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

Friday, 26 July 2024	
Project number:	ED_2406CR1
Client:	Hornsby Shire Council
Prepared for:	Petra Holland, David Bolton
Report prepared by:	Emma Walker, Dr Reid Tingley
Report approved by:	Josh Griffiths
Lab analyst:	Dr Rachael Impey
Assay(s):	*Vertebrate (12S), Decapod*
Filter used:	1.2 µm EnviroDNA manual syringe disc filter

Highlights

- At each of the 41 sites, 2 water samples were collected in Spring 2023 and Autumn 2024.
- Spring 2023 results:
    - 67% (vertebrate assay) and 80% (decapod assay) of taxa were resolved at the species level.
    - Across all 41 sites, 97 vertebrate taxa, including 1 threatened species *(Pteropus poliocephalus)* and 5 decapod taxa were detected.
    - Taxon richness at the site level ranged from 3 to 29 (vertebrate assay) and from 0 to 2 (decapod assay).
    - Longfin eel *(Anguilla reinhardtii)* (vertebrate assay) and Sydney spiny crayfish *(Euastacus australasiensis)* (decapod assay) was the most commonly detected taxon.
    - >5000 reads were obtained for 96% (vertebrate assay) and 41% (decapod assay) of metabarcoding samples.
- Autumn 2024 results:
    - 78% (vertebrate assay) and 80% (decapod assay) of taxa were resolved at the species level.
    - Across all sites, 79 vertebrate taxa, including 3 threatened species; Powerful owl *(Ninox strenua),* Grey-headed flying-fox *(Pteropus poliocephalus)* and Pilotbird, *(Pycnoptilus floccosus)* and 5 decapod taxa were detected.
    - Taxon richness at the site level ranged from 0 to 17 (vertebrate assay) and from 0 to 2 (decapod assay).
    - Longfin eel *(Anguilla reinhardtii)* (vertebrate assay) and Sydney Spiny Crayfish *(Euastacus australasiensis)* (decapod assay) was the most commonly detected taxon.

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 both a vertebrate and decapod 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 and decapod species during Spring 2023 and Autumn 2024.

Methods

Sampling

During both Spring 2023 and Autumn 2024, 82 water samples were collected from 41 sites (Figure 1) 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) in the Spring sampling round and 4,000 mL (mean = 1513 mL) in the Autumn sampling round 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.

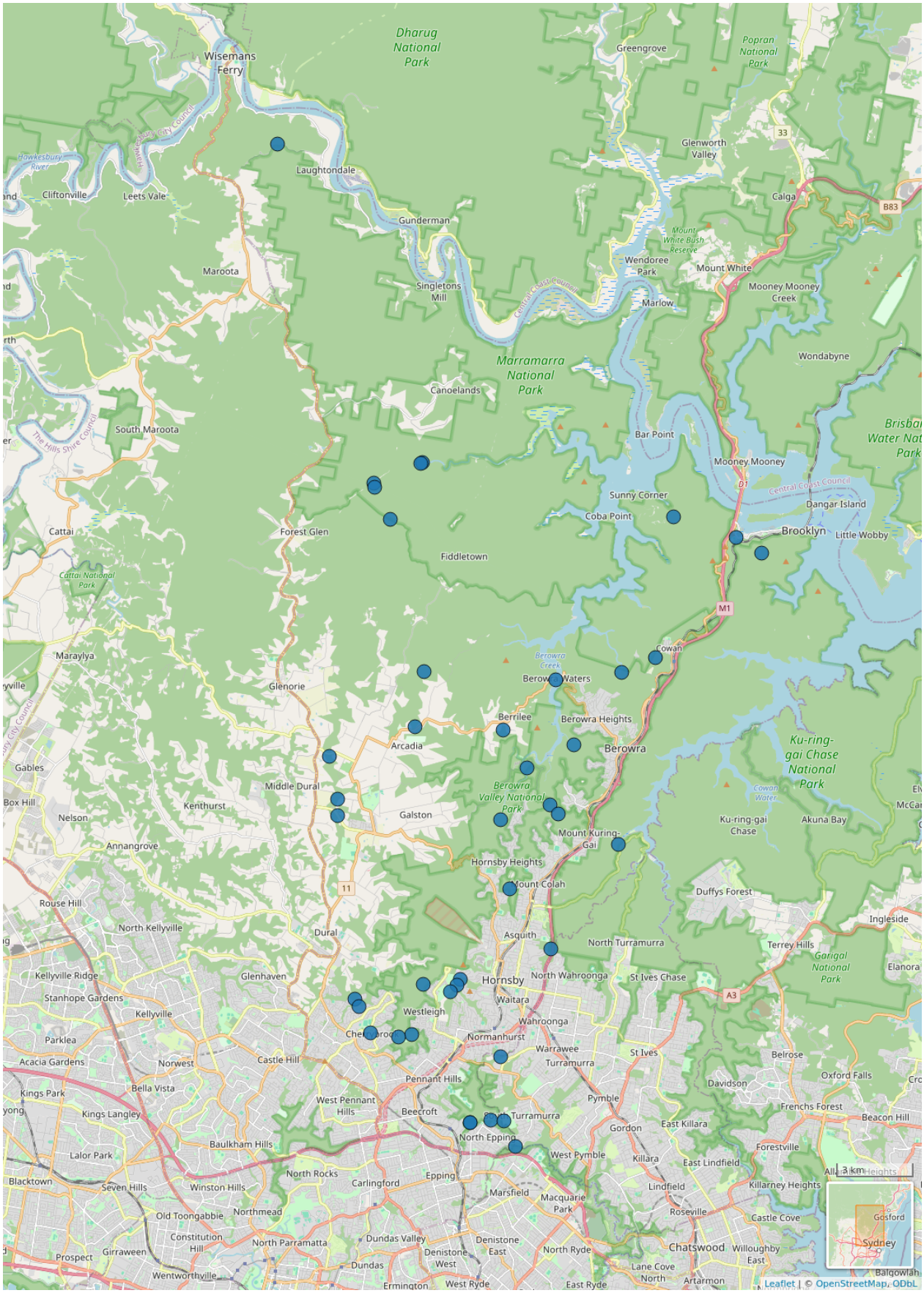

Figure 1. Locations of the 41 sites sampled in both Spring 2023 and Autumn 2024 in the Hornsby Council region, NSW.

EnviroDNA

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_VertDeca_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.

Spring 2023 sampling results

Vertebrate Metabarcoding

Overall, 97 vertebrate taxa were detected, including 13 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.

Most taxa were resolved at the species level (vertebrate assay =67%, decapod assay = 80% 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 during the Spring 2023 sampling season.

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

Group	Taxa	Common name	N detections	N sites
	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
	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

Group	Taxa	Common name	N detections	N sites
	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
	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

Group	Taxa	Common name	N detections	N sites
	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
	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 2, 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.

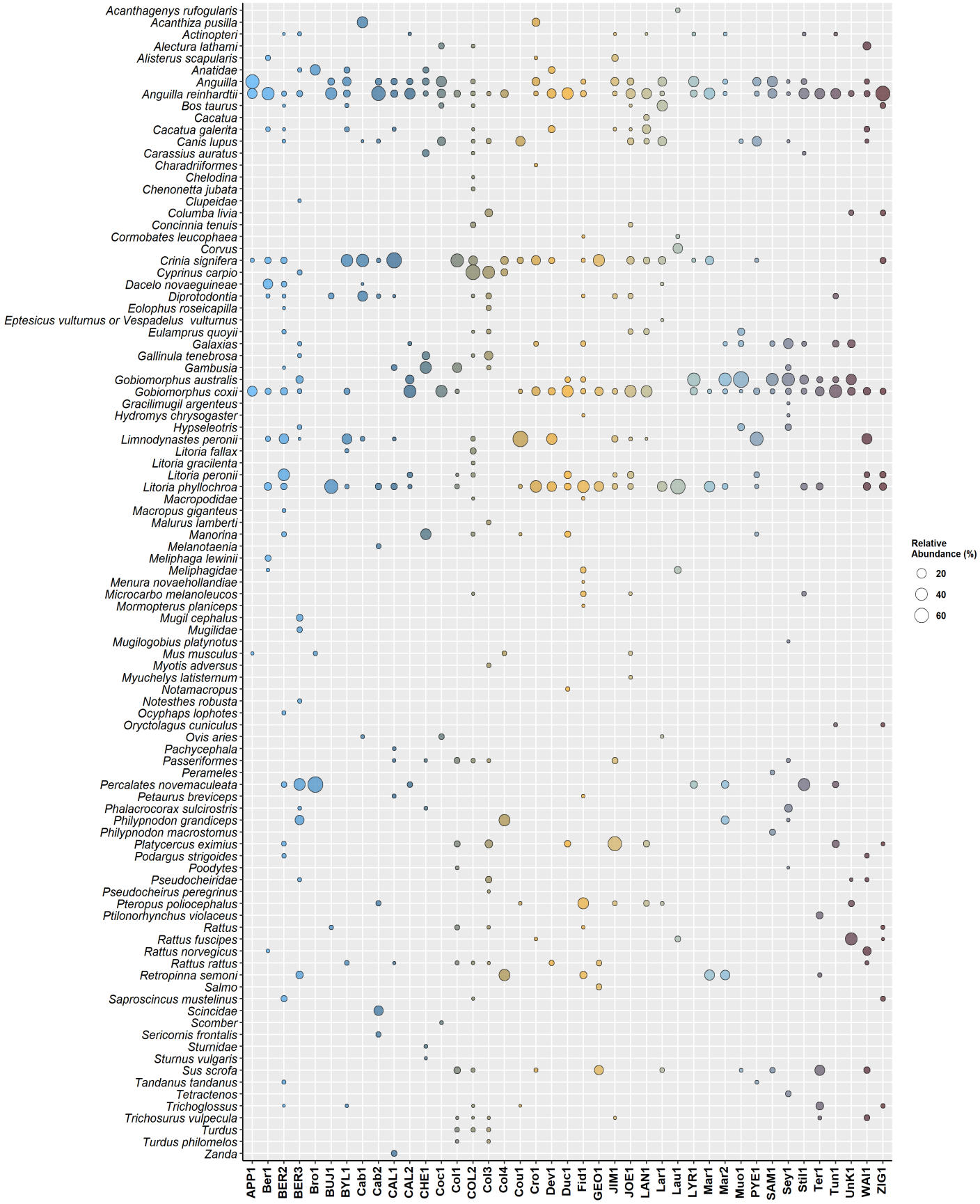

Figure 2. Percentage of reads assigned to each vertebrate taxon at each site in Spring 2023.

Decapod Metabarcoding

Overall, five decapod taxa were detected.The number of decapod taxa at each site (across all replicate samples) ranged from 0 to 2.

The lower detection levels present in the decapod assay in comparison to the vertebrate assay may be due to factors such as differences in eDNA shedding and decay rates as welll as high environmental flows and diluted DNA concentration present in the waterways.

A summary of the frequency of occurrence of each decapod species across the 41 sites is provided in Table 2.

Table 2. Number of detections and number of occupied sites for each decapod taxon during the Spring 2023 sampling season.

Family	Species	Common name	N detections	N sites
Atyidae	*Australatya striolata*	Riffle shrimp	2	2
	Paratya australiensis	Australian glass shrimp	7	6
Parastacidae	*Euastacus*	Genus of freshwater crayfish	3	2
	Euastacus australasiensis	Sydney spiny crayfish	4	3
	Euastacus spinifer	Giant spiny crayfish	7	6

The figure 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 in a sample.

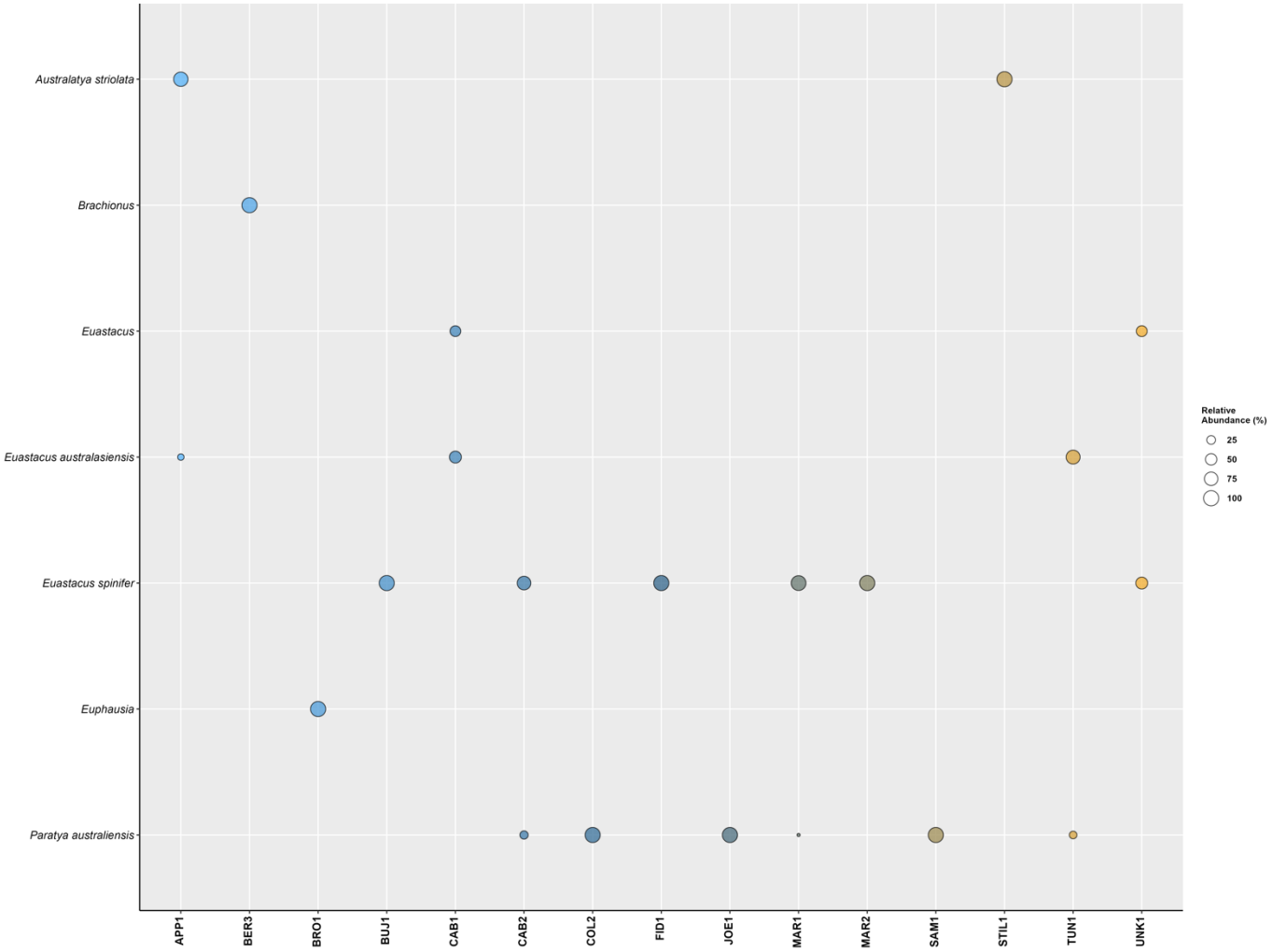

Figure 3. Percentage of reads assigned to each decapod taxon at each site in Spring 2023.

Autumn 2024 sampling results

Most taxa were resolved at the species level (vert assay = 78%; decapod assay = 80% of all taxa).

Vertebrate Metabarcoding

Overall, 79 vertebrate taxa were detected, including 12 vertebrate species that are not native to Australia and 3 vertebrate species Powerful owl *(Ninox strenua),* Grey-headed flying-fox *(Pteropus poliocephalus)* and Pilotbird, *(Pycnoptilus floccosus)* listed at the Federal and/or State level (listing data from https://www.environment.gov.au/sprat-public/action/report). Three frog, 19 fish, 4 reptile, 34 bird, and 19 mammal taxa were detected. The number of vertebrate taxa at each site (across all replicate samples) ranged from 1 to 17. The number of native taxa per site varied from to 0 to 15.

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

Table 3. Number of detections and number of occupied sites for each vertebrate taxon during the Autumn 2024 sampling season.

Group	Species	Common name	N detections	N sites
Birds	*Acanthizidae*	Family of Australian warblers	2	2
	Alectura lathami	Australian brush-turkey	1	1
	Alisterus scapularis	Australian king-parrot	11	7
	Anatidae	Genus of waterbirds that includes ducks, geese and swans	9	6
	Anthochaera	Genus of honeyeaters and wattlebirds	1	1
	Cacatua	Genus of cockatoos	1	1
	Cacatua galerita	Sulphur-crested cockatoo	13	9
	Chenonetta jubata	Australian wood duck	1	1
	Columba livia	Domestic pigeon	1	1
	Corvus	Genus of crows and ravens	1	1
	Dacelo novaeguineae	Laughing kookaburra	3	3
	Eopsaltria australis	Eastern yellow robin	3	3
	Gallinula tenebrosa	Dusky moorhen	8	5
	Lichenostomus	Genus of honeyeaters	2	2
	Macropygia	Genus of cuckoo-doves	2	2
	Manorina	Genus of honeyeaters	1	1
	Meliphaga lewinii	Lewin's honeyeater	2	2
	Meliphagidae	Family of honeyeaters	4	4
	Menura novaehollandiae	Superb lyrebird	2	1
	Microcarbo melanoleucos	Little pied cormorant	5	4
	Ninox	Genus of owls	1	1
	Ninox novaeseelandiae	Morepork	1	1

Group	Species	Common name	N detections	N sites
	Ninox strenua	Powerful owl	3	2
	Passeriformes	Order of perching birds	7	6
	Phalacrocorax sulcirostris	Little black cormorant	1	1
	Platycercus eximius	Eastern rosella	3	3
	Ptilonorhynchus violaceus	Satin bowerbird	2	1
	Ptilotula penicillata	White-plumed honeyeater	1	1
	Pycnoptilus floccosus	Pilotbird	1	1
	Sericornis frontalis	White-browed scrubwren	2	2
	Trichoglossus haematodus	Rainbow lorikeet	11	8
	Turdus	Genus of true thrushes	1	1
	Zanda	Genus of cockatoos	1	1
	Zosterops lateralis	Silvereye	2	2
Fishes & eels	*Afurcagobius tamarensis*	Tamar goby	2	1
	Aldrichetta forsteri	Yellow-eye mullet	1	1
	Anguilla australis	Shortfin eel	33	23
	Anguilla reinhardtii	Longfin eel	48	28
	Carassius auratus	Goldfish	2	2
	Cyprinus carpio	European carp	9	5
	Galaxias maculatus	Common galaxias	11	7
	Gambusia holbrooki	Eastern gambusia	12	7
	Gobiomorphus australis	Striped gudgeon	20	13
	Gobiomorphus coxii	Cox's gudgeon	37	22
	Hypseleotris	Genus of carp gudgeons	3	2
	Mugil cephalus	Sea mullet	2	1
	Percalates novemaculeata	Australian bass	11	7
	Philypnodon grandiceps	Flatheaded gudgeon	7	4
	Philypnodon macrostomus	Dwarf flathead gudgeon	4	2
	Pseudomugil signifer	Pacific blue-eye	2	1
	Retropinna semoni	Australian smelt	7	4

Group	Species	Common name	N detections	N sites
	Tandanus tandanus	Eel-tailed catfish, freshwater catfish	3	2
	Trachystoma petardi	Pinkeye mullet	2	1
Frogs	*Crinia signifera*	Common froglet	9	5
	Limnodynastes peronii	Striped marsh frog	3	3
	Litoria phyllochroa	Leaf-green tree frog	7	5
Mammals	*Acrobates pygmaeus*	Narrow-toed feather-tailed glider	1	1
	Antechinus stuartii	Brown antechinus	2	2
	Bos taurus	Cattle	9	9
	Canis lupus	Dog or dingo	20	17
	Miniopterus	Genus of bats	1	1
	Mus musculus	House mouse	1	1
	Ovis aries	Sheep	2	2
	Perameles nasuta	Long-nosed bandicoot	1	1
	Petaurus breviceps	Sugar glider	7	5
	Pseudocheirus peregrinus	Common ringtail possum	17	14
	Pteropus poliocephalus	Grey-headed flying-fox	10	9
	Rattus	Genus of rats	3	3
	Rattus fuscipes	Bush rat	6	5
	Rattus norvegicus	Brown rat	1	1
	Rattus rattus	Black rat	9	6
	Sus scrofa	Pig	13	10
	Trichosurus vulpecula	Common brush-tailed possum	7	7
	Vulpes vulpes	Red fox	4	4
	Wallabia bicolor	Swamp wallaby	3	3
Reptiles	*Eulamprus quoyii*	Eastern water skink	2	1
	Lampropholis delicata	Delicate skink	2	2
	Myuchelys latisternum	Saw-shelled turtle	1	1
	Scincidae	Genus of skinks	2	2

The figure 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.

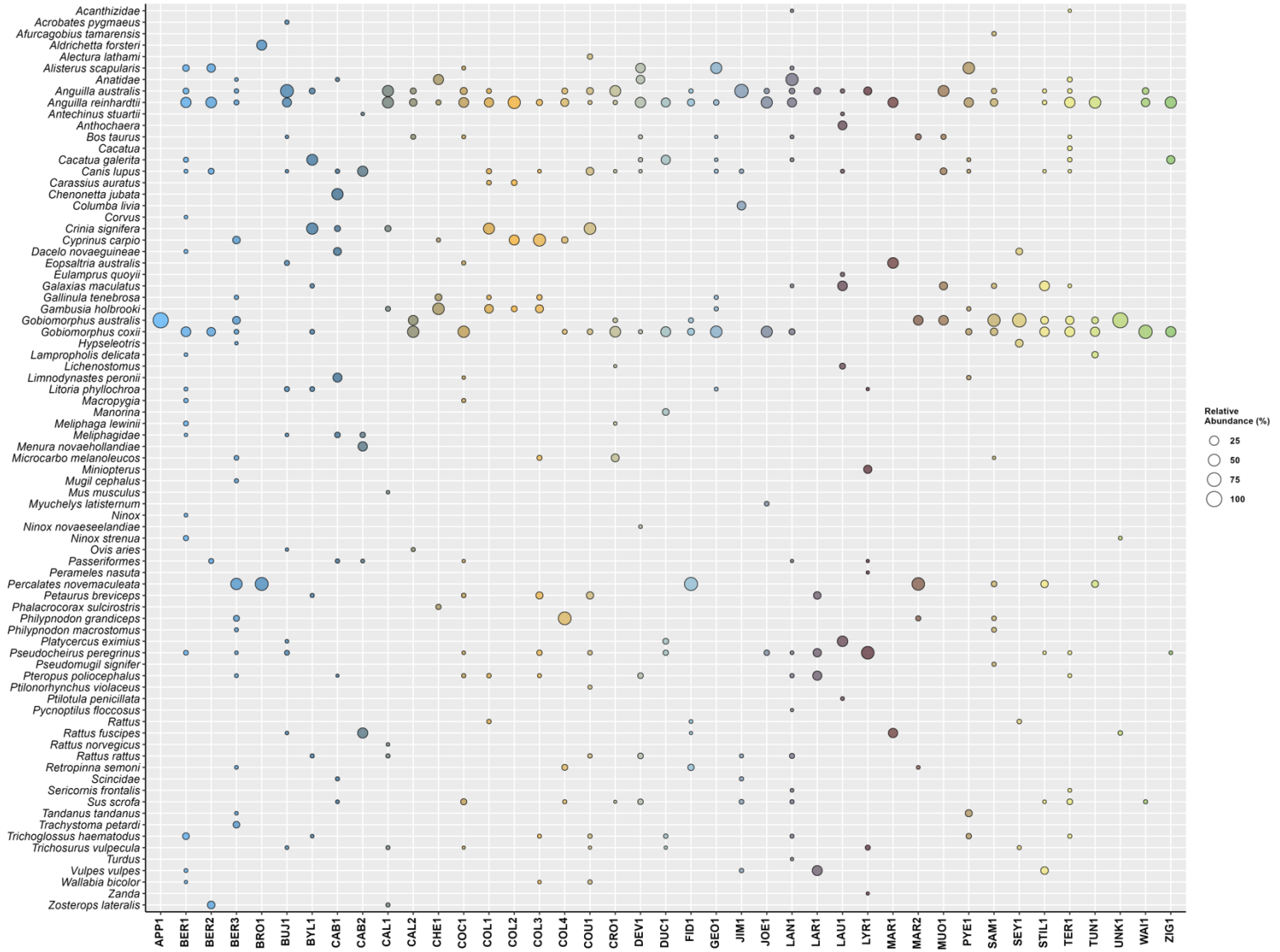

Figure 4. Percentage of reads assigned to each vertebrate taxon at each site in Autumn 2024.

Decapod Metabarcoding

Overall, five decapod taxa were detected in the Autumn 2024 sampling round. The number of decapod taxa at each site (across all replicate samples) ranged from 0 to 2.

A summary of the frequency of occurrence of each decapod species across the 41 sites is provided in Table 2.

Table 4. Number of detections and number of occupied sites for each decapod taxon in Autumn 2024.

Family	Species	Common name	N detections	N sites
Atyidae	*Australatya striolata*	Riffle shrimp	3	3
Parastacidae	*Cherax destructor*	Common yabby	10	7
	Euastacus	Genus of Australian freshwater crayfish	10	9
	Euastacus australasiensis	Sydney spiny crayfish	11	10
	Euastacus spinifer	Giant spiny crayfish	8	5

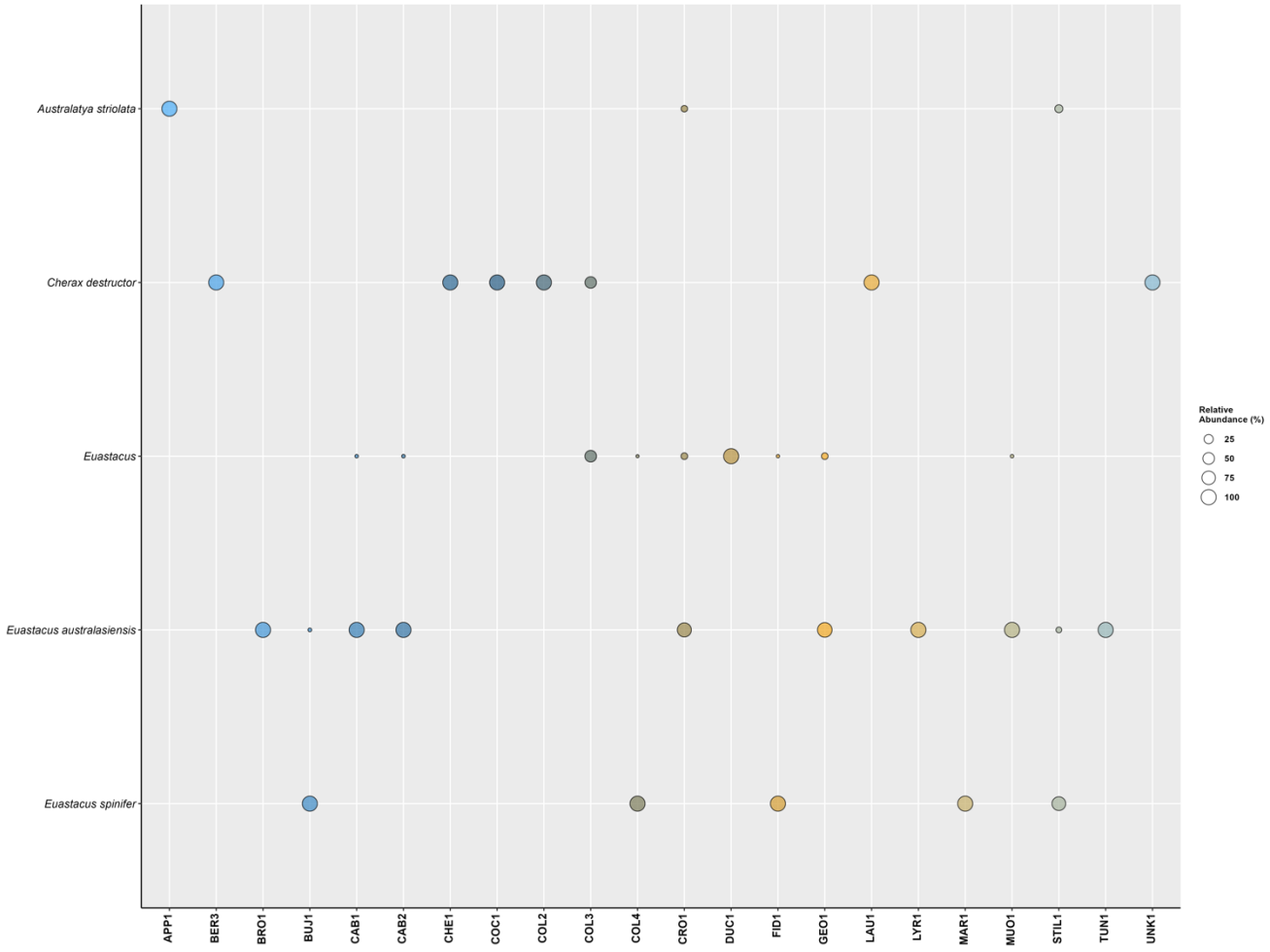

Figure 5. Percentage of reads assigned to each decapod taxon in Autumn 2024.

Quality Control

- Amplification success was confirmed by gel electrophoresis.
- 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.

Spring 2023:

- The following controls were used:
    - 4 extraction controls
    - 4 mock communities
- The total number of reads was 4,884,394 (vertebrate assay) and 1,138,492 (decapod assay) .
- The median number of reads per sample was 59,458.5 (range = 0 – 92,983) (vertebrate assay) and 2,837 (range = 0 – 114,911) (decapod assay).
- Out of 82 samples analysed, 3 samples were labelled dropouts (fewer than 5,000 non-human reads).

Autumn 2024:

- The following controls were used:
    - 12 extraction controls
    - 4 mock communities
- The total number of reads was 15,432,129 (vertebrate assay) and 9,660,320 (decapod assay).
- The median number of reads per sample was 73,261.5 (range = 0 – 245,534) (vertebrate assay) and 19,514.5 (range = 0 – 687,089) (decapod assay).

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.