CONFIDENTIAL REPORT

EnviroDNA

Ginninderry Conservation Trust

Report

Version [1.0]
July 2021

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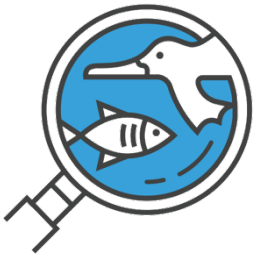
Wildlife Biodiversity on Ginninderry Conservation Trust Land Report July 2021

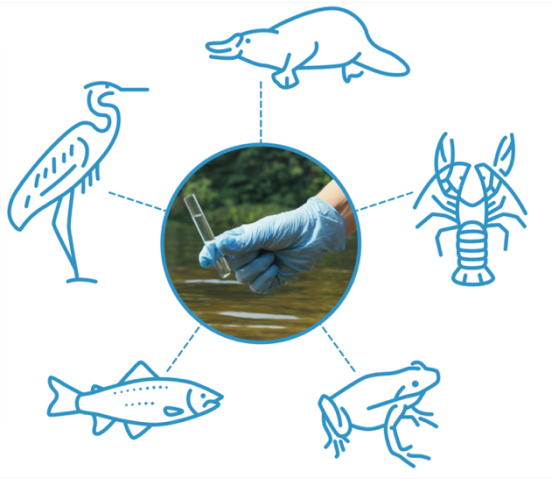
Project summary

In May 2021, water samples were collected from 7 sites along the Murrumbidgee River, an adjacent stream, and several dams and ponds near Strathnairn, ACT (Appendix: Table 1). Samples were collected by Ginninderry Conservation Trust members following standard protocols developed by EnviroDNA. At each site, duplicate samples were taken by passing between 20 – 300ml of water through a 0.22 μm filter (Sterivex).



What is eDNA?

All creatures shed DNA into their surrounding environment via skin cells, hair, scales and more – this is known as environmental DNA.



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EnviroDNA

Wildlife biodiversity study using eDNA

Overview of results

7 sampling locations

2 samples per site

14 eDNA samples

25 native wildlife

9 invasive wildlife

3 livestock animals

2 domestic animals

Number of wildlife detected per site in this project:
7 native species / 6 invasive species

Figure 1. Map of Ginnenderry Conservation Trust sampling locations

Wildlife Detected

Birds
11 species

Species name	Common name
Anas superciliosa	Pacific black duck
Cacatua galerita	Sulphur-crested cockatoo
Chenonetta jubata	Australian wood duck
Fulica atra	Eurasian coot
Menura novaehollandiae	Superb lyrebird
Microcarbo melanoleucos	Little pied cormorant
Phalacrocorax carbo	Great cormorant
Porphyrio melanotus	Australasian swamphen
Tachybaptus novaehollandiae	Australasian grebe
Gallus gallus	Chicken - Invasive
Turdus merula	Blackbird - Invasive

Mammals
10 species

Species name	Common name
Ornithorhynchus anatinus (6)	Platypus
Rattus fuscipes	Bush rat
Vombatus ursinus	Common wombat
Bos taurus	Cow - Invasive
Canis lupus	Dog - Invasive
Felis catus	Cat - Invasive
Oryctolagus cuniculus	Rabbit - Invasive
Rattus rattus	Black rat - Invasive
Rusa unicolor	Sambar Deer - Invasive
Sus scrofa	Pig - Invasive

Fish
7 species

Species name	Common name
Galaxias sp. (1)	Genus of native freshwater fish
Hypseleotris sp. (2)	Genus of carp gudgeons
Maccullochella peelii (3)	Murray cod
Macquaria ambigua	Golden perch, yellowbelly
Retropinna semoni	Australian smelt
Perca fluviatilis	Redfin perch - Invasive
Salmo trutta	Brown trout - Invasive

Reptiles
2 species

Species name	Common name
Chelodina longicollis	Eastern long-necked turtle
Lampropholis guichenoti	Pale-flecked garden sunskink

Frogs
6 species

Species name	Common name
Crinia signifera	Common froglet
Limnodynastes dumerilii	Pobblebonk
Limnodynastes sp. (4)	Genus of Australian swamp frogs
Litoria lesueurii	Lesueur's frog
Litoria peronii	Emerald-spotted tree frog
Litoria sp. (5)	Genus of Australian treefrogs

Domestic & Livestock
5 species

Species name	Common name
Bos taurus	Cow
Canis lupus	Dog
Felis catus	Cat
Gallus gallus	Chicken
Sus scrofa	Pig

Notes

1. Based on occurrence data, this is likely to be G. olidus (mountain galaxias).
2. Based on occurrence data, this is likely to be H. klunzingeri (Western carp gudgeon).
3. This species is listed on the EPBC Act.
4. These may be uncharacterised haplotypes of L. dumerilii or other Limnodynastes species.
5. These may be uncharacterised haplotypes of L. peronii and L. lesueurii or other Litoria species.
6. . This species is listed as Vulnerable in Victoria.

The list of species above includes species that were positively detected in at least 1 replicate. For complete results, as well as eDNA analysis methodology, please see the report Appendix.

Insights

Most commonly detected native wildlife

1. **Australian smelt**
2. **Common froglet**
3. **Pacific black duck**

Most commonly detected invasive wildlife

1. **Brown Trout**
2. **Carp or Goldfish**
3. **Cow**

Listed wildlife detected

Murray Cod –detected at one site in the Murrumbidgee River (Bidgee001), providing important resources for this species, which is listed as 'vulnerable' under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act).

Platypus – detected in the Belconnen Farm Dam BFD001. While this incredibly unique species is not technically considered threatened in Australia (e.g., under the Environment Protection and Biodiversity Conservation Act or the Fauna and Flora Guarantee Act), it is listed as 'vulnerable' in Victoria and the International Union of Conservation of Nature lists platypus as 'near threatened'. It is expected that Australian environmental authorities will be reviewing platypus conservation status given increasing evidence of fragmented habitat and decreasing populations

Non-aquatic or semi-aquatic species

There were many terrestrial bird and mammal species detected that are not considered aquatic or semi aquatic in their ecologyand behaviour. While they are not necessarily living in within the waterway, they could be passing by, using the waterway for drinking, bathing, or using the habitat surrounding it. While eDNA water sampling may not be the most reliable tool for monitoring all birds and terrestrial species, the results help to demonstrate the sensitivity of the technique. Not surprisingly, several livestock species were also detected.

Interpreting your eDNA results

There are animals that I know are around – why haven't they been detected in the results?

They may not have visited the waterway in the days leading up to the eDNA sampling event (or only visited briefly) and therefore no DNA or not enough of their DNA was present for detection. This occurs more frequently with terrestrial species (e.g., reptiles, birds, and mammals) as these species may not visit waters ways often if at all, limiting the amount of DNA that makes it into the water.

Additionally, the animal may be present in and around the waterway at particular times of the year (e.g., migratory birds), which means their DNA may not have been present at the time of sampling.

Why are there some animals that cannot be distinguished from each other?

Some animals share very similar DNA sequences, and the particular eDNA test used in this project has not been able to distinguish between some. For example, carp and goldfish, two invasive species of fish, are genetically very similar. Further analysis would need to be conducted to confirm, however in this instance it is likely that both fish species are present in the area.

I thought I had a particular species, but it didn't show up in results.

If it is a native species and is in very low abundance (particularly likely if there are invasive species present), there is a possibility that its DNA reads were not strong enough to show in results.

If it was an invasive species, it is highly possible that there is not an established population, or it may no longer be present in the waterway. If there is enough food, invasive fish species tend to establish and have high abundance compared to other native fish, and thus we would expect to detect them with eDNA if they are present.

I have less frog detections than I expected

Many frog species breed in and around spring, at which time there is more activity in waterways as well as tadpoles. Outside of breeding season we expect to detect less DNA in waterbodies, which may help to explain these results.

Thank you!

Our services are available in Australia and New Zealand from our home in Melbourne.

We would love to hear from you and discuss how we can help with your next eDNA project.

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Resources & References

Online Resources

eDNA Video: What is Environmental DNA (eDNA)? YouTube

EnviroDNA eDNA White Paper: Online

Scientific Literature

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Appendix

Methods

DNA was extracted from the filters using a DNeasy Blood and Tissue Kit (Qiagen). Biodiversity assessments were performed with a universal vertebrate assay (Riaz et al. 2011) targeting a small region of the mitochondrial DNA. 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 control samples 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 100 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 (Rognes et al. 2016) whereby each OTU was assigned a species identity using a threshold of 95% by comparing against a reference sequence database. In cases where an OTU could not be adequately assigned to a species (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). Where an OTU 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.

Tables

Table 1. Details of water sampling sites.

Site Code	Waterway	Location	Latitude	Longitude	Date sampled
B8001	HES Pond B8	GCT Development	-35.226291	148.987566	27/5/21
BD001	Murrumbidgee Dam	GCT Corridor	-35.231101	148.971407	28/5/21
Bidgee001	Murrumbidgee River	GCT Corridor	-35.232038	148.969934	28/5/21
BFD001	Belconnen Farm Dam	Belconnen Farmhouse	-35.216998	148.974920	28/5/21
LD001	Link Dam	Link Building	-35.231842	148.994823	27/5/21
PP001	Residential Pond	Paddy's Park	-35.228481	148.993690	27/5/21
SE001	Stream E	GCT Corridor	-35.227240	148.981514	28/5/21

Table 2. Summary of results from the vertebrate assay with taxa identified at each site.

Scientific names	Common names	B8001	BD001	Bidgee001	BFD001	LD001	PP001	SE001
FISH								
Carassius auratus or Cyprinus carpio	carp* or goldfish*	+	+	+	+	+	+	/
Galaxias sp. (1)	genus of native freshwater fish	/	/				/	
Gambusia holbrooki	Eastern mosquitofish*	/		/		+		
Hypseleotris sp. (2)	genus of carp gudgeons			/	/	/		
Maccullochella peelii (3)	Murray cod			+				
Macquaria ambigua	golden perch, yellowbelly				/			
Oncorhynchus mykiss	rainbow trout*				/	/	+	
Perca fluviatilis	redfin perch*					/		
Retropinna semoni	Australian smelt	/	/	+	+	/	/	
Salmo trutta	brown trout*		/	/	/	/	+	/
FROGS								
Crinia signifera	common froglet	/	/	/	/	/	+	
Limnodynastes dumerilii	pobblebonk		/		/			
Limnodynastes sp. (4)	genus of Australian swamp frogs		/		/			
Litoria lesueurii	Lesueur's frog						/	
Litoria peronii	emerald-spotted tree frog						/	
Litoria sp. (5)	genus of Australian treefrogs						/	
BIRDS								
Anas superciliosa	Pacific black duck	/	/	+	+	/	+	
Cacatua galerita	sulphur-crested cockatoo	/					/	
Chenonetta jubata	Australian wood duck		/		/	+	/	
Fulica atra	Eurasian coot			/				
Gallus gallus	chicken*	/	/		/	/	/	
Menura novaehollandiae	superb lyrebird	/						

Microcarbo melanoleucos	little pied cormorant						/	
Phalacrocorax carbo	great cormorant			/				
Porphyrio melanotus	Australasian swamphen				/	/		/
Tachybaptus novaehollandiae	Australasian grebe				/			
Turdus merula	blackbird*	/						
MAMMALS								
Bos taurus	cow*		+	+	+		/	/
Canis lupus	dog*					/		
Felis catus	cat*	/						
Ornithorhynchus anatinus (6)	platypus				+			
Oryctolagus cuniculus	rabbit*		/					
Rattus fuscipes	bush rat				/			
Rattus rattus	black rat*	/		+	+			
Rusa (Cervus) sp.	sambar deer*	/				/		
Sus scrofa	pig*	/				/	/	
Vombatus ursinus	common wombat							/
REPTILES								
Chelodina longicollis	eastern long-necked turtle		/					
Lampropholis guichenoti	pale-flecked garden sunskink						/	
Number of species detected		14	13	12	18	15	17	5

* *indicates introduced species*

\+ *indicates positive detections in at least 2 replicate samples at that site*

/ *indicates positive detections in only 1 replicate sample at that site*

Notes:

1. Based on occurrence data, this is likely to be G. olidus (mountain galaxias).

2. Based on occurrence data, this is likely to be H. klunzingeri (Western carp gudgeon).

3. This species is listed on the EPBC Act.

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