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Long-term eDNA monitoring program for the Ginninderry Conservation Trust.

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Summary

A key challenge for biodiversity conservation is the ability to detect species. Determining the presence or absence of a species is integral to making informed management decisions. Unfortunately, detecting species, particularly in an aquatic environment, can be difficult, time consuming, expensive, and often highly invasive. Analysis of environmental DNA (eDNA) is a relatively new, cheap, quick and non-invasive method for detecting species (Rees *et al.* 2014; McColl-Gausden *et al.* 2019; Thomsen and Willerslev 2015). As the name suggests, eDNA refers to the genetic material that an organism leaves behind in its environment. Quantitative comparisons with traditional sampling methods indicate that eDNA methods can be superior in terms of sensitivity and cost efficiency, particularly for scarce, elusive or cryptic species (Biggs *et al.* 2015; Lugg *et al.* 2018; Smart *et al.* 2015; Thomsen *et al.* 2012; Valentini *et al.* 2016), enabling effective detection of species at low densities.

Environmental DNA methods are being used routinely to monitor aquatic animals including fish, amphibians and mammals across waterways, estuaries and wetlands throughout Australian catchments. Here we aim to undertake a baseline vertebrate assessment within the Ginninderry Conservation Trust's study area using an Environmental DNA (eDNA) metabarcoding biodiversity assessment approach.

Method

Sampling

During May and June 2022, water samples were collected from 10 waterway, dam and pond sites by Ginninderry Conservation Trust staff following sampling protocols developed by EnviroDNA. At each site, between three and 10 replicate samples were collected by passing between 26-1320 ml (average 316 mL) water through a 1.2 µm syringe filter. A summary of site and replicate location information is provided in Appendix A, Table A1. Filtration was undertaken on-site to reduce DNA degradation that may occur during transport of whole water samples (Yamanaka *et al.* 2016). Clean sampling protocols were employed to minimise contamination including new sampling equipment at each site, not entering water, and taking care not to transfer soil, water or vegetation between sites. A preservative was added to the filters after filtering to minimise DNA degradation. Filters were stored out of sunlight and kept at ambient temperature before being transported to the laboratory for processing. It should be noted that some samples were stored up to one month before being transported to the laboratory and therefore did not meet the preservative requirement of ambient temperature storage up to 10 days only. This delay in getting the samples to the laboratory has resulted in significant degradation of the eDNA samples.

Analysis – biodiversity (vertebrate) assessment

DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen Power Soil Pro) that minimizes compounds that can inhibit PCR reactions. Biodiversity assessments were performed on all samples using a universal vertebrate assay targeting a small region of the 12S

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mitochondrial genome (Riaz et al. 2011). Library construction involved two rounds of PCR whereby the first round employed gene-specific primers to amplify the target region and the second round incorporated sequencing adapters and unique barcodes for each sample-amplicon combination included in the library. Negative controls were also included during library construction. Negative controls consisted of the extraction negative as well as PCR negatives where nuclease-free water was used in place of DNA during both rounds of PCR. Sequencing was carried out on an Illumina iSeq machine.

Following quality control filtering to remove primer sequences, truncated reads and low-frequency reads, DNA sequences were clustered into Operational Taxonomic Units (OTUs) on the basis of sequence similarity. Taxonomic assignment was performed with VSEARCH software (Rognes et al. 2016) whereby each OTU cluster was assigned a species identity using a threshold of 95% by comparing against a reference sequence database. Where a species could not be assigned (i.e. reference database was deficient and/or taxa were poorly-characterised), taxonomic assignments were manually vetted by first obtaining a list of possible species through BLASTN searches against the public repository Genbank (www.ncbi.nlm.nih.gov), then eliminating species on the basis of their geographic distribution using information from the Atlas of Living Australia (ALA). In cases where an OTU could not be adequately 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. Detection of species in multiple replicates from a site increases the confidence that the eDNA detection represents actual presence of the species. Detection of a species in a single replicate may indicate species presence at low abundance but can also arise from site level (in field) or sample level (sampling or laboratory protocols) contamination (Darling *et al.* 2021).

Results

A summary of the vertebrate species detected at each site is provided in Table 1 along with notes where species could not be adequately assigned. At least 38 taxa were detected across all sites – six fish, three amphibians, 11 birds, 17 mammals and one reptile species which is comparable to the surveys undertaken at some of the same sites by the Ginninderry Conservation Trust in 2021 (39 taxa were detected).

Results show five samples with no detections and a number of samples and sites overall with significantly lower detections than the previous 2021 surveys. Those sites with a significantly reduced number of taxa (e.g. one to four taxa in total) were those sites sampled in May 2022. EnviroDNA sampling protocols were not followed for these samples, with the samples being stored at an ambient temperature for more than the 10 day limit (estimated to be around one month). The results clearly indicate sample DNA degradation, a result of prolonged storage at ambient temperature, and therefore results of this analysis do not represent the true biodiversity of these sites.

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Table 1- Summary of results from the vertebrate assay with taxa identified at each site.

Scientific names	Common names	LD^	BFD	BD	Bidgee	SE	DD1	TD	WD	LD^^	HES
FISH											
Cyprinus carpio	common carp, European carp				+			+			
Galaxias olidus/ ornatus (1)	mountain galaxias				+						
Macquaria ambigua	golden perch, yellowbelly				/						
Misgurnus anguillicaudatus	oriental weatherloach							+			
Oncorhynchus mykiss	rainbow trout				/						
Retropinna semoni	Australian smelt							/			
AMPHIBIANS											
Crinia signifera	common froglet			/	/					/	
Limnodynastes tasmaniensis	spotted marsh frog					/					/
Litoria verreauxii	Verreaux's frog									/	/
BIRDS											
not classified further			+	+	/	/				/	+
_f:Meliphagidae	family that includes honeyeaters				/						
Anas platyrhynchos or A. superciliosa	mallard or Pacific black duck		+	+	+		+	+			/
Anas sp.	genus of dabbling ducks		/	+	+			+	/		+
Cereopsis novaehollandiae	Cape Barren goose										/
Chenonetta jubata	Australian wood duck		+	+	+	+	/	+	+		+
Columba livia	rock dove, rock pigeon				/						
Microcarbo melanoleucos	little pied cormorant							+			
Ocyphaps lophotes	crested pigeon				/						/
Tachybaptus novaehollandiae	Australasian grebe		+								
Turdus merula or T. philomelos	blackbird or song thrush					+		+			
MAMMALS											
_f:Macropodidae	family of marsupials				/	/		/	/	+	
Bos taurus	cow		/	+	+	+	+	+	+	+	+
Canis lupus	dog or dingo				/						
Dama dama	fallow deer							/		/	
Felis catus	cat									/	
Hydromys chrysogaster	rakali, water rat							+			
Macropus giganteus	Eastern grey kangaroo				+					+	
Mus musculus	house mouse	/			+	/				+	+
Notamacropus rufogriseus	red-necked wallaby				/						
Ornithorhynchus anatinus	platypus				+						
Ovis aries	sheep				+			/		/	
Rattus rattus	black rat					+				+	
Rattus sp.	genus of rats				+	+		/		+	+
Rusa (Cervus) unicolor	sambar deer				/						
Vombatus ursinus	common wombat				/	/		/		/	
Vulpes vulpes	red fox					/					
Wallabia bicolor	swamp wallaby					/					
REPTILES											
Chelodina longicollis	Eastern long-necked turtle						/			/	
Number of taxa detected		**1**	**6**	**6**	**23**	**12**	**4**	**15**	**4**	**14**	**11**

^ Sites sampled in May

^^ Sites sampled in June

+ indicates positive detections in 2 or more replicate samples from that site

/ indicates positive detections in only 1 replicate sample from that site

Taxa prefixed with an underscore cannot be classified further. Abbreviations: f-family

1) The mountain galaxias complex may comprise up to 15 species, including *G. ornatus* (ornate galaxias) and *G. olidus* (mountain galaxias). Based on occurrence data, this is likely to be G. olidus (mountain galaxias).

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References

Biggs J., Ewald N., Valentini A. *et al.* (2015) Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biol. Conserv.* doi: 10.1016/j.biocon.2014.11.029. [online].

Connelly F., Impey R., Song S. (2021) *Vertebrate biobiversity of Ginninderry Conservation Trust land*. A report prepared for Ginninderry Conservation Trust by EnviroDNA, Brunswick, VIC

Darling J. A., Jerde C. L. & Sepulveda A. J. (2021) What do you mean by false positive? *Environ. DNA* n/a. [online].

Lugg W. H., Griffiths J., van Rooyen A. R., Weeks A. R. & Tingley R. (2018) Optimal survey designs for environmental DNA sampling. *Methods Ecol. Evol.* 9 , 1049–1059.

McColl-Gausden E. F., Weeks A. R. & Tingley R. (2019) A field ecologist's guide to environmental DNA sampling in freshwater environments. *Aust. Zool.* doi: 10.7882/az.2019.025.

Rees H. C., Maddison B. C., Middleditch D. J., Patmore J. R. M. & Gough K. C. (2014) REVIEW: The detection of aquatic animal species using environmental DNA – a review of eDNA as a survey tool in ecology. *J. Appl. Ecol.* 51 , 1450–1459. [online].

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*(21), e145-e145

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 4: e2584.

Smart A. S., Tingley R., Weeks A. R., Van Rooyen A. R. & McCarthy M. A. (2015) Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecol. Appl.* 25 , 1944–1952.

Thomsen P. F., Kielgast J., Iversen L. L. *et al.* (2012) Monitoring endangered freshwater biodiversity using environmental DNA. *Mol. Ecol.* 21 , 2565–2573.

Thomsen P. F. & Willerslev E. (2015) Environmental DNA - An emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv.* doi: 10.1016/j.biocon.2014.11.019. [online].

Valentini A., Taberlet P., Miaud C. *et al.* (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol.* 25 , 929–942.

Yamanaka H., Motozawa H., Tsuji S., Miyazawa R. C., Takahara T. & Minamoto T. (2016) On-site filtration of water samples for environmental DNA analysis to avoid DNA degradation during transportation. *Ecol. Res.* 31 , 963–967.

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Appendix A: Sample metadata

Table A1. Summary of sample metadata

Site Code	Replicate	Latitude	Longitude	Collection Date	Sample Volume
LD001	1	-35.2316262	148.994346	17/5/22	180
LD002	2	-35.2318258	148.994479	17/5/22	250
LD003	3	-35.2318565	148.994974	17/5/22	68
LD004	4	-35.2317149	148.995097	17/5/22	130
BFD01	1	-35.217372	148.97493	17/5/22	720
BFD02	2	-35.2168708	148.974776	17/5/22	510
BFD03	3	-35.2176394	148.974891	17/5/22	460
BFD04	4	-35.2169689	148.975008	17/5/22	380
BD01	1	-35.2312899	148.971386	2/6/22	480
BD02	2	-35.2309895	148.971169	2/6/22	600
BD03	3	-35.2307819	148.971451	2/6/22	540
BD04	4	-35.231119	148.971626	2/6/22	30
BD05	5	-35.231209	148.971585	2/6/22	480
Bidgee001	1	-35.231242	148.969554	7/6/22	600
Bidgee02	2	-35.231242	148.969554	7/6/22	300
Bidgee03	3	-35.231242	148.969554	7/6/22	240
SE001	1	-35.2271583	148.981297	2/6/22	180
SE002	2	-35.2271583	148.981297	2/6/22	360
SE003	3	-35.2271583	148.981297	2/6/22	240
SE004	4	-35.2271583	148.981297	2/6/22	300
DD01	1	-35.2379059	148.989117	17/5/22	110
DD02	2	-35.237933	148.989444	17/5/22	120

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Site Code	Replicate	Latitude	Longitude	Collection Date	Sample Volume
DD03	3	-35.2378273	148.989542	17/5/22	93
DD04	4	-35.2377052	148.989612	17/5/22	94
DD05	5	-35.2374875	148.989778	17/5/22	120
DD06	6	-35.2378656	148.98994	17/5/22	70
DD07	7	-35.2378949	148.989786	17/5/22	110
DD08	8	-35.2380163	148.989604	17/5/22	26
DD09	9	-35.2380853	148.989349	17/5/22	114
DD10	10	-35.238002	148.989213	17/5/22	120
TD01	1	-35.2278616	148.971887	2/6/22	240
TD02	2	-35.2277	148.97184	2/6/22	1320
TD03	3	-35.2277085	148.971477	2/6/22	770
TD04	4	-35.2278769	148.971299	2/6/22	420
TD05	5	-35.2279649	148.971616	2/6/22	480
WD01	1	-35.232802	148.988297	17/5/22	120
WD02/ WAN2	2	-35.2330259	148.988116	17/5/22	124
WD03	3	-35.2327156	148.988067	17/5/22	175
WD04	4	-35.2328725	148.987896	17/5/22	220
WD05	5	-35.2330872	148.987843	17/5/22	200
LD01	1	-35.2375869	148.980166	2/6/22	600
LD02	2	-35.2376192	148.979969	2/6/22	460
LD03	3	-35.2374877	148.980414	2/6/22	540
LD04	4	-35.2376381	148.980208	2/6/22	660
HES001	1	-35.2287322	148.990934	2/6/22	190
HES002	2	-35.2292049	148.99092	2/6/22	400
HES003	3	-35.2298924	148.991557	2/6/22	192

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Site Code	Replicate	Latitude	Longitude	Collection Date	Sample Volume
HES004	4	-35.2292049	148.99092	2/6/22	180
HES005	5	-35.228752	148.992887	2/6/22	180

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