Community baseline vertebrate biodiversity surveys in the Hornsby Council region using eDNA - Spring 2023

Wednesday, 20 December 2023	
Project number:	ED_2406CR1
Client:	Hornsby Shire Council
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Assay(s):	*Vertebrate (12S)*
Filter used:	1.2 µm EnviroDNA manual syringe disc filter

Highlights

- At each of the 41 sites, 2 water samples were collected.
- 67% of taxa were resolved at the species level.
- Across all sites, 97 taxa were detected, including 1 threatened species *(Pteropus poliocephalus)*.
- Taxon richness at the site level ranged from 3 to 29.
- *Anguilla reinhardtii* was the most commonly detected taxon.
- >5000 reads were obtained for 96% of metabarcoding samples.

Background

Environmental DNA (eDNA) methods are being used routinely to monitor aquatic animals including fish, amphibians and mammals across waterways, estuaries and wetlands throughout Australian catchments. Here we use a vertebrate eDNA metabarcoding assay to screen 82 eDNA samples taken from 41 sites throughout the Hornsby council region, New South Wales to provide a baseline biodiversity assessment of vertebrate species during Spring, 2023.

Methods

Sampling

During Spring 2023, 82 water samples were collected from 41 sites by Hornsby council staff and citizen scientists. At each site, 2 replicate samples were collected by passing up to 2,000 mL of water (mean = 907 mL) through a 1.2 μm EnviroDNA manual syringe disc filter. Filtration was undertaken on-site to reduce DNA degradation during transport of water samples. Filters were stored out of sunlight and at ambient temperature before being transported to the laboratory for processing.

Analysis

DNA was extracted from filters using a Qiagen PowerSoil Kit that minimises compounds that can inhibit PCR reactions in environmental samples. 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 included during library construction. Negative controls consisted of the extraction negative as well as PCR negatives, in which nuclease-free water was used in place of DNA during both rounds of PCR. Sequencing was carried out on an Illumina sequencing platform.

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), followed by elimination of species on the basis of their geographic distributions, using information from the Atlas of Living Australia and other relevant data sources. In cases where an OTU could not be adequately resolved to a single species (e.g., due to shared haplotypes), either a list of multiple species is included, or the OTU is assigned to the lowest taxonomic rank without further classification.

Results

A total of 82 samples were analysed from 41 sites across the Hornsby Council region, NSW using a 1.2 µm EnviroDNA manual syringe disc filter. Raw data on per-sample detections can be found in accompanying spreadsheet (ED_2406CR1_Hornsby_Vert_Data). The spreadsheet provides the taxa detected in each sample, as well as the number of sequence reads for each taxon. Reads should not be directly interpreted as taxa abundance. While some studies have shown a positive correlation between read numbers and abundance, reads can also be influenced by a number of other variables. Reads may be used to help assign a level of confidence in species detection along with the number of replicates in which the species was detected.

Overall, 97 vert taxa were detected, including 12 introduced species and 1 species listed at the Federal and/or State level; Grey-headed flying-fox *(Pteropus poliocephalus)* (listing data from https://www.environment.gov.au/sprat-public/action/report). Six frog, 25 fish, 6 reptile, 36 bird, and 24 mammal taxa were detected. The number of vertebrate taxa at each site (across all replicate samples) ranged from 3 to 29. The number of native taxa per site varied from 1 to 16 (Figure 1).

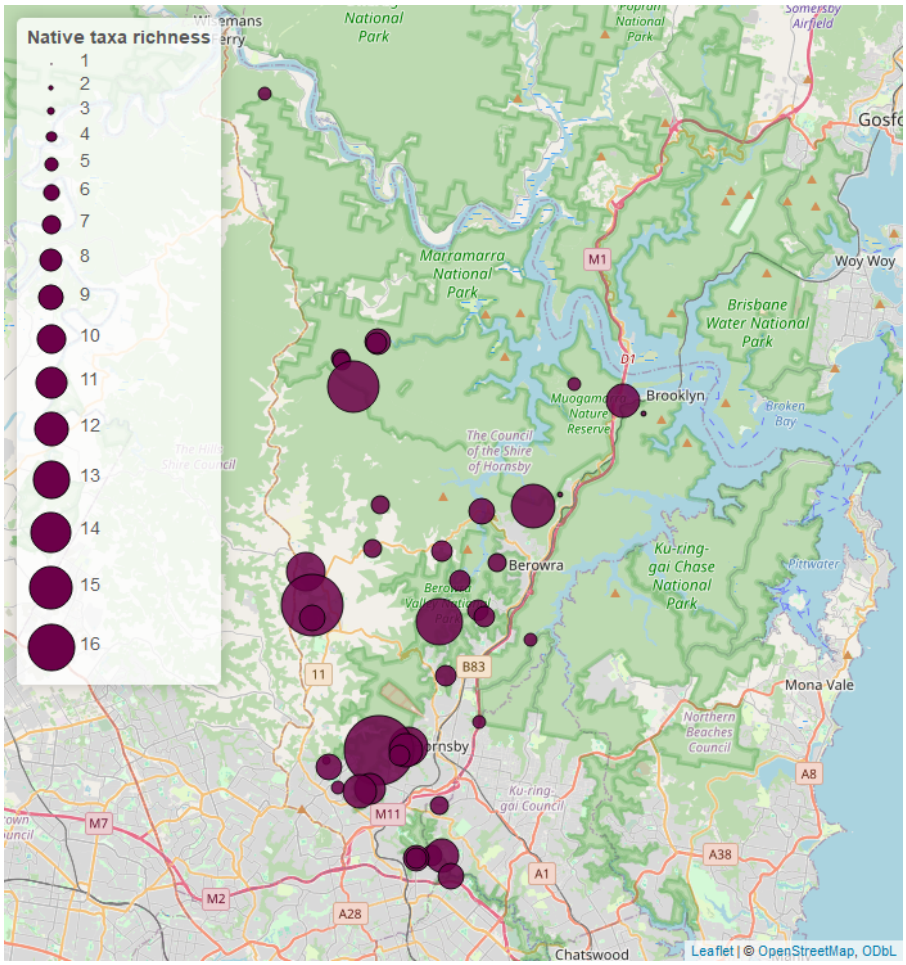

Figure 1. Native vertebrate species richness at the 41 sampled sites. Marker size is proportional to detected species richness. Note that mapped native richness only includes taxa resolved at the species level.

Most taxa were resolved at the species level (67% of all taxa). The fact that some taxa could not be resolved at the species level is likely due to inadequate genetic sequence data available in the reference library for the region. Further reference sequences for species that are not currently captured in the reference database are needed to fully evaluate the potential for the 12S region to resolve these taxa to a species or genus level. Unresolved taxa can also arise due to limitations with the target region (e.g., 12S, 16S) and metabarcoding assays in general, whereby only a very small subset of the entire genome is interrogated for the purpose of species identification. Consequently, there is not always enough genetic variation in that short marker sequence to definitively assign it to a species.

A summary of the frequency of occurrence of each vertebrate species across all samples and sites is provided in Table 1.

Table 1. Number of detections and number of occupied sites for each vertebrate taxon.

Group	Taxa	Common name	N detections	N sites
Birds	*Acanthagenys rufogularis*	Spiny-cheeked honeyeater	1	1
	Acanthiza pusilla	Brown thornbill	2	2
	Alectura lathami	Australian brush-turkey	3	3
	Alisterus scapularis	Australian king-parrot	3	3
	Anatidae	Family of waterbirds that includes ducks, geese and swans	6	5
	Cacatua	Genus of cockatoo	1	1
	Cacatua galerita	Sulphur-crested cockatoo	13	10
	Charadriiformes	Order of shorebirds	1	1
	Chenonetta jubata	Australian wood duck	1	1
	Columba livia	Domestic pigeon	3	3
	Cormobates leucophaea	White-throated treecreeper	2	2
	Corvus	Genus of crows and ravens	1	1
	Dacelo novaeguineae	Laughing kookaburra	6	4
	Eolophus roseicapilla	Galah	2	2
	Gallinula tenebrosa	Dusky moorhen	6	4
	Malurus lamberti	Variegated fairy-wren	1	1
	Manorina	Genu s of australian honeyeaters and miners	9	6
	Meliphaga lewinii	Lewin's honeyeater	1	1
	Meliphagidae	Family of honeyeaters	4	3
	Menura novaehollandiae	Superb lyrebird	1	1
	Microcarbo melanoleucos	Little pied cormorant	5	4

Group	Taxa	Common name	N detections	N sites
	Ocyphaps lophotes	Crested pigeon	1	1
	Pachycephala	Genus of whistlers	1	1
	Passeriformes	Order of perching birds	8	7
	Phalacrocorax sulcirostris	Little black cormorant	4	3
	Platycercus eximius	Eastern rosella	9	8
	Podargus strigoides	Tawny frogmouth	2	2
	Poodytes	Genus of grassbirds	2	2
	Ptilonorhynchus violaceus	Satin bowerbird	1	1
	Sericornis frontalis	White-browed scrubwren	1	1
	Sturnidae	Family of starlings	1	1
	Sturnus vulgaris	Common starling	1	1
	Trichoglossus	Genus of lorikeet	6	6
	Turdus	Genus of thrush	4	3
	Turdus philomelos	Song thrush	2	2
	Zanda	Genus of black cockatoo	2	1
Fishes & eels	*Actinopteri*	Class of fish, unassigned	14	9
	Anguilla	Genus of freshwater eels	34	21
	Anguilla reinhardtii	Longfin eel	60	35
	Carassius auratus	Goldfish	3	3
	Clupeidae	Family of herrings and sprats	1	1
	Cyprinus carpio	European carp	7	4
	Galaxias	Genus of galaxiids	17	11
	Gambusia	Genus of mosquitofish	10	6
	Gobiomorphus australis	Striped gudgeon	24	13
	Gobiomorphus coxii	Cox's gudgeon	48	30
	Gracilimugil argenteus	Flat-tail mullet	2	1
	Hypseleotris	Genus of carp gudgeons	5	3
	Melanotaenia	Genus of rainbowfish	1	1
	Mugil cephalus	Sea mullet	2	1
	Mugilidae	Family of mullet	1	1
	Mugilogobius platynotus	Flatback mangrovegoby	1	1
	Notesthes robusta	Bullrout	2	1
	Percalates novemaculeata	Australian bass	14	8
	Philypnodon grandiceps	Flatheaded gudgeon	7	4
	Philypnodon macrostomus	Dwarf flathead gudgeon	2	1
	Retropinna semoni	Australian smelt	10	6

Group	Taxa	Common name	N detections	N sites
	Salmo	Genus of salmon and trout	1	1
	Scomber	Genus of mackerels	1	1
	Tandanus tandanus	Eel-tailed catfish, freshwater catfish	2	2
	Tetractenos	Genus of toadfish	2	1
Frogs	*Crinia signifera*	Common froglet	34	22
	Limnodynastes peronii	Striped marsh frog	23	14
	Litoria fallax	Eastern dwarf tree frog	3	2
	Litoria gracilenta	Dainty green tree frog	1	1
	Litoria peronii	Peron's tree frog	12	10
	Litoria phyllochroa	Leaf green tree frog	40	25
Mammals	*Bos taurus*	Cattle	8	7
	Canis lupus	Dog/dingo	18	14
	Diprotodontia	Order of marsupials that includes kangaroos, wallabies, possums	15	12
	Eptesicus vulturnus or Vespadelus vulturnus	Little forest bat	1	1
	Hydromys chrysogaster	Rakali	3	2
	Macropodidae	Family of marsupials that includes kangaroos, wallabies	3	2
	Macropus giganteus	Eastern grey kangaroo	1	1
	Mormopterus planiceps	Southeastern free-tailed bat	1	1
	Mus musculus	House mouse	4	4
	Myotis adversus	Large foot bat	1	1
	Notamacropus	Genus of wallaby	1	1
	Oryctolagus cuniculus	European rabbit	2	2
	Ovis aries	Sheep	4	3
	Perameles	Genus of bandicoots	1	1
	Petaurus breviceps	Sugar glider	2	2
	Pseudocheiridae	Family of ring-tailed possums	5	4
	Pseudocheirus peregrinus	Eastern ring-tailed possum	1	1
	Pteropus poliocephalus	Grey-headed flying-fox	9	7
	Rattus	Genus of rodents	5	5
	Rattus fuscipes	Bush rat	5	4
	Rattus norvegicus	Brown rat	3	2
	Rattus rattus	Black rat	9	8
	Sus scrofa	Pig	11	9
	Trichosurus vulpecula	Common brush-tailed possum	6	6
Reptiles	*Chelodina*	Genus of snake necked turtles	1	1

Group	Taxa	Common name	N detections	N sites
	Concinnia tenuis	Barred-sided skink	3	2
	Eulamprus quoyii	Eastern water skink	8	6
	Myuchelys latisternum	Saw-shelled turtle	1	1
	Saproscincus mustelinus	Weasel skink	4	3
	Scincidae	Genus of skinks	2	1

Figure 3, below, shows similar data to those presented in the table above. Rather than focusing on the number of detections, however, this figure shows the percentage of reads assigned to each taxon.

Quality Control

- Amplification success was confirmed by gel electrophoresis.
- The following controls were used:
    - 2 extraction controls
    - 4 mock communities
- The total number of reads was 4,884,394.
- The median number of reads per sample was 59,458.5 (range = 0 – 92,983).
- Out of 82 samples analysed, 3 samples were labelled dropouts (fewer than 5,000 non-human reads).
- Numbers of reads in negative controls were below the acceptable threshold.
- All mock community positive controls produced reads of expected species, with no contamination from other species.

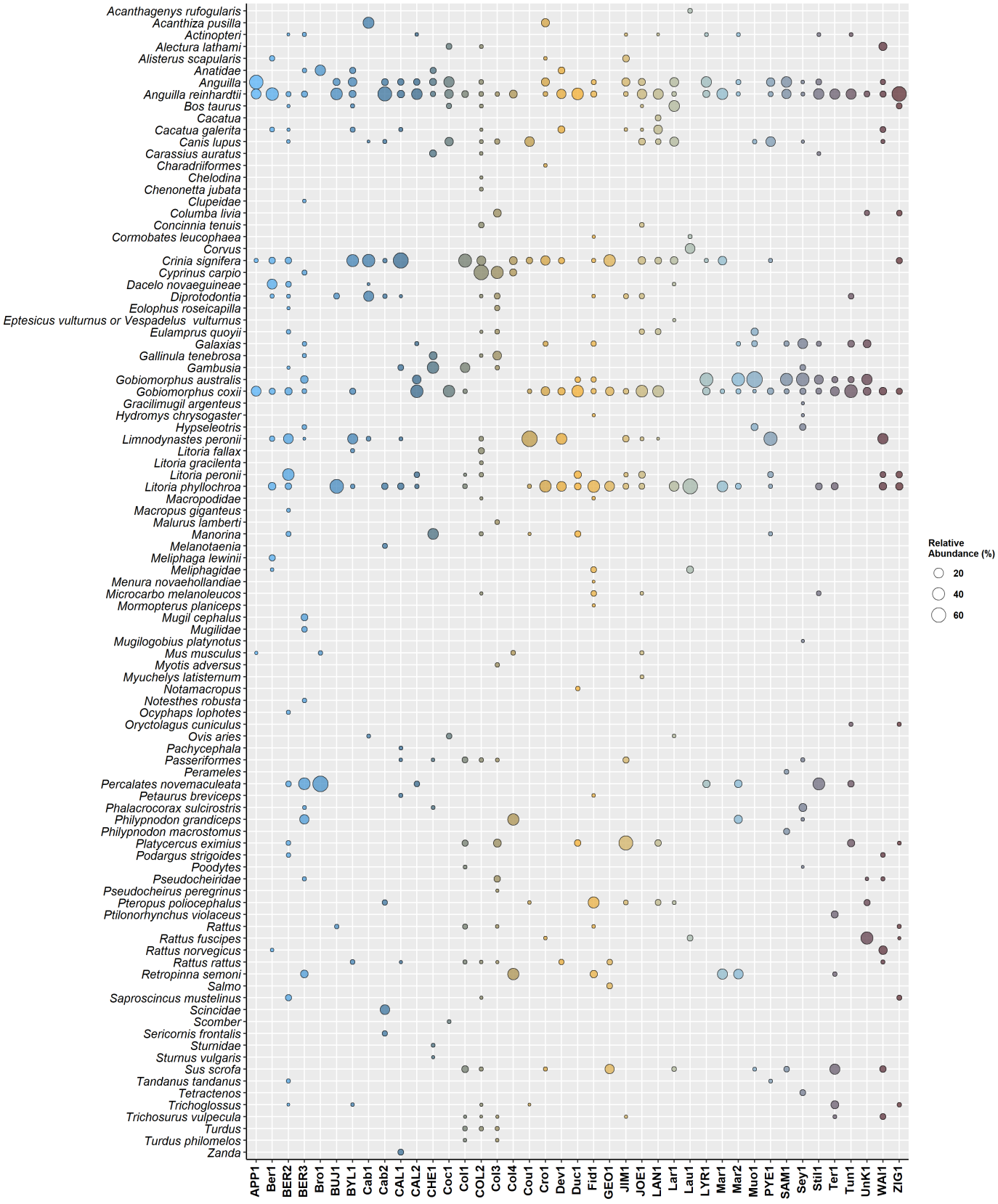

Figure 3. Percentage of reads assigned to each vertebrate taxon.

References

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. PeerJ. 2016 Oct 18;4:e2584. doi: 10.7717/peerj.2584. PMID: 27781170; PMCID: PMC5075697.