UNIVERSITY OF CANBERRA

National eDNA Reference Centre

PROJECT: ENVIRONMENTAL DNA METABARCODING OF VETERBRATE BIODIVERSITY THROUGHOUT THE GINNINDERRY CONSERVATION CORRIDOR.

Date: 31/August/2023

Prepared by:

EcoDNA group-National eDNA Reference Centre

University of Canberra

EcoDNA

EcoDNA@canberra.edu.au

EXECUTIVE SUMMARY

EXECUTIVE SUMMARY

The National eDNA Reference Centre (NRC) was contracted by the Ginninderry Conservation Trust (GCT) to undertake environmental DNA-based surveys for vertebrate fauna within the Ginninderry Conservation Corridor within the Australian Capital Territory and New South Wales. Ten sites were sampled across two different time periods (Summer: 6/12/2022 and Autumn: 26/04/2023) by GCT officers following training by the NRC. All samples were analysed using broad spectrum primers designed to amplify a 125 bp fragment within the conserved 12SV5 gene region.

Testing showed positive detections for 14 birds, 11 mammals, eight fish, two amphibians, and one turtle species. Of importance, platypus DNA was detected during Autumn in the Murrumbidgee River, frog DNA was only detected in Dams during Summer and detections for carp and golden perch corresponded to known and confirmed prior reports, as well as stocking of fish in corresponding sites.

The Murrumbidgee River showed the highest diversity for fish species in Summer and Autumn, while no fish species were detected in Goodamon creek, Leaky Dam, Double Dam, site B5 and Woodland Dam. High bird diversity was mostly associated to dam sites sampled in this study. All sites showed significant differences in alpha diversity with the exemption of Belconnen farm dam and Goodamon Creek, and sites B5, Link dam, Murrumbidgee dam, Murrumbidgee River and Woodland dam showed significantly higher species diversity in samples collected during Autumn compared to samples collected during Summer. Contrastingly, Double dam, Leaky dam and Triangle dam showed significantly higher species diversities in samples collected during Summer compared to Autumn.

Future sampling should consider increasing sequencing depth to improve detection of rare species across sites, increase sampling for winter and spring, and increase sampling effort in the Murrumbidgee River to better assess ecosystem use by important species such as the resident platypus within the Ginninderry Conservation Corridor.

TABLE OF CONTENT

TABLE OF CONTENT
CONTENTS

BACKGROUND

BACKGROUND AND REPORT OBJECTIVE

BACKGROUND

The Ginninderry Conservation Corridor is a 242-ha section of land spanning the ACT/NSW border and Murrumbidgee River and Ginninderra Creek (Ginninderry (2023) *Conservation*). The land is managed by the Ginninderry Conservation Trust (GCT) which is comprised of community representatives. Biodiversity conservation and management is a significant component of GCT initiatives, and the trust engage a variety of stakeholders and volunteers to survey and manage biodiversity throughout the corridor. Opportunities to conduct research with various research sectors are encouraged and this aims to promote, conserve, and manage the biodiversity found throughout the diverse and culturally significant ecosystems that the land is situated on.

The corridor offers a unique mix of ecosystem services for both human activities and biodiversity conservation. Pink Tailed Worm Lizard (*Aprasia parapulchella*) and Golden Sun Moth (*Synemon plana*) are prioritised for biodiversity surveys and habitat preservation, while the Murrumbidgee River corridor is habitat for aquatic fauna such as the Macquarie Perch, (*Macquaria australasica)*, Two-Spined Blackfish, (*Gadopsis bispinosus)* and Murray Crayfish (*Euastacus armatus)*. Invasive species such as foxes (*Vulpes vulpes*) and deer species are notable occupants throughout the corridor while historical sightings of endangered species such as the Platypus have been reported to the trust. While the current land size is 242-ha, expansion of the corridor is planned to continue with an estimated size of 596-ha once fully developed alongside continued urban development (Ginninderry Interim Management Plan, 2018). Survey methods that can accurately monitor and manage biodiversity throughout the expanding corridor are needed especially those that are efficient with resources available to the trust.

Environmental DNA (eDNA) surveys were proposed as being useful to monitoring corridor biodiversity by providing a tool for GCT personnel to employee at their discretion. Environmental DNA (eDNA) based approaches utilise shed DNA from organisms to detect species presence from environmental samples such as water or soil or air (Pawlowski et al., 2020, Ficetola et al., 2008). They have been shown to be highly effective at detecting a range of biodiversity across various ecosystems whilst resource efficient. Single-target testing is often employed to detect presence of invasive and endangered species, especially when other survey methods are limited in their detection. Broader assessment of taxa and communities through detection of multiple species eDNA can also be employed with, termed ‘metabarcoding’ analysis.

Environmental DNA-based surveys could offer GCT with complementary monitoring tools to assess diversity. With on-going management of the GCC conducted by GCT personnel, the purpose of this project was for the NRC to provide technical and on-ground support that facilitates appropriate eDNA sample collection and confidence in eDNA surveys through the detection of vertebrate biodiversity detected in samples collected throughout the GCC over two sampling occasions.

BACKGROUND

REPORT OBJECTIVE

The NRC (University of Canberra) was asked to collect and analyse environmental DNA (eDNA) water samples from sites collected throughout the Ginninderry Conservation Corridor. Surveys were conducted on two occasions, in December 2022 and April 2023, with eDNA metabarcoding employed to analyse vertebrate biodiversity detected in samples. The NRC also provided equipment and training for the surveys and engaged GCT field officers to facilitate the collection of samples.

The project had three overall objectives:

- Undertake eDNA-based analysis using metabarcoding techniques for a total of 20 samples (on each sampling occasion), which will be collected by the Client and provided to the University.

- Provide technical support and provide the Client with a Smith-Root eDNA sampler for the purpose of sample collection.

- Provide insights into the vertebrate biodiversity detected from samples such as fish, mammal and bird, amphibian, and reptile fauna.

ENVIRONMENTAL DNA METABARCODING OF VERTEBRATE BIODIVERSITY IN THE GINNINDERRY CONSERVATION CORRIDOR

AIM

Perform eDNA metabarcoding analysis of 40 filter collected eDNA samples and field controls, over Spring and Autumn sampling events, and provide insights into vertebrate biodiversity DNA detected in samples. Provide appropriate equipment and support to GCT personnel for the purpose of sample collection.

METHODS

Site selection

Ten sites were selected for eDNA sampling based on consultation between GCT field officers and NRC researchers. A range of sites were selected considering ecosystem dynamics such as ecosystem type and water availability, previous and current knowledge of biodiversity presence at sites and future management of sites (i.e., corridor expansion). A total of two samples were collected per site at two different time periods: Summer (06/12/2022) and Autumn (26/04/2023).

Table 1: Overview of sites sampled for eDNA metabarcoding surveys. Two samples were collected at sites by NRC and GCT personnel.

Sample site	Latitude	Longitude	Sampling Date
Triangle Dam (TD)	-35.230647	148.97507	06/12/2022, 26/04/2023
Goodamon Creek (GC)	-35.229032	148.97741	06/12/2022, 26/04/2023
Murrumbidgee Dam (MD)	-35.227841	148.971141	06/12/2022, 26/04/2023
Murrumbidgee River (MR)	-35.237674	148.976188	06/12/2022, 26/04/2023
Leaky Dam (LD)	-35.248488	148.964647	06/12/2022, 26/04/2023
Belconnen Farm Dam (BF)	-35.216809	148.974609	06/12/2022, 26/04/2023
Double Dam (DD)	-35.237923	148.989518	06/12/2022, 26/04/2023
B5	-35.232191	148.992356	06/12/2022, 26/04/2023
Woodland Dam (WD)	-35.232191	148.99616	06/12/2022, 26/04/2023
Link Dam (LD)	-25.231689	148.994722	06/12/2022, 26/04/2023

Sample collection and processing

The NRC provided field officers of the GCT with a Smith-Root eDNA sampling backpack and Smith-Root self-preserving filter papers for surveys (5.0uM). Briefly, officers were trained on using the sampling backpack and filters for sample collection. Sampling was undertaken by the NRC researchers and GCT officers who were advised by the NRC as required (Figure 1).

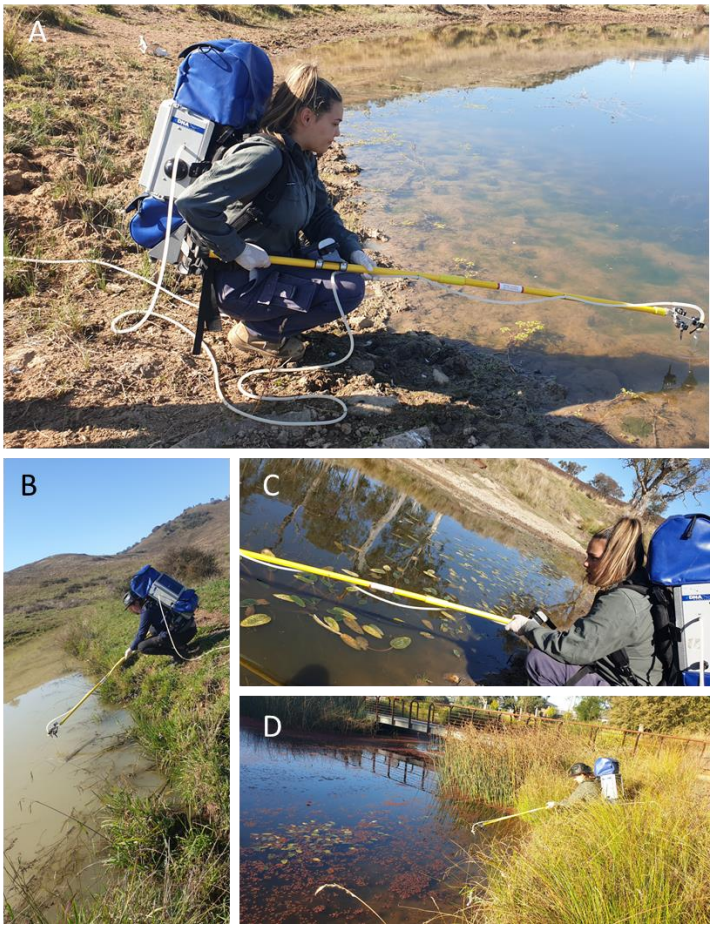

Figure 1. GCT field officers collecting samples using provided eDNA equipment at sites: A) Murrumbidgee Dam B) Triangle Dam C) Woodland Dam and D) Link Dam.

The NRC also provided technical and on-ground support for the sampling occasions. At each site, two samples were filtered and processed using the eDNA sampling equipment which was performed by filtering water directly from the water body using the sampling pole and attached filter housing and nozzle. Continuous filtering occurred using the manual setting of the sampling backpack until the filter clogged (i.e., no more filtrate moving through the system), and the final sample volume was recorded. The filter housing was then removed from the sampling pole and stored in the original packaging for further processing. Between each sampling site, sampling equipment was lightly sterilised using bleach to further reduce contamination risk between sites. Field negative controls were taken at two of the sites during for each sampling event where 1L of previously UV treated water was submerged closed in a Nalgene bottle, exposed to the air for one minute before being filtered using the same method as for eDNA samples. All

packages containing the filter papers were stored in an esky and frozen at -20 °C for later extraction and processing at the NRC Trace DNA Laboratory.

eDNA extraction

Prior to extraction, filter housing packaging was wiped with a 10 % bleach solution to limit potentially contaminating DNA from entering the NRC Trace DNA Laboratory. Filter housings were then removed from packaging and the filters were exposed by carefully opening the filter housing. Sterile forceps were used to transfer the filter paper into a new tube. Extraction was then performed using a modified Qiagen Blood and Tissue Kit protocol where each filter was lysed in 360 µL of ATL buffer and 40 µL of Proteinase K and incubated at 56 °C for one hour (Hinlo et al., 2017). Following incubation 400 µL of AL buffer and 400 µL of 100 % ethanol was added to the sample tubes. The Qiagen protocol was then followed as prescribed, and samples were eluted in 100 µL of buffer AE Samples were quantified using a Nanodrop One C (Thermofisher) and 1:10 and 1:100 dilutions were created for downstream analyse and optimisation.

Library construction, PCR amplification and analyses

Each dilution was tested for optimal amplification using the 12SV5 primer set from Riaz et al. (2011). The effect of PCR inhibition in samples was evaluated by performing a single qPCR replicate for the neat and diluted samples. Reaction components and concentrations are reported below (Table 2).

Table 2. qPCR reaction components and volumes for the 12SV5 metabarcoding primer set, targeting the 12S gene region.

Component	Reaction volume (µL)
DNase/RNase-free UltraPure ™ dH2O	12.85
GeneAmp ™ 10X Gold Buffer	2.5
$MgCl_2$ [25 mM]	2
UltraPure ™ BSA [50 mg/mL]	0.2
SYBR ™ Green I Nucleic Acid Gel Stain [5x, 1:2000 dilution water]	0.6
GeneAmp ™ dNTP Blend [10 mM]	0.65
Amplitaq Gold DNA Polymerase	0.2
12SV5-F [10 µM]	1
12SV5-R [10 µM]	1
DNA Template	4
Total volume	25

Quantitative PCR reactions were performed using a QuantStudio™7 Pro (Applied Biosystems) with thermal cycling profiling comprised of an initial activation step at 95 °C for 5 min, followed by 50 X 3-step cycles of 30 s at 95 °C, 30 s at 57 °C and 1 min at 72 °C. This was followed by a final extension step of 10 min at 72 °C and a melting curve with a continuous increase of 0.05

°C/sec from 60 °C to 95 °C. The most optimal dilution (i.e., based on Cq-value and melt curve analysis) of each sample was selected for library preparation.

All eDNA samples and controls were included for construction of high-throughput sequencing libraries using a one-step PCR amplification workflow. Combinatorial dual indexing (CDI) of forward and reverse fusion tagged primers (FTP) was employed to amplify and tag samples for identification purposes in downstream high-throughput sequencing (HTS). Fusion-tagged primers were comprised of a flow-cell binding region, a read1/read2 metabarcoding primer sequence, a unique 7bp in-line Multiplex Identification (MID)tag, and the target-specific 12SV5 primer sequence. Triplicate qPCR reactions were performed using the previously described conditions and thermal cycling profile. After qPCR amplification, amplicon pools were generated by combining 11 - 12 uniquely labelled amplicon libraries based on their average Cq-values. The volumes used to construct amplicon pools were determined based on the total number of positive PCR replicates to ensure an equal representation of the amplicons from each sample. Samples where no amplification occurred (i.e., field or extraction controls) were not included in pools but if amplification was observed in any replicate, then these samples were included in the pooling stage. Amplicons were cleaned using Agencourt AMPure XP Beads (Beckman Coulter) in a 1.8:1 bead to sample volume ratio and amplicon concentrations for each pool were determined using a Qubit™ HS Assay. Purified amplicon pools were normalised, and a single amplicon library was constructed by combining equal quantities from each pool followed by a final clean-up step as described previously. Finally, this single library was combined in ratio with other projects also targeting the 12SV5 primer set and other gene regions. The final library containing was sequenced on a MiSeq sequencing machine (Illumina, San Diego, CA) using a V3 2 x 300 bp sequencing kit.

The raw sequence data was filtered and analysed using a bio-informatics pipelines developed at the University of Canberra for sample demultiplexing and then following the DaDa2 general workflow (Callahan et al., 2016) using RStudio (v. 2021.09.1). Briefly, forward, and reverse sequences were assessed for quality (Phred>35) and length (120 bp target amplicon length). Forward and reverse sequences were then paired and any sequences that did not match perfectly were removed. Lastly, chimeric sequences (i.e., artifact sequences formed by two or more biological sequences incorrectly joined together) were removed from all samples. Taxonomic identity was completed using a custom reference database for the 12SV5 primers used in this study using the CRABS bioinformatic package (Jeunen et al., 2023) and confirmed by BLASTn (Altschul et al., 1990). Taxonomic identity was only accepted when pairwise similarity achieved >99 % across the entirety of the sequence length.

RESULTS AND DISCUSSION

A total of 40 samples were collected across the two sampling occasions (20 each occasion). Samples were collected by both NRC and GCT personnel using the Smith-Root eDNA Sampler backpack and self-preserving filter papers. All eDNA samples showed amplification during the library preparation stage and subsequently were included for sequencing. A total of 1,395,911 reads were achieved across all samples in this study for the target gene region of 12SV5, with an average of 31,032 ± 5048 SD fully curated reads per sample. All field controls, as expected, showed variable amplification for human, dog, chicken and cow DNA. No other species were detected in any of the four field controls collected in this study.

A total of 677 Amplicon Specific Variances representing 37 species were sequenced in this study. Specifically, 14 birds (Table 3), 11 mammals (Table 4), eight fish (Table 5), two amphibians, and one turtle (Table 6) were detected.

Detection of bird species

Bird species were detected across all sites, except for Goodamon Creek in which no birds were detected (Table 3). Ducks, coots and grebe species were the most common groups of birds detected in this study, with mallard ducks (*Anas platyrhynchos*) being the most common bird species, detected across all sites except for Goodamon creek, followed by Australian woodland ducks (*Chenonetta jubata*), which were detected during both sampling events in Triangle Dam, Belconnen Farm Dam, site B5, Woodland Dam, and Link Dam, while it was only detected in Autumn at Murrumbidgee Dam. Australian coots (*Fulica atra*) were consistently detected during Autumn at Murrumbidgee Dam, Belconnen Farm Dam and site B5 (Table 3). Australian grebe (*Tachybaptus novaehollandiae*) was detected mostly in dam locations (Murrumbidgee Dam, Leaky_Dam, Double_Dam, site B5, Woodland Dam and Link Dam, Table 3).

Detection of mammal species

As expected, human DNA was detected across all sites. Cattle DNA was detected across all sites with very low (<250 reads) detections in the Murrumbidgee River and Link Dam (Table 4). Sheep, pig, dog, mouse, and rat DNA were detected sporadically across sites. Of importance, Platypus (*Ornithorhynchus anatinus*) DNA was detected in both Murrumbidgee River samples collected during Autumn (Table 4).

Detection of fish species

The Murrumbidgee River was by far the most diverse sampling site for fish species. Carp (*Cyprinus carpio*) and Murray cod (*Maccullochella peelii*) were detected during both sampling events at this site. Western carp gudgeon (*Hypseleotris klunzingeri*) was only detected in low abundance during Summer, while mosquito fish (*Gambusia holbrooki*), mountain galaxias (*Galaxias olidus*), and Australian smelt (*Retropinna semoni*), were only detected during Autumn (Table 5). Triangle Dam showed positive detections for carp and pond loach (*Misgurnus anguillicaudatus*) during both sampling events. Murrumbidgee Dam showed positive detection for Macquarie perch (*Macquaria ambigua*) during both sampling events, while Link Dam showed high detection for Macquarie perch in Autumn (Table 5). Low detections were detected for mountain galaxias in Belconnen farm Dam during Autumn, while no fish species were

detected in Goodamon Creek, Leaky Dam, Double Dam, site B5 and Woodland Dam.

Detection of Amphibian and turtle species

Eastern long-necked turtle (*Chelodina longicollis*) DNA was detected across all sites except for the Murrumbidgee River (Table 6). Of importance, high detections were found at Triangle Dam, Leaky Dam, Double Dam, and Link Dam during Summer compared to Autumn, while no major differences were observed in the number of reads for the species at Woodland Dam (Table 6). Frog species (*Litoria* and *Limnodynastes* and *Uperoleia* spp.) were only detected in Summer within dam sites (Murrumbidgee Dam, Leaky Dam, Double Dam, Link Dam).

Table 3. Total sequences reads for Bird species detected across 10 sites within the Ginninderry conservation corridor.

Species	Triangle_Dam		Goodamon_Creek		Murrumbidgee_Dam		Murrumbidgee_River		Leaky_Dam		Belconnen_Farm_Dam		Double_Dam		B5		Woodland_Dam		Link_Dam	
	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023
Anas sp.	0	0	0	0	0	0	0	0	0	0	0	106	0	316	335	2336	0	312	0	0
Anas platyrhynchos	638	0	0	0	31	13826	0	3041	10505	21724	377	2625	269	3139	2020	730	628	1457	3375	2696
Anser anser	0	0	0	0	0	0	0	0	0	0	0	0	0	0	80	0	0	0	0	0
Aythya nyroca	0	0	0	0	0	0	0	0	0	0	0	132	0	0	0	0	0	0	0	0
Chenonetta jubata	6410	338	0	0	0	1093	0	0	0	0	544	72	0	0	13364	508	169	8001	6311	8129
Geophaps lophotes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1006	0	0	0	0
Gallus gallus	0	0	0	0	21	0	30883	0	0	569	54	0	1213	0	31176	67	26797	0	89	73
Fulica atra	0	0	0	0	0	2202	0	0	0	0	0	2027	0	0	0	22595	0	0	0	0
Gallinula sp.	0	0	0	0	0	0	0	0	0	1	0	1392	0	0	0	0	0	0	0	0
Gallinula chloropus	0	141	0	0	0	0	0	0	0	0	0	878	0	0	0	0	0	0	0	439

Zosterops japonicus	0	0	0	0	0	0	0	0	168	0	0	0	0	0	0	0	0	0	0	0
Tachybaptus novaehollandiae	0	0	0	0	93	0	0	0	0	103	0	0	743	0	200	94	0	147	610	0
Cacatua sp.	0	0	0	0	0	0	355	0	0	0	0	0	0	0	201	637	0	382	0	0
Phalacrocorax sp.	0	0	0	0	0	126	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. Total sequences reads for mammal species detected across 10 sites within the Ginninderry conservation corridor.

	Triangle_Dam		Goodamon_Creek		Murrumbidgee_Dam		Murrumbidgee_River		Leaky_Dam		Belconnen_Farm_Dam		Double_Dam		B5		Woodland_Dam		Link_Dam	
Species	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023
Bos sp.	408	1151	210	280	1124	407	0	0	412	15	224	221	187	830	15	18	1297	892	2	0
Bos indicus	4337	38349	51900	48157	51133	26780	0	0	25892	1608	37527	35266	2944	43135	0	3424	20597	21348	42	0
Bos taurus	7949	5220	8587	7832	7251	4000	0	204	3854	243	6366	5670	4069	6511	704	579	2034	2605	3	129
Ovis aries	0	0	0	0	0	0	126	0	0	0	0	0	0	0	0	0	0	0	0	0
Ovis vignei	0	0	0	0	0	0	0	0	0	0	0	129	0	0	0	0	0	0	0	0
Sus scrofa	0	0	0	73	0	0	0	0	0	199	0	0	0	0	0	0	0	0	0	0
Canis familiaris	0	0	0	0	0	0	162	0	0	0	0	0	106	639	0	0	0	85	0	0
Macropus robustus	0	64	53	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	0	0

Ornithorhynchus anatinus	0	0	0	0	0	0	0	2308	0	0	0	0	0	0	0	0	0	0	0	0
Homo sapiens	444	389	250	321	740	404	1169	11916	634	25667	4122	1584	16334	4988	26726	10968	1620	18178	2878	23973
Mus musculus	0	0	0	0	0	0	0	0	0	0	0	91	0	0	0	0	0	0	0	0
Rattus rattus	0	0	0	199	0	0	0	0	0	0	0	58	0	0	0	0	0	0	561	457

Table 5. Total sequences reads for fish species detected across 10 sites within the Ginninderry conservation corridor.

	Triangle_Dam		Goodamon_Creek		Murrumbidgee_Dam		Murrumbidgee_River		Leaky_Dam		Belconnen_Farm_Dam		Double_Dam		B5		Woodland_Dam		Link_Dam	
Species	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023
Misgurnus anguillicaudatus	11847	274	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyprinus carpio	24199	16999	0	0	0	0	30007	34013	0	0	0	0	0	0	0	0	0	0	0	0
Gambusia holbrooki	0	0	0	0	0	0	0	4299	0	0	0	0	0	0	0	0	0	0	0	0
Hypseleotris klunzingeri	0	0	0	0	0	0	157	0	0	0	0	0	0	0	0	0	0	0	0	0
Galaxias olidus	0	0	0	0	0	0	0	162	0	0	0	77	0	0	0	0	0	0	0	0
Retropinna semoni	0	0	0	0	0	0	0	741	0	0	0	0	0	0	0	0	0	0	0	0
Maccullochella peelii	0	0	0	0	0	0	554	643	0	0	0	0	0	0	0	0	0	0	0	0
Macquaria ambigua	0	0	0	0	730	2258	0	0	0	0	0	0	0	0	0	0	0	0	0	16858

Table 6. Total sequences reads for amphibians and turtle species detected across 10 sites within the Ginninderry conservation corridor.

Species	Triangle_Dam		Goodamon_Creek		Murrumbidgee_Dam		Murrumbidgee_River		Leaky_Dam		Belconnen_Farm_Dam		Double_Dam		B5		Woodland_Dam		Link_Dam	
	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023	6/12/2022	26/04/2023
Uperoleia laevigata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	438	0
Litoria sp.	0	0	0	0	0	0	0	0	231	0	0	0	934	0	0	0	0	0	167	0
Litoria spp.	0	0	0	0	188	0	0	0	3031	0	0	0	351	0	0	0	0	0	3530	0
Limnodynastes sp.	0	0	0	0	0	0	0	0	0	0	0	0	127	0	0	0	0	0	0	0
Chelodina longicollis	1208	115	125	0	1266	415	0	0	15149	3217	764	0	20147	2356	893	0	2200	1318	28842	0

Species diversity

There were significant differences in species diversity and abundance between locations and date of collection (Figure 2). All sites showed significant differences in alpha diversity with the exemption of Belconnen farm dam and Goodamon Creek (Figure 2). Moreover, sites B5, Link dam, Murrumbidgee dam, Murrumbidgee River and Woodland dam showed significantly higher species diversity in samples collected during Autumn compared to samples collected during Summer (Figure 2). Contrastingly, Double dam, Leaky dam and Triangle dam showed significantly higher species diversities in samples collected during Summer compared to Autumn (Figure 2).

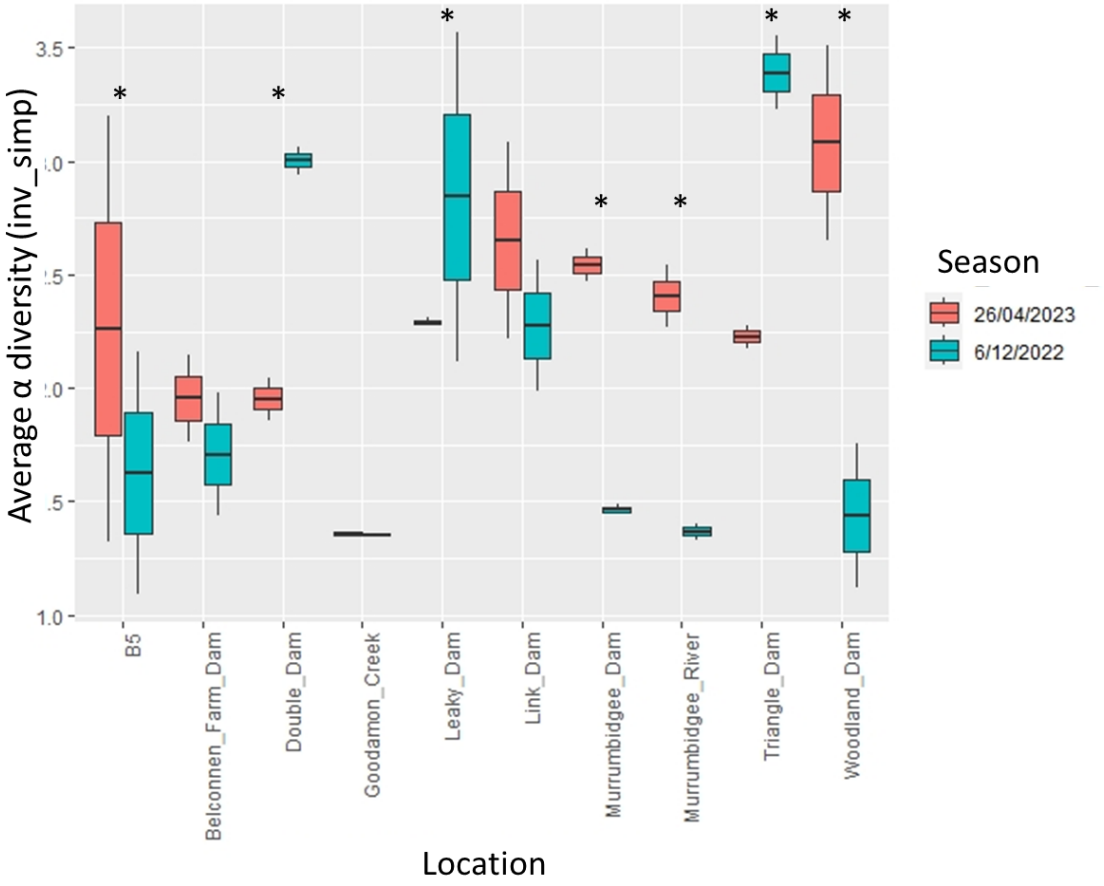

Figure 2. Alpha diversity for each site and season. There are significant differences in alpha diversity between sites and date of collection (ANCOVA, $F_{9,20}$=3.574, P<0.05) *=statistical significance within sites was examined using post hoc Tukey HSD.

BRIEF SUMMARY

A total of 14 birds, 11 mammals, eight fish, two amphibians, and one turtle were detected in this study. Although a majority of detections were expected (e.g., human, cattle, carp, ducks), there are important trends that must be highlighted:

1. Platypus DNA was detected during Autumn in the Murrumbidgee River.
2. Frog DNA was only detected in Dams during Summer.

FUTURE RECOMMENDATIONS

1. Future sampling events could include a much larger range of sites and seasons to enable inference on yearly diversity and abundance of species.
2. High throughput sequencing can be improved by increasing sequencing depth (more reads per site) to enable greater detection probability of potentially rare species, as well as gene coverage (use multiple gene regions) to improve detection accuracy of species.
3. The use of passive surveillance is recommended for exploration in future sampling events to encompass more time frames to assess species presence

REFERENCES

ALTSCHUL, S. F., GISH, W., MILLER, W., MYERS, E. W. & LIPMAN, D. J. 1990. Basic local alignment search tool. *Journal of molecular biology,* 215**,** 403-410.

CALLAHAN, B. J., MCMURDIE, P. J., ROSEN, M. J., HAN, A. W., JOHNSON, A. J. & HOLMES, S. P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods,* 13**,** 581-3.

FICETOLA, G. F., MIAUD, C., POMPANON, F. & TABERLET, P. 2008. Species detection using environmental DNA from water samples. *Biology letters,* 4**,** 423-425.

HINLO, R., FURLAN, E., SUITOR, L. & GLEESON, D. 2017. Environmental DNA monitoring and management of invasive fish: comparison of eDNA and fyke netting. *Management of Biological Invasions,* 8**,** 89.

JEUNEN, G. J., DOWLE, E., EDGECOMBE, J., AMMON, U., GEMMELL, N. J. & CROSS, H. 2023. crabs—A software program to generate curated reference databases for metabarcoding sequencing data. *Molecular ecology resources,* 23**,** 725-738.

PAWLOWSKI, J., APOTHÉLOZ‐PERRET‐GENTIL, L. & ALTERMATT, F. 2020. Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. *Molecular Ecology,* 29**,** 4258-4264.

RIAZ, T., SHEHZAD, W., VIARI, A., POMPANON, F., TABERLET, P. & COISSAC, E. 2011. ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic acids research,* 39**,** e145-e145.