc. This environmental DNA (eDNA) can be collected and sequenced to provide information on the organisms that are present within an area (Taberlet et al. 2012; Rees et al. 2014; Valentini et al. 2016). Comparisons with traditional sampling methods indicate sampling of eDNA is an effective and cost-efficient approach to biodiversity monitoring, and can be superior in terms of effectively detecting rare, cryptic or elusive species (Biggs et al. 2015; Valentini et al. 2016).

Once received, samples were stored at -20°C until processing and DNA sequencing. DNA was extracted from water filters; and a vertebrate-specific region of DNA, suitable for species identification, was then targeted and sequenced. Negative controls were included in all steps to monitor for contamination. The resultant DNA sequences were compared to a local reference dataset, comprised of relevant sequence data. For a detailed overview of methods, see the Appendix.

Sample Summary

Water samples were collected from three sites, in 2021 and 2022 from the Ginninderry Conservation Corridor (Figure 1 & Appendix). Between five and ten samples were collected from each site and between 70 and 720 ml of water were filtered per sample and supplied for processing (Appendix 1).

Figure 1: Locations of sampling sites in the Ginninderry Conservation Corridor

Results Summary

Overall, 44 species were identified in the water samples, including both native and non-native species.

Table 1: Summary of groups identified at each site. *Due to limitations of identification among dabbling duck species (*Anas*) this group may include introduced species and therefore ranges are supplied.

	Bidgee Dam 2022	Double Dam 2022	Triangle Dam 2022	Double Dam 2021	Triangle Dam 2021	Total
NATIVE						
Mammals	6	3	6	3	4	**10**
Fish	0	0	1	0	0	**1**
Reptiles	0	1	1	1	1	**1**
Frogs	0	0	0	1	0	**1**
Birds*	8-9	11-12	8-9	3-4	8-9	**18-19**
NON-NATIVE						
Mammals	5	5	5	3	3	**8**
Fish	2	2	2	2	2	**2**
Birds*	1-2	1-2	1-2	0-1	0-1	**2-3**

Species detected

The most common native species detected:

1. Australian wood duck
2. *Anas* sp – which may include pacific black duck and/or domestic mallard
3. Wedge tailed eagle
4. Eastern long-necked turtle

The most common non-native species detected:

1. Domestic cow
2. Common carp / goldfish
3. Oriental weatherloach

Potentially listed/vulnerable species

1. **Murray cod** was detected in one sample from Triangle Dam in 2022

Several terrestrial species were detected in the water samples. While these are not necessarily aquatic or semi-aquatic in behaviour, many species will interact with waterways, either in moving through it, or using the waterways for drinking or bathing. Use of the surrounding habitat can also lead to shed DNA entering the waterways and being detected, particularly after rains. This demonstrates the sensitivity of the approach; however, while these interactions allow for terrestrial species to be detected in water samples, it may not be the most reliable tool for monitoring terrestrial species. These samples were sequenced with a high level of sequencing

coverage (higher than normal for our process because of the number of samples) which can allow for less abundant DNA to be detected and increase the diversity of species detected. Consequently, single sample identifications here should be treated with caution. Equally, there may be species that have been observed in the area but not detected. There are several reasons why this can occur. Interactions with the water may have been limited prior to sampling – DNA only lasts in the environment for a limited period of time and so no DNA, or not enough to allow detection, was present in the samples. Similarly, even aquatic or semi-aquatic species in low abundance may go undetected as there is insufficient DNA present in the sample to confirm detections using this approach.

A small number of species are difficult to distinguish using this method, such as common carp and goldfish, or species of *Anas*, which include both pacific black duck and domestic mallards (and their hybrids). More detailed and targeted approaches would be required to confirm the presence of both species, and/or hybrids.

Table 2: Detailed list of species at each site. X denotes detected in multiple samples (i.e., at least 2), x denotes detection in a single sample; * denotes introduced species.

Species	Common name	Bidgee Dam 2022	Double Dam 2022	Triangle Dam 2022	Double Dam 2021	Triangle Dam 2021
MAMMALS						
*Bos taurus**	Domestic cattle	X	X	X	X	X
*Canis lupus**	Domestic dog or dingo	-	-	X	-	-
*Dama dama**	European fallow deer	-	X	x	-	-
*Felis catus**	Domestic cat	x	-	-	-	-
*Sus scrofa**	Feral/ Domestic pig	-	x	x	-	X
*Vulpes vulpes**	European red fox	x	-	-	-	-
*Mus musculus**	House mouse	X	x	-	x	x
*Rattus rattus**	Black rat	x	x	x	X	-
Macropus giganteus	Eastern grey kangaroo	x	-	X	x	-
Macropus robustus	Common wallaroo	x	-	-	-	-
Vombatus ursinus	Common wombat	x	X	x	-	x
Antechinus sp	Antechinus species	x	-	-	-	x
Rhinolophus megaphyllus[1]	Eastern Horseshoe Bat	-	-	-	-	X
Pteropus sp	Flying fox species	-	x	-	-	x

Pseudocheirus peregrinus	Common ringtail possum	-	-	x	-	-
Petaurus breviceps	Sugar glider	-	-	x	x	-
Ornithorhynchus anatinus	Platypus	X	-	x	-	-
Hydromys chrysogaster	Rakali (Australian water-rat)	x	X	x	x	-
FISH						
Cyprinus carpio/ Carassius auratus**	Common carp / goldfish	X	X	X	X	X
*Misgurnus anguillicaudatus**	Oriental weatherloach	X	X	X	x	X
Maccullochella peelii	Murray cod	-	-	x	-	-
REPTILES						
Chelodina longicollis	Eastern long-necked turtle	-	X	X	X	X
FROGS						
Crinia signifera	Common eastern froglet	-	-	-	x	-
BIRDS						
Aquila audax	Wedge-tailed eagle	X	X	X	X	x
Anas sp	Species of dabbling ducks (including *A. superciliosa:* Pacific black duck and *A. platyrhynchos** domestic mallard)	X	x	X	X	X
Aythya australis	Hardhead	-	-	x	-	-
Charadrius/ Vanellus sp	Plover species	x	-	-	-	-
Chenonetta jubata	Australian wood duck	X	X	X	X	X
Phalacrocorax melanoleucos	Little pied cormorant	x	-	x	-	-
Acanthiza pusilla	Brown thornbill	-	-	x	-	-

Acanthiza reguloides	Buff-rumped thornbill	X	x	X	x	x
Anthochaera carunculata	Red wattlebird	-	-	x	-	-
Ceyx azureus	Azure Kingfisher	-	x	-	-	x
*Columba livia**	Rock dove/pigeon	x	x	-	-	-
Cormobates leucophaeus	White-throated treecreeper	x	x	-	-	-
Gymnorhina tibicen	Australian magpie	-	x	-	-	-
Dacelo novaeguineae	Laughing kookaburra	-	x	-	-	-
Malurus cyaneus	Superb fairywren	-	x	-	-	x
Ninox novaeseelandiae	Southern boobook	-	-	-	-	x
Philemon corniculatus	Noisy friarbird	-	X	x	-	X
Tyto sp. (likely T. alba)	Barn/Grass owl species (most likely Barn Owl)	-	x	-	-	x
*Acridotheres tristis**	Common myna	-	-	x	-	-
Smicrornis brevirostris	Weebill	X	X	-	-	-
Estrildidae	Family of finches	x	-	-	-	-

[1] *Rhinolophus megaphyllus* was the mostly likely match identified and has been reported in the ACT, but not recently. Thus, this identification should be treated with caution and it may represent another species. These bats are not handled in the lab and so contamination is unlikely.

Details of methods applied

Samples were frozen at -20°C upon receipt. The Conservation and Environmental Genomics labs maintain physical separation of pre- and post- PCR amplification processes to minimise contamination. All surfaces are sterilised with bleach and UV light before use, and gloves are worn at all times to minimise contamination. In addition, negative controls, consisting of extraction and PCR controls, which are processed as samples but with no sample included, were included in all steps to monitor for contamination.

DNA extraction was performed using the New England Biolabs (NEB) Monarch DNA extraction kit, using the manufacturer's protocol with overnight lysis. DNA libraries for assessing vertebrate biodiversity were constructed in a two-step process. Firstly, a universal PCR assay (Riaz et al. 2011) targeting a small region of the mitochondrial DNA was amplified using Q5 Taq (New England Biolabs). If a sample produced a product, unique indexes for each sample were incorporated via a second round of PCR. Individual amplicon-sample combinations were pooled in equimolar concentrations, library quality was assessed and sequencing was then performed on an Illumina MiSeq at the Biomolecular Resource Facility, Australian National University.

The sequences from the pooled sample were demultiplexed based on their unique indexes. Quality control filtering removed primer sequences (cutadapt; Martin 2011), truncated reads, and chimeric sequences following the DADA2 pipeline (Callahan et al. 2016). The sequences were then clustered into Amplicon Sequence Variants (ASV: Callahan et al. 2017), these are sequences with 100% identity (i.e. DNA sequence matches). Amplicon Sequence Variants were then assigned to a species (or higher taxonomic level) using a Naive Bayesian classifier (Wang et al. 2007) to a local reference set. The local reference dataset was generated using publicly available sequence data from GenBank (www.ncbi.nlm.nih.gov), for species recorded in the Ginninderry Conservation Corridor, and more broadly across the ACT. For ASVs that did not produce a match, these were manually tested using BLASTN (Altschul et al. 1990; McGinnis & Madden 2004) searches against the entire Genbank repository (May 2023; Sayers et al. 2023), and then adding species to the local reference data set based on their reported geographic distribution using information from the Atlas of Living Australia (ALA) and Canberra Nature maps (https://canberra.naturemapr.org/). Where an ASV could not be resolved to a single species (due to shared haplotypes, for instance), either a list of multiple species was included, or it was assigned to the lowest taxonomic rank without further classification.

If you require any additional information, don't hesitate to get in touch with us.

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Appendix 1: Detailed information on individual samples.

#Sample code	Waterway	Sample	Collection date	Latitude	Longitude	Volume	Person collected
ABD01	Bidgee Dam	1	6/02/2022	-35.2313	148.9714	420ML	NA
ABD02	Bidgee Dam	2	2/06/2022	-35.231	148.9712	720ML	NA
ABD03	Bidgee Dam	3	6/02/2022	-35.2308	148.9715	340ML	NA
ABD04	Bidgee Dam	4	2/06/2022	-35.2311	148.9716	300ML	NA
ABD05	Bidgee Dam	5	2/06/2022	-35.2312	148.9712	300ML	NA
ADD01	Double Dam	1	5/17/2022	-35.2379	148.9891	102ML	NA
ADD02	Double Dam	2	5/17/2022	-35.2379	148.9894	103ML	NA
ADD03	Double Dam	3	5/17/2022	-35.2378	148.9895	70ML	NA
ADD04	Double Dam	4	5/17/2022	-35.2377	148.9896	116ML	NA
ADD05	Double Dam	5	17/05/2022	-35.2375	148.9898	70ML	NA
ADD06	Double Dam	6	17/05/2022	-35.2379	148.9899	120ML	NA
ADD07	Double Dam	7	17/05/2022	-35.2379	148.9898	107ML	NA
ADD08	Double Dam	8	17/05/2022	-35.238	148.9896	40ML	NA
ADD09	Double Dam	9	5/17/2022	35.23809	148.9893	120ML	NA
ADD10	Double Dam	10	5/17/2022	-35.238	148.9892	120ML	NA
ATD01	Triangle Dam	1	6/02/2022	-35.2279	148.9719	300ML	NA
ATD02	Triangle Dam	2	6/02/2022	-35.2277	148.9718	1180ML	NA
ATD03	Triangle Dam	3	6/02/2022	-35.2277	148.9715	720ML	NA
ATD04	Triangle Dam	4	2/06/2022	-35.2279	148.9713	480ML	NA
ATD05	Triangle Dam	5	6/02/2022	-35.228	148.9716	720ML	NA
DD01A	Double Dam	1	11/10/2021	-35.2379	148.9893	200ML	Tyson
DD02A	Double Dam	2	11/10/2021	-35.2379	148.9893	250ML	Tyson
DD03A	Double Dam	3	11/10/2021	-35.2376	148.9897	400ML	Tyson
DD04A	Double Dam	4	11/10/2021	-35.2376	148.9898	300ML	Tyson
DD05A	Double Dam	5	11/10/2021	-35.2377	148.99	70ML	Bridie
DD06A	Double Dam	6	11/10/2021	-35.2379	148.9899	250ML	Tyson
DD07A	Double Dam	7	11/10/2021	-35.2379	148.9897	250ml	Tyson
DD08A	Double Dam	8	10/11/2021	-35.238	148.9896	250ml	Tyson
DD09A	Double Dam	9	11/10/2021	-35.238	148.9894	210ML	Tyson
DD10A	Double Dam	10	11/10/2021	-35.238	148.9892	210ml	Tyson
TD01A	Triangle Dam	1	11/08/2021	-35.2279	148.9718	350ml	Rachel
TD02A	Triangle Dam	2	11/08/2021	-35.2277	148.9713	150ML	Rachel

TD03A	Triangle Dam	3	11/08/2021	-35.2277	148.9714	700ML	Rachel
TD04A	Triangle Dam	4	11/08/2021	-35.2276	148.9718	156ML	Bridie
TD05A	Triangle Dam	5	11/08/2021	-35.2278	148.9719	350ML	Bridie
TD06A	Triangle Dam	6	11/08/2021	-35.228	148.9719	150ML	Tyson
TD07A	Triangle Dam	7	11/08/2021	-35.228	148.9718	200ML	Bridie
TD08A	Triangle Dam	8	11/08/2021	-35.2279	148.9715	54ML	Bridie
TD09A	Triangle Dam	9	11/08/2021	-35.2279	148.9714	174ML	Bridie
TD10A	Triangle Dam	10	11/08/2021	-35.2279	148.9713	250ml	Bridie