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RESEARCH

Establishing biotic baselines in urban catchments using eDNA

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Abstract

Urban catchments provide valuable habitat for threatened species and host unique biodiversity that supports critical ecosystem services. Persistent anthropogenic stressors threaten the integrity of urban lotic and lentic systems, causing ecological degradation that impacts this biodiversity. Several methods exist for monitoring biodiversity in urban catchments; however, these fail to capture the full biological community due to inherent biases and logistical constraints. Tree of Life (ToL) metabarcoding presents a new approach for addressing challenges associated with establishing biotic baselines in the most underrepresented urban catchments. In this study, we used ToL-metabarcoding of filtered water samples collected from freshwater lotic and lentic systems in the Eastern Suburbs of Sydney, Australia to characterise whole ecosystem biodiversity, test for fluctuations in taxonomic diversity, and explore the effectiveness of this approach for future monitoring of these catchments. In total, we detected 1038 total taxa using this approach, and taxonomic richness as well as community structure appeared to fluctuate significantly in response to season, rainfall, and site condition. This variability was correlated with taxonomic subgroup, suggesting that micro-organisms, photosynthetic organisms and invertebrates may be optimal indicators of urban ecosystem health given their shorter generation times and much higher turnover rates. Based on these findings, we suggest adapting the site condition index used here, developed in New Zealand freshwater environments, to be more responsive to fluctuations in aquatic taxa typical of, or unique to, Australian urban catchments. We also suggest that ToL-metabarcoding can provide a promising tool for rapidly establishing biotic baselines in urban catchments, particularly when targeted monitoring programs are limited or absent.

Keywords Biodiversity · Environmental DNA · Freshwater · Metabarcoding · Monitoring · Urbanisation

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Introduction

Urban catchments refer to the water and surface water run-off found inside an urban drainage basin. This includes rivers and streams connecting to the ocean, as well as permanent and ephemeral freshwater ponds and creeks. As opposed to natural catchments, the landscape of urban catchments and their water cycle is greatly affected by anthropogenic activity, resulting in stormwater and urban run-off causing flooding and pollution of urban waterways. When this influx is not managed, it can negatively impact the catchment and its resident biodiversity (Davies et al. 2010).

Urban freshwater biodiversity maintains catchment integrity and provides valuable ecosystem services including recreational, cultural, and educational assets, as well as material goods, health resources, food, climate regulation, and nutrient cycling (Lynch et al. 2023). Because cities can serve as refuges for threatened species, urban catchments also act to preserve at risk biodiversity (Ives et al. 2016).

Despite their social and environmental value, urban catchments are under persistent anthropogenic stress resulting in ecological degradation, a condition termed urban stream syndrome (Walsh et al. 2005). Biodiversity in these ecosystems is impacted by factors characteristic of anthropogenic development including catchment imperviousness, invasive vegetation, clearing of riparian zones, as well as wastewater and stormwater inputs (Davies et al. 2010; Lake and Bond 2007; Oertli and Parris 2019).

Anthropogenic stressors on lotic and lentic systems in highly urbanised areas can reduce native biodiversity and taxonomic richness (McKinney 2008). Macroinvertebrates are commonly used as bioindicators of stream health because their ecology is well understood and many species are sensitive to these stressors (Davies et al. 2010; Gresens et al. 2007; Hill et al. 2017; Van den Broeck et al. 2015). Interestingly, Hill et al. (2017) found that macroinvertebrate diversity did not differ between urban and non-urban ponds, which they attributed to the intensive management of specific urban ponds to promote biodiversity. An investigation into aquatic macroinvertebrate biodiversity among seventeen urban creeks and rivers in Northern Sydney (i.e., Cowan Creek Catchment and Middle Harbour Catchment), Australia demonstrated a lack of sensitive species, loss of invertebrate biodiversity, and a simplified community structure, including fewer trophic levels, a loss of functional groups, and lower ecosystem resilience (Davies et al. 2010).

Within the Eastern Suburbs of Sydney (including the local government areas [LGA] of Woollahra Municipal Council, Waverley Council, the City of Randwick, and parts of Bayside Council), close to Sydney's central business district, there are several persistent urban freshwater lotic and lentic systems of high ecological and social significance whose biodiversity is under threat. Parsley Bay Reserve, Cooper Park, and Trumper Park within the Woollahra Municipal Council LGA are key reclaimed recreational areas, which act as migratory corridors for animals and are essential to supporting biodiversity in the Eastern Suburbs, including vulnerable grey-headed flying fox (*Pteropus poliocephalus*) and powerful owl (*Ninox strenua)* populations (Woollahra Municipal Council 2015; Biodiversity Conservation Act 2016; also see Fig. 1). However, increased recreational use since the establishment of these areas over 100 years ago, as well as related infrastructure, stormwater incursion, introduced flora, water pollution and debris, threaten its resident and migratory biodiversity (Woollahra Municipal Council 2015).

As part of their biodiversity conservation strategy, the Woollahra Municipal Council runs regular biodiversity assessments; however, no comprehensive biotic baselines in their urban catchments have been established due to funding limitations and lack of available survey methods; existing surveys have been limited to a small range of terrestrial and freshwater taxa (Woollahra Municipal Council 2015). Moreover, annual aquatic faunal assessments do not test for seasonal effects or the impacts of rainfall on taxa. It should be noted that biological water quality assessments have not been conducted at these sites since 2005 and therefore do not provide a metric for site condition that can quantify extent of biotic change (Woollahra Municipal Council 2015); a requirement to measure the success of conservation efforts.

Environmental DNA (eDNA) metabarcoding is an emerging biological monitoring approach that provides a sensitive tool to address these challenges by generating biotic baselines. Given that flora and fauna are constantly shedding genetic material into the surrounding environment, water samples can be collected, DNA in each sample isolated and sequenced, and sequences matched to known taxa to provide a snapshot of the biological community associated with the sampling site. Combining multiple metabarcoding assays further allows the simultaneous targeting of a broad range of taxonomic groups (Tree of Life or ToL-metabarcoding; Stat et al. 2017; Macé et al. 2024; DiBattista et al. 2024). We hypothesise that ToL-metabarcoding can provide a solution to rapidly establish biotic baselines for urban catchments lacking this information and benchmark future changes.

The aims of our study were therefore to (1) characterise the biota present in urban lotic and lentic systems in the Eastern Suburbs of Sydney using ToL-metabarcoding, (2) opportunistically identify patterns in taxonomic richness associated with season, rainfall, or site condition, and (3) discuss the suitability of ToL-metabarcoding to support future monitoring programs in urbanised ecosystems with little a priori information on aquatic biodiversity.

Methods

Sample collection

Water was collected and filtered from five freshwater sites in the Woollahra Municipal Council LGA across the Eastern Suburbs of Sydney, Australia. This included one site at Trumper Park Duck Pond (Fig. 1c), two sites at Cooper Park (Fig. 1d and e), and two sites in Parsley Bay Reserve Creek (Fig. 1f and g). The latter site (Parsley Bay Reserve Creek) was opportunistically sampled on multiple occasions to provide a time series of eDNA detections (see below). We generated maps of the region with ArcGIS (Esri 2024), and land use as reported by ABARES (2024) was overlayed on the detailed map (Fig. 1b).

All three sample sites (Fig. 1) sit within popular recreational areas, with significant economic and ecological

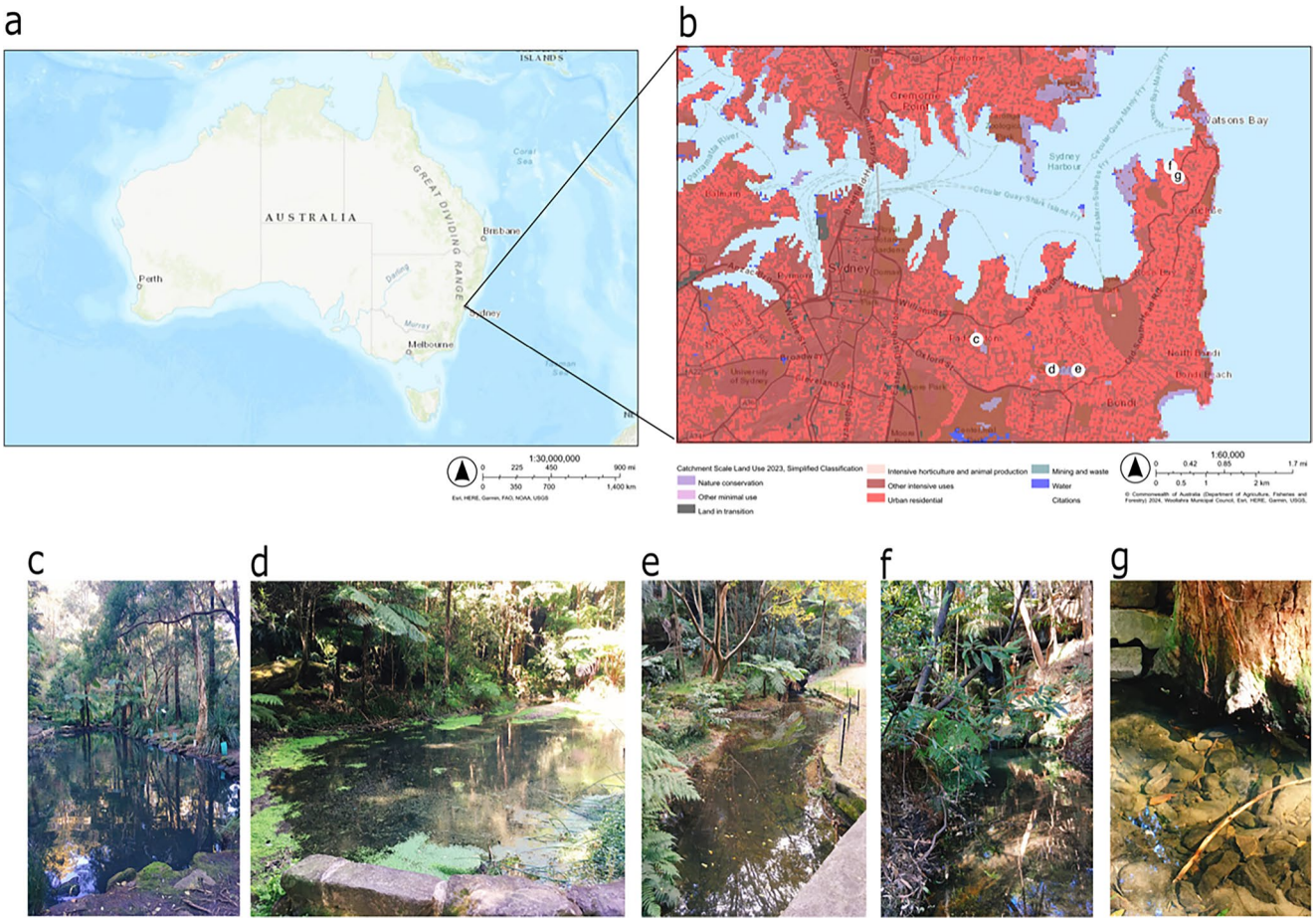

Fig. 1 Location of water sampling sites across the Eastern Suburbs of Sydney, Australia. (**a**) Location in Southeast Australia, (**b**) location of sites in the Eastern Suburbs of Sydney with shading in the legend indicating land use as reported by ABARES (2024), (**c**) Trumper Park Duck Pond in Paddington, (**d**) Cooper Park Pond in Bellevue Hill, (**e**) Cooper Park Creek in Bellevue Hill, (**f**) Parsley Bay Reserve Creek low elevation site in Vaucluse, and (**g**) Parsley Bay Reserve Creek high elevation site in Vaucluse. See the Woollahra Municipal Council Biodiversity Conservation Strategy for complete mapping of the Woollahra historic vegetation types, vegetation communities, known flora, known fauna, key local habitat areas, wildlife corridors, and land managers, current as of 2014 (Woollahra Municipal Council 2015). Note all maps were generated with ArcGIS

value. Previous water quality assessments at Parsley Bay Reserve Creek and Cooper Park Creek concluded that the overall quality was not ideal, and based on limited biotic surveys, the macroinvertebrate community was moderately impaired (Woollahra Municipal Council 2015). Encroachment, mountain biking, and the proliferation of invasive aquatic weeds (*Alternanthera philoxeroides* and *Ludwigia peruviana*) are also threatening the integrity of these aquatic sites (Woollahra Municipal Council 2015). Management strategies to address these threats include bush and riparian vegetation regeneration at all sites, more regular aquatic faunal assessments, ongoing weed monitoring, macrophyte planting, and stormwater harvesting (Woollahra Municipal Council 2015).

To assess our ability to detect seasonal fluctuations in biodiversity and site condition from at least one location, samples were collected every four months in Parsley Bay Reserve Creek between June 2022 and February 2024, both from high elevation and low elevation areas of the creek. Samples from all other sites were collected only once between June and October 2022, though these samples allowed us to capture a broader snapshot of the diversity of persistent urban catchments in the Woollahra Municipal Council LGA. In addition to filtering water samples, we recorded collector, date, GPS coordinates, environment type, and general observations for each replicate. Note that two sewage incursion events into Parsley Bay Reserve Creek occurred during the study (December 20, 2022 and February 8, 2023; Environmental Protection Agency, pers. comm., May 28, 2024) due to heavy rain overloading the drainage system.

For each water replicate, a sterile eDNA syringe mini kit containing an encapsulated 30 mm diameter by 1.2 µm cellulose acetate syringe filter with luer-lock inlet and outlet

fitting was used (Wilderlab, Wellington, New Zealand). Each kit additionally included sterile nitrile gloves, a 60 cc luer-lock syringe for aspirating and pumping water over the filter membrane, and 400 µl of DNA/RNA Shield preservative (Zymo Research Cat. No. R1200-125) pre-loaded into a 3 cc luer-lock syringe. In brief, for each water replicate, the 60 cc syringe was used to filter up to 1 L of water through the encapsulated filter (or a smaller volume if the filter clogged) by repeated aspiration and dispensing through the filter. Residual water in the filter was then purged by forcing 60 cc of air through the filter, using the emptied 60 cc syringe. The material collected on the filter was then preserved by injecting the 400 µl DNA/RNA Shield preservative from the pre-loaded 3 cc syringe into the filter inlet. Syringe filters were sealed and kept at ambient temperature following preservation and shipped to Wilderlab NZ Ltd in Wellington, New Zealand within six weeks of collection for further processing. Note that at one site the filter clogged prematurely, and so 500 mL of water was collected for that replicate (Trumper Park Duck Pond; Online Resource 1), but due to consistency in the number and diversity of detections, the data was retained (see below).

Sample processing

The preservation syringe was used to extract filtrates at Wilderlab NZ Ltd. Once extracted, sample lysates were transferred to low-bind microcentrifuge tubes (Eppendorf, Germany) and stored at -20˚C. For DNA extraction, 200 µl of lysate was loaded into a Genolution GD141 cartridge extracted on the Genolution Nextractor NX-48 S system (Genolution, South Korea) a magnetic bead-based extraction instrument, using default settings (Colman et al. 2020). Single step quantitative Polymerase Chain Reaction (PCR) reactions were carried out for DNA quality/quantity analysis, adapter-fusion, indexing, and amplification. The applied Biosystems QuantStudio 1 qPCR instrument was used for the BE and CI assays, and the Applied Biosystems ProFlex PCR System was used for all other assays (Online Resource 2 and below).

PCR reactions were carried out in duplicate, and extracts were amplified using fusion-tag mitochondrial and nuclear rRNA assays for the detection of vertebrate, invertebrate, plant, microeukaryote, and microbial DNA. The protocol included a negative control reaction of 200 µl of deionised water (IDT; Singapore) instead of a DNA template included in each PCR run; these results are included in the NCBI Sequence Read Archive (BioProject Accession Number: PRJNA1241474) of raw .fastq files (see below). A Qubit 4 Fluorometer (ThermoFisher Scientific) was used to determine the final DNA library concentrations. These libraries were then loaded onto an iSeq V2 reagent cartridge with a 300-cycle flow cell (Illumina) with 5% Phi X and run for 290 cycles in a single direction on an Illumina iSeq 100 instrument. Short amplicon metabarcoding libraries (~100 base pairs) were targeted in each assay due to the increase in sensitivity from amplifying highly fragmented and degraded DNA typical of aquatic samples.

The following nine assays were consistently used to target different taxonomic groups: eukaryotes (BE assay), eukaryotes and bacteria (BU assay), aquatic insects (CI assay), fish (LG assay), vascular plants (MZ assay), vertebrates (RV assay), vascular plants (TP assay), bacteria (UM assay), and fish (WV assay). The detections recorded from all assays are available in Online Resource 1 and methodological details of each assay are outlined in Online Resource 2.

Output .fastq files were demultiplexed and trimmed using the insect package (v 1.4.0; Wilkinson et al. 2024) in R (R Core Team 2023), sequences were filtered to produce amplicon sequence variants (ASVs) using the DADA2 package (Callahan et al. 2016). Sequence run outputs are provided for each sample (UID or sample ID, sequence run number, target or assay, number of raw reads, number of reads after quality filtering, number of reads after DADA2 denoising, and number of reads after chimera removal) in Online Resource 1. ASVs were then taxonomically assigned by searching exact matches, matching to a global reference sequence database from GenBank (Benson et al., 2011), BOLD (Ratnasingham and Hebert 2007), and the RDP reference database (v18; Cole et al., 2013; UM assay only), and querying any unmatched sequences against the local reference database compiled by Wilderlab using the SINTAX classification algorithm (Edgar 2016). Species level assignments were only made based on 100% identity and query cover match, whereas unmatched sequences were assigned using the SINTAX classification algorithm with a conservative 99% threshold, restricting them to genus or higher taxonomic level assignments (i.e., no species level assignments). Singletons were removed along with any ASVs with sequence read count < 5. Given that there were no metazoan ASVs in the negative extraction controls, no further removal of data was carried out. The bioinformatics R script that codes for these steps was published in Wilkinson et al. (2024) and can also be found at https://zenodo.org/records/7768777.

Taxon-independent community index (TICI) score

To correlate biodiversity that we detected in our water samples using eDNA with site condition, we used a taxon-independent community index (TICI) that integrates health indicator scores assigned to common freshwater ASVs, thus providing a measure of ecological health from across the tree of life (Wilkinson et al. 2024). This taxon-independent

approach reduces assignment bias associated with incomplete DNA reference libraries. Each of our samples was therefore assigned a TICI score, which was developed using the Chessman learning algorithm (Chessman 2003) that assigns health indicator scores to 3000 of the most common DNA sequences encountered in freshwater sampling across a wide geographic range in New Zealand (Wilkinson et al. 2024). We used this index to estimate site condition in Woollahra Municipal Council urban catchments given that no equivalent TICI has been calibrated in Australia. We note however that more than 1967 of the 3000 indicator ASVs that make up the New Zealand TICI model are also found in Australia based on the 1825 samples submitted to the commercial sequencing provider Wilderlab at the time of publication (Wilderlab, unpubl. data). Moreover, the TICI provides a non-invasive, cost-effective, and faster method for detecting a wider range of biodiversity, including cryptic or morphologically similar taxa (Wilkinson et al. 2024), compared to the Macroinvertebrate Community Index (MCI) upon which it was trained (Begum et al. 2023). The application of the TICI therefore allows for standardisation across different monitoring efforts in Australia and the reuse of our data.

A possible TICI score ranging from 60 to 140 was assigned to each sample and used to additionally bin samples into condition categories ranging from "very poor" to "pristine". Each TICI score had an associated reliability score based on the number of indicator sequences identified in the sample (Wilkinson et al. 2024). For the Trumper Park Duck Pond sample only, the TICI score was not calculated as the number of indicator sequences identified was less than the threshold of 100 (Online Resource 1); this sample was excluded from statistical analysis focused on TICI values and categories, but included in analyses related to other ecosystem qualities.

Data curation

Characterisation of biological detections

Based on our ToL-metabarcoding data, sequences classified as "root" or "environmental sample" were flagged and removed from further analysis after TICI calculation as they either could not be identified at the highest level of taxonomic organisation (i.e., unassignable even at the superkingdom level), or their exact taxonomic identity could not be inferred. The scientific names assigned to ASVs were cross-referenced with the World Register of Marine Species (WoRMS) (WoRMS 2024; accessed March-May 2024) to determine appropriate taxonomic nomenclature. The WoRMS database was also used to determine the habitat(s) associated with each taxa (terrestrial, marine, freshwater, or brackish). Strictly terrestrial taxa were excluded from statistical analysis. Accepted taxonomic names could not be found for all taxa on WoRMS; in cases where taxonomic information was limited (most frequently for bacteria and plants), taxa were assigned to all habitat types. The Atlas of Living Australia (ALA) biodiversity database (accessed March-May 2024) was also used to assess whether identified taxa had previously been recorded in Australia, and specifically, in the Woollahra Municipal Council LGA. Distribution of occurrence records were noted, and in cases where records did not overlap with the study region, taxon distribution was further investigated to ensure the range included New South Wales (NSW) to identify potential erroneous assignments.

For each replicate water sample, the cumulative seven-day rainfall (mm) prior to sampling was estimated from the Bureau of Meteorology (BoM) daily rainfall statistics for Rose Bay (33.88ºS, 151.27ºE), representing a midpoint of the Woollahra Municipal Council administrative boundaries. Visualisation of spatial data was done using 'ggplot2' and 'tidyverse' in R v 4.3.0 (R Core Team 2023; Wickham 2016). Cladograms of all named taxa were visualised using the Shiny Wheels app (https://shinywheels.wilderlab.co/). The backbone of the cladogram is created in the app using the hierarchical NCBI taxonomy database. While NCBI is not considered the authoritative taxonomy for many groups, it is the only backbone that encompasses all groups in our study.

Data analysis

Several factors can impact the estimation of organismal abundance from eDNA metabarcoding sequence data including the amount of DNA released from the organism, DNA degradation rate, and removal of DNA from the environment (Stewart 2019). Additionally, several steps in field sampling and DNA metabarcoding in the laboratory, including choice of filter size, water filtration volume, preservative used, PCR primer bias, and the number of replicates can introduce bias to the detection data (van der Loos and Nijland 2021; Zinger et al. 2019). Therefore, as a conservative measure, statistical inference in our study is limited to presence or absence for detected taxa, rather than considering relative abundance as a function of read number.

Statistical analyses were run with all taxa detected in each sample, as well as for the following taxonomic subgroups separately: micro-organisms (defined as bacteria, ciliates, archaea, amoeba, fungi), photosynthetic organisms (defined as plants, cryptomonads, diatoms, green algae, heterokont algae, red algae, mosses), fish, invertebrates (defined as bryozoans, cnidarians, crustaceans, molluscs, ribbon worms, rotifers, flatworms, mites and ticks, springtails), a combination of birds, mammals, and herpetofauna

(hereafter BMH), and "other" denoting organisms that were identified at levels spanning multiple taxonomic subgroups. Taxonomic subgroup classifications were assigned using the more refined categories in Online Resource 1 to allow for analysis of broad groups. The R script used for all statistical analysis here and below is available as Online Resource 3.

Multivariate analysis

A permutational multivariate analysis of variance (PERMANOVA) was used to test for statistical differences in eDNA community structure between water samples assigned TICI scores and thus different condition categories. These analyses were conducted using Jaccard dissimilarity values and a Principal Coordinate Analysis (PCoA) plot was used to visualise the results. An Indicator Species Analysis (ISA) was also used to identify taxa contributing significantly to the three condition categories (very poor, poor, average) represented in our data set using the 'labdsv' package (Roberts 2023) given that the TICI is focused on ASVs (taxonomy independent) and not the taxa assigned to these ASVs (taxonomy dependent). This analysis was repeated using only the two most common condition categories (poor, average) represented in our data set (see Online Resource 4) to confirm the generality of our findings.

Univariate analysis

For the Parsley Bay Reserve Creek replicates ($N=12$) collected at six different time points across 20 months, a Generalised Additive Model (GAM) was run using the 'mgcv' package in R (Wood 2011) with a Quasipoisson distribution and a log link. Sampling site (high versus low elevation), season (spring, summer, winter), and cumulative 7-day rainfall (high > 20 mm versus low < 20 mm) were included as categorical predictors. TICI score (range: 81.33–94.88) was included as a continuous predictor and modelled using a smoothing spline (basis dimension $k=6$) to allow for potential nonlinear relationships with taxonomic richness. A separate analysis considered each taxonomic subgroup as distinct datasets (micro-organisms, photosynthetic organisms, invertebrates, fish, BMH, and other). The 'emmeans' package in R was then used for pairwise comparisons between levels in each factor (Lenth and Piaskowski 2026). All GAMs were conducted in R v.4.3.0.

Results

Characterisation of biological detections

Across all water samples and assays, a total of 5760 ASVs were detected. Of those detections, 2534 ASVs were assigned ambiguous scientific names and therefore removed from further analysis; this included 2533 ASVs assigned to "root" and one ASV assigned to an "environmental sample". Of the nine assays consistently applied across all samples, 43% of the ASVs were detected by the CI assay, 17% by the UM assay, 15% by the BU assay, 12% by the BE assay, and 13% by all remaining assays combined (LG, MZ, RV, TP, WV).

Once all assays and ASVs were aggregated into assigned taxa, there were 1038 unique taxa identified from all water samples (Fig. 2). This included 154 strictly terrestrial taxa, which were excluded from statistical analysis (but reported in Online Resource 1). Of the 1038 detected taxa, 3 were assigned at the level of kingdom (0.3%; plus 2 or 0.2% assigned to superkingdom), 37 were assigned to phyla (3.6%; plus 2 or 0.2% assigned to subphyla), 49 were assigned to class (4.7%; plus 9 or 0.9% assigned to subclass), 105 were assigned to order (10.1%; plus 2 or 0.2% assigned to suborder and 1 or 0.1% assigned to infraorder), 199 were assigned to family (19.2%; plus 4 or 0.4% assigned to superfamily and 21 or 2.0% assigned to subfamily), 353 were assigned to genera (34.0%; plus 2 or 0.2% assigned to subgenera), and 173 were assigned to species (16.7%). An additional 45 or 4.3% of taxa were assigned at the level of "clade", "cohort", "subtribe", or "tribe", and 30 or 2.9% were assigned "no rank" (Fig. 3).

Most of the identified taxa were bacteria (24%) and plants (19%) (Fig. 4). Other notable identified taxonomic groups included insects (9%), ciliates (7%), worms (5%), algae (4%), fungi (4%), and fish (2%). Amoeba, amphibians, archaea, birds, bryozoans, cnidarians, crustaceans, cryptomonads, diatoms, flatworms, lizards and snakes, mammals, mites and ticks, molluscs, mosses, oomycetes, ribbon worms, rotifers, roundworms, spiders, and springtails all contributed less than 2% each to the overall data set of aggregated ASVs (19% in total); the remaining 8% of taxa were identified as "other".

Of the 1038 detected taxa, 700 (67%) had previously been recorded on ALA (accessed March-May 2024). ALA records were available for 87% of the fish, 93% of the BMH, 83% of the invertebrates, 73% of the photosynthetic organisms, and 51% of the micro-organisms in the dataset. Of the 338 taxa not previously recorded on ALA, 19 taxa were instead identified on ALA at a higher level of classification. Note that six taxa had records on ALA strictly from Antarctica, and one taxa only had records from South Africa.

Of the 1038 detected taxa, only 248 had occurrence records on ALA across the Woollahra Municipal Council region of Sydney (accessed March-May 2024). Of the 790 "new" taxa detected in the Woollahra Municipal Council region (Fig. 4), the majority were bacteria (30%). Other

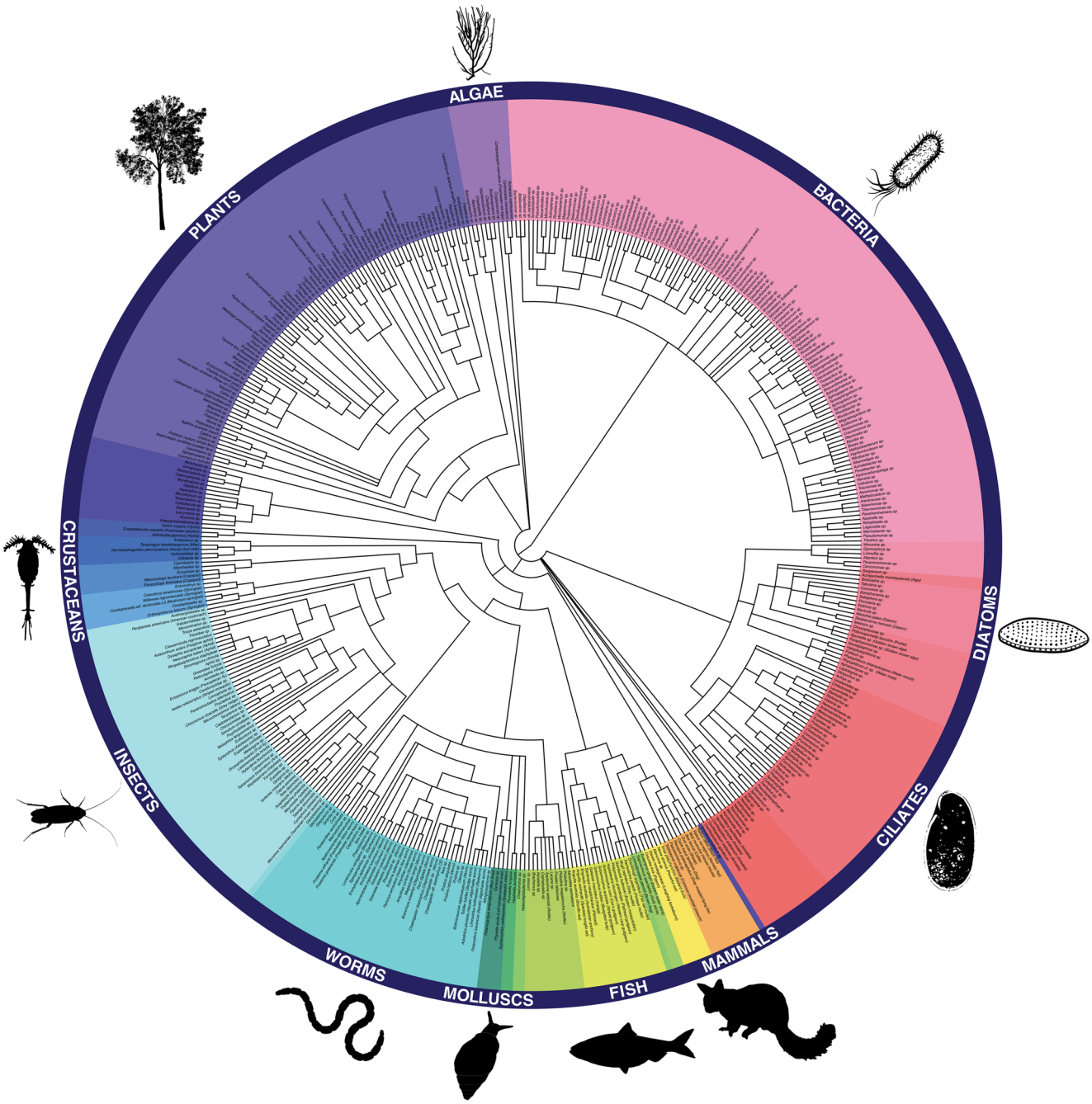

Fig. 2 Cladogram showing all taxonomic detections from tree of life (ToL) metabarcoding of filtered water samples ($N=15$) collected from Parsley Bay Reserve Creek, Cooper Park Creek and Pond, and Trumper Park Duck Pond in the Eastern Suburbs of Sydney. The backbone of the cladogram is created in the Shiny Wheels app (https://shinywheels.wilderlab.co/) using the hierarchical NCBI taxonomy database

new taxa included ciliates (9%), insects (8%), plants (8%), worms (6%), algae (5%), fungi (4%), fish (2%), and diatoms (2%). Amoebae, archaea, bryozoans, cnidarians, crustaceans, cryptomonads, flatworms, mammals, mites and ticks, molluscs, mosses, oomycetes, ribbon worms, rotifers, spiders, and springtails were also included in the new taxa, but contributed less than 2% each (18% in total); 8% of the "new" taxa were identified as "other".

Tracking temporal patterns in taxonomic richness

Over the 20-month sampling period in Parsley Bay Reserve Creek, taxonomic richness per sample ranged from 168 to 313 taxa at the high elevation site and 190 to 302 taxa at the low elevation site (Fig. 5). At both the high and low elevation sites, the fewest taxa were detected on February 21, 2023, which immediately followed a sewage incursion event on

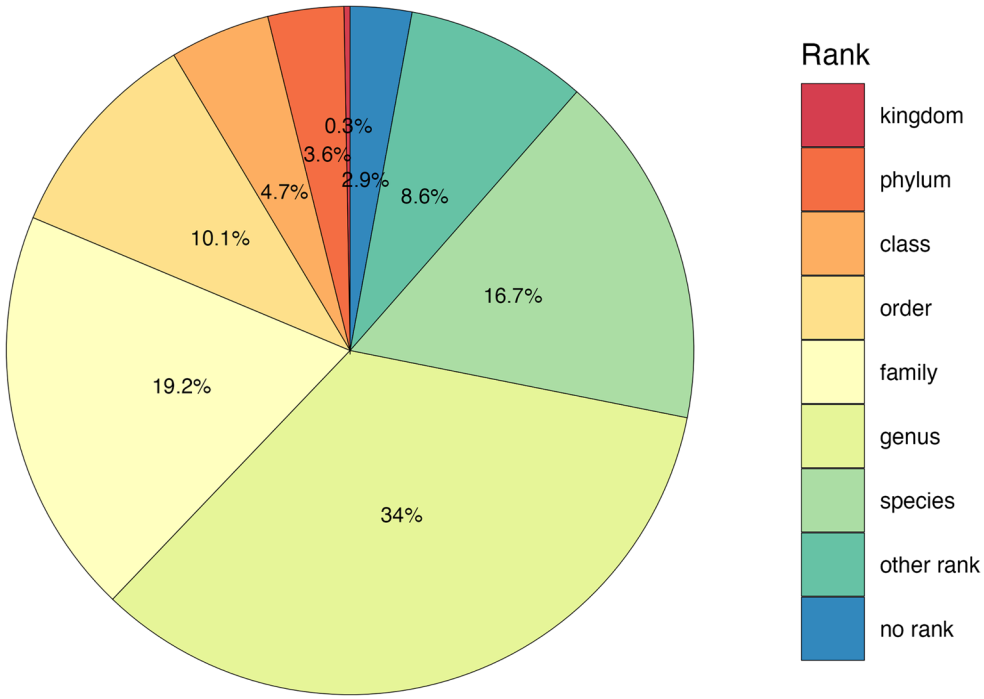

Fig. 3 Pie chart of taxonomic ranks assigned to amplicon sequence variants (ASVs) from filtered water samples ($N=15$) collected from Parsley Bay Reserve Creek, Cooper Park Creek and Pond, and Trumper Park Duck Pond in the Eastern Suburbs of Sydney. "Other rank" includes ASVs assigned to the level of clade, cohort, subtribe, and tribe

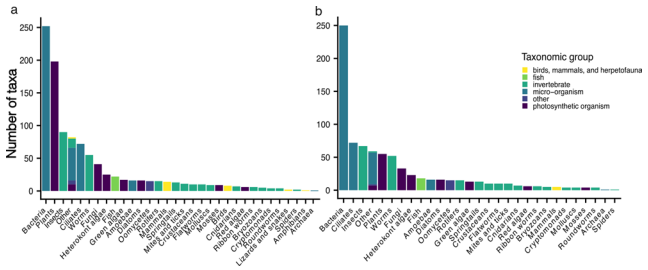

Fig. 4 Bar charts showing **a**) all taxa detected and **b**) "new" taxa, not previously recorded on Atlas of Living Australia in the Woollahra Municipal Council region from filtered water samples (N = 15). Colour denotes taxonomic subgroups used in statistical analysis and the common names for groupings on the x-axis are assigned to each ASV by inputting and interpreting a vector of full lineage strings delimited with semicolons

February 8, 2023 (Environmental Protection Agency, personal communication, May 28, 2024).

Characterising biological communities and taxa underpinning site condition

As expected, PERMANOVA indicated that the biological community structure based on the ASVs underlying the detected taxa was significantly different between samples with different TICI condition categories (very poor, poor, average) given that this forms the basis for validation of the index (Table 1). Specifically, significant differences were identified for the entire taxonomic community ($p=0.01$), as well as some of the taxonomic subgroups (micro-organisms, $p=0.013$; invertebrates, $p=0.005$). There were no significant differences in community structure however among condition categories for the taxonomic subgroups BMH ($p=0.596$), fish ($p=0.308$), photosynthetic organisms ($p=0.056$), and other taxa ($p=1.000$). Based on only one sample being assigned to the "very poor" TICI condition category ($N=1$), we repeated the PERMANOVA while excluding this TICI condition category from the data set, noting that only invertebrates were significantly different between the two remaining TICI condition categories (poor vs. average, $p=0.044$).

A Principal Coordinate Analysis (PCoA) plot was used to visualise the Jaccard dissimilarity matrix of community structure for each sample defined by condition category, which did suggest some clustering within each category (Fig. 6a, b). The cumulative variation explained by PC1 and PC2 was 26% (Fig. 6a) and 27% (Fig. 6b), respectively, highlighting the complexity of the community structure. Despite significant differences in community structure among condition categories in the overall PERMANOVA, pairwise comparisons between categories for the full community and all taxonomic subgroups did not show any significant differences ($p>0.05$ in all cases) (Online Resource 4).

An Indicator Species Analysis (ISA) identified 8 taxa that contributed significantly ($p<0.05$) to the TICI condition category for each sample, when restricted to the two most robust categories (poor vs. average; Fig. 7, Online Resource 4). Of the significant taxa in our ISA, 4 were micro-organisms, 3 were invertebrates, and 1was assigned an "other" taxonomic classification. These indicators included

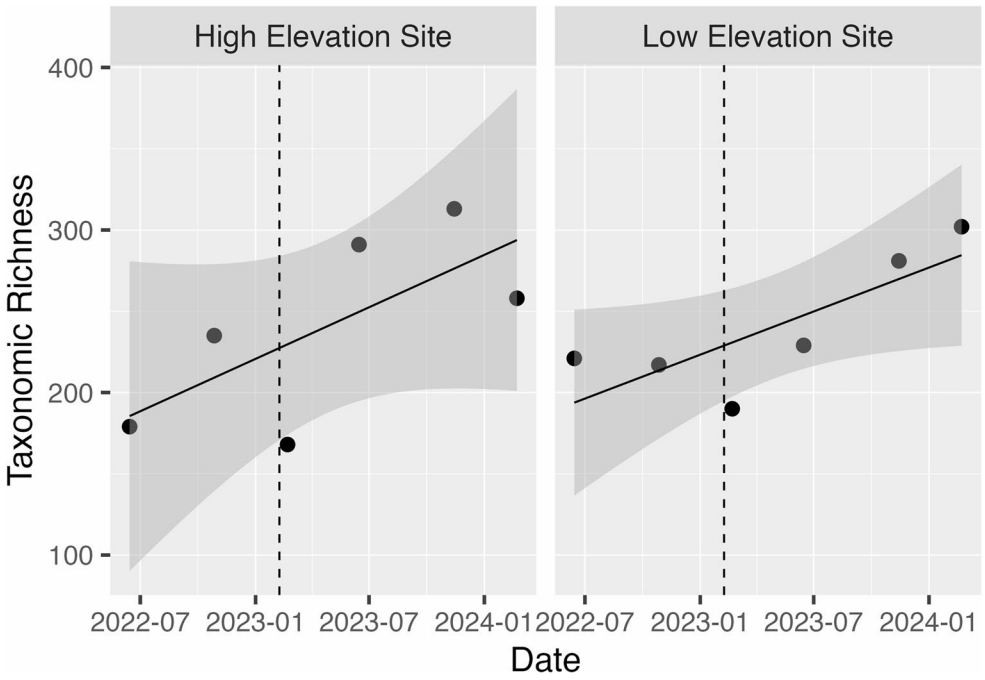

Fig. 5 Scatter plot of total taxonomic richness for each sampling date at the high elevation site (left) ($N=6$) and low elevation site (right) ($N=6$) in Parsley Bay Reserve Creek with the trend line in black (high elevation site $R^2 = 0.47$, low elevation site $R^2 = 0.64$). The approximate date of a sewage incursion event is indicated by the dotted vertical line

Table 1 Results from PERMANOVA of community structure from water samples collected at sites with different assigned TICI condition categories (very poor, poor, average). Analyses were run for the entire taxonomic community ($N=884$), as well as separately for micro-organisms ($N=423$), birds, mammals, and herpetofauna (BMH; $N=14$), invertebrates ($N=209$), fish ($N=21$), photosynthetic organisms ($N=195$), and all "other" taxa ($N=16$). The analysis was repeated with the very poor (vp) category excluded due to low sample size in that category ($N=1$). Significant values ($p<0.05$) are in bold

Group	R^2	F	*p* value all	*p* value vp excluded
Full community	0.221	1.561	**0.01**	0.132
Micro-organisms	0.200	1.372	**0.013**	0.108
BMH	0.137	0.874	0.596	0.524
Invertebrates	0.213	1.492	**0.005**	**0.044**
Fish	0.162	1.059	0.308	0.112
Photosynthetic organisms	0.184	1.239	0.056	0.478
Other taxa	0.190	0.106	1.000	0.103

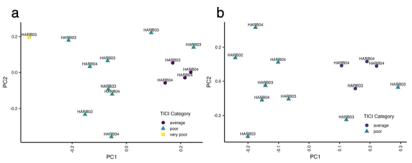

Fig. 6 Principal coordinate analysis (PCoA) plot of the tree of life (ToL) metabarcoding detections across (a) 14 filtered freshwater samples collected in the Eastern Suburbs of Sydney, and (b) 13 filtered freshwater samples collected in the Eastern Suburbs of Sydney, excluding the one sample assigned a "very poor" TICI score. Symbols represent TICI condition categories: average (N=4), poor (N=9), and very poor (N=1). HARB02=Cooper Park Creek; HARB03=Parsley Bay Reserve Creek, high elevation; HARB04=Parsley Bay Reserve Creek, low elevation; HARB05=Cooper Park Pond

Phytophthora (genus of plant-damaging water mold), Chaetothyriales (fungus), *Potamopyrgus* (mud snail), *Vannella* (amoeba), Dugesiidae (freshwater flatworm), *Hydra vulgaris* (freshwater hydroid), *Cyclidium* (free-living ciliate), and Frontoniidae (free-living ciliate).

Testing the effects of season, rainfall, and site condition on taxonomic richness

A Generalised Additive Model (GAM) testing for the effects of month, season, TICI value, and cumulative 7-day rainfall prior to sampling on taxonomic richness in Parsley Bay Reserve Creek indicated that most factors significantly impacted richness (Table 2). The GAM suggested a strong fit (adjusted $R^2= 0.963$) and a significant non-linear relationship between TICI values and taxonomic richness. Pairwise comparisons from the GAM demonstrated that community richness in the full dataset was significantly different among some seasons ($p=0.006$ to 0.986), as well as between sites ($p=0.006$) and across TICI values ($p<0.001$) (Online Resource 4). Rainfall on the other hand was not a significant predictor of taxonomic richness within the full dataset. No significant differences were found in taxonomic richness for BMH or invertebrates and these models had a poor fit (adjusted $R^2= -0.421$ and 0.157, respectively).

Pairwise comparisons within the significant taxonomic subgroups indicated that micro-organisms had significantly lower richness in winter compared to the other seasons ($p=0.012$–0.013) and photosynthetic organisms had significantly lower richness in winter versus spring ($p=0.040$).

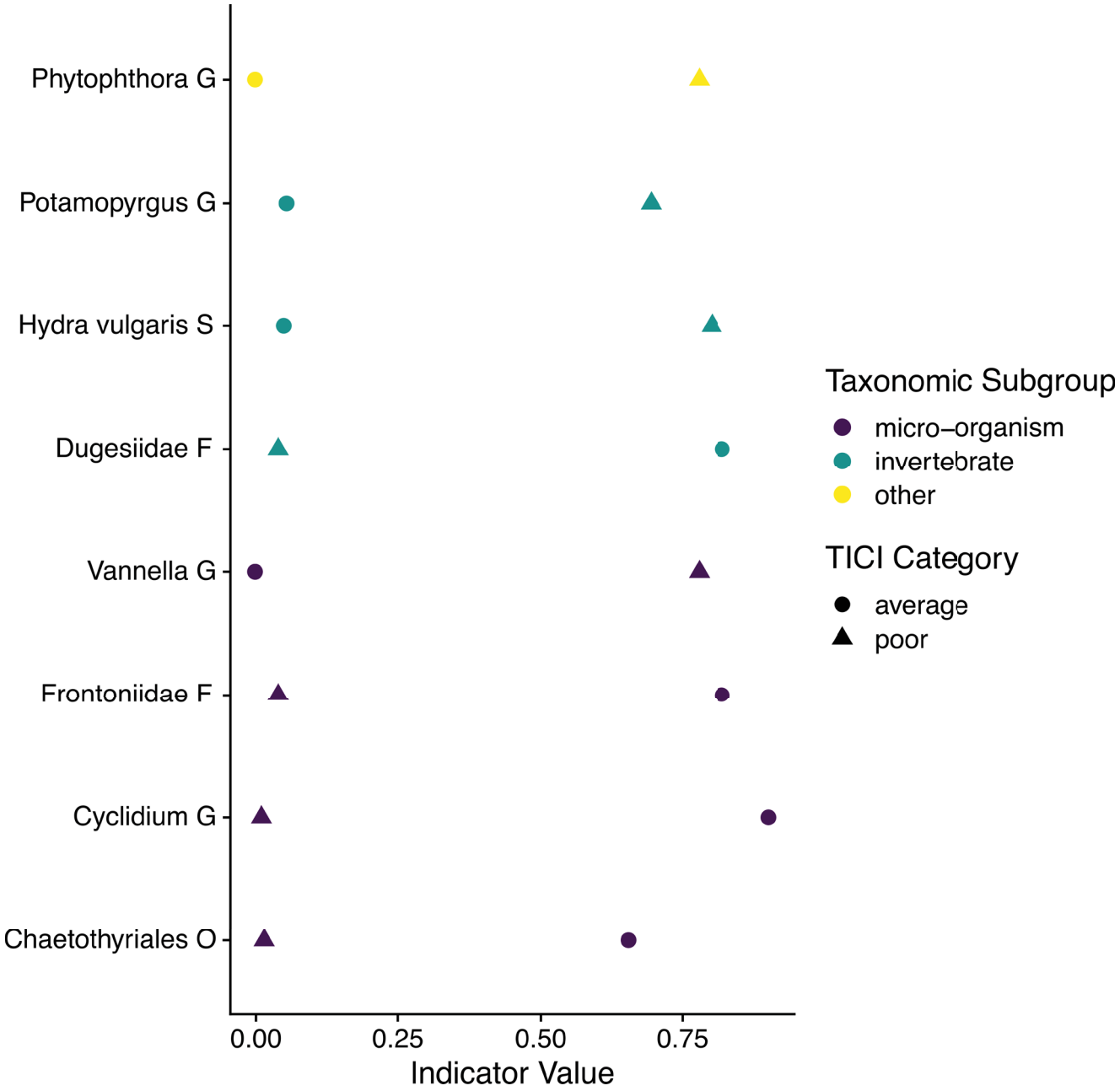

Fig. 7 Significant taxa ($p<0.05$) from the Indicator Species Analysis (ISA) based on TICI condition category (circles = average, triangles = poor) and taxonomic subgroup across 14 filtered freshwater samples. Micro-organisms are coloured purple, invertebrates are coloured aqua, and photosynthetic organisms are coloured yellow. Letter following taxa name represents their taxonomic rank (G = 5, F = 5, O = 3, C = 2, PP = 3). G = genus, F = family, O = order, C = class, P = phylum

For both micro-organisms and photosynthetic organisms, richness was significantly higher when sampling followed low rainfall (micro-organisms, $p=0.0248$; photosynthetic organisms, $p=0.021$). Micro-organism and photosynthetic organism richness also showed a significant non-linear response to TICI values ($p=0.002$–0.003). Month, cumulative rainfall, and TICI values were not significant indicators for fish richness ($p=0.716$–0.986), however fish richness was significantly higher at the low elevation site in Parsley Bay Reserve Creek ($p=0.0004$). For visual display of these outcomes, refer to Figs. 8 and 9.

Discussion

In this study, ToL-metabarcoding was used to address several major challenges associated with monitoring aquatic organisms in urban freshwater lotic and lentic systems

Table 2 Results from a generalised additive model (GAM) testing for the effects of season (spring, summer, and winter), site (low versus high elevation), cumulative 7-day rainfall prior to sampling (high > 20 mm versus low < 20 mm), and TICI value (range = 81.33–94.88) on the number of detected taxa from Parsley Bay Reserve Creek samples ($N = 12$). Significant values ($p < 0.05$) are in bold. Note that we did not sample in the fall season at this site

Factor	Level	Std. Error	T value	*p* value
Season	Spring vs. Summer	0.041	-0.159	0.9860
	Spring vs. Winter	0.038	-6.700	**0.0028**
Site	High elevation vs. Low elevation	0.026	-5.473	**0.0060**
Rainfall	High vs. Low	0.051	1.232	0.2870
		edf	*F statistics*	*p value*
TICI Value		3.10.064	42.43	**0.0006**

(Radinger et al. 2019; Seymour et al. 2020; Turunen et al. 2021). Previous work has shown that molecular approaches can provide a cost-effective, non-invasive sampling method with greater sensitivity than traditional approaches, specifically in high diversity regions (Bálint et al. 2018; Blattner et al. 2021). As demonstrated by our study, ToL-metabarcoding identified changes in taxonomic richness and biological community structure influenced by a number of environmental factors, and we generated a biotic baseline that can now be used to benchmark future change at these locations. With further ground truthing and the adaptation of New Zealand TICIs to new environments, ToL-metabarcoding can provide a means to rapidly assess freshwater urban catchment health in at least parts of eastern Australia.

Characterisation of biota

Our ToL-metabarcoding data provided insights into some of the vertebrate taxa present in small urban catchments across the Eastern Suburbs of Sydney. The Eastern Mosquitofish (*Gambusia holbrooki)* for example, which is considered a restricted noxious fish (Biosecurity Act 2014), was detected using eDNA at both Cooper Park sites and Trumper Park Duck Pond in June 2022. Previously, there was only a single published record for *Gambusia holbrooki* in the Woollahra Municipal Council region on ALA (Cooper Park, 33.887175ºS, 151.2558ºE, observed on December 24, 2023). Additionally, the range-restricted

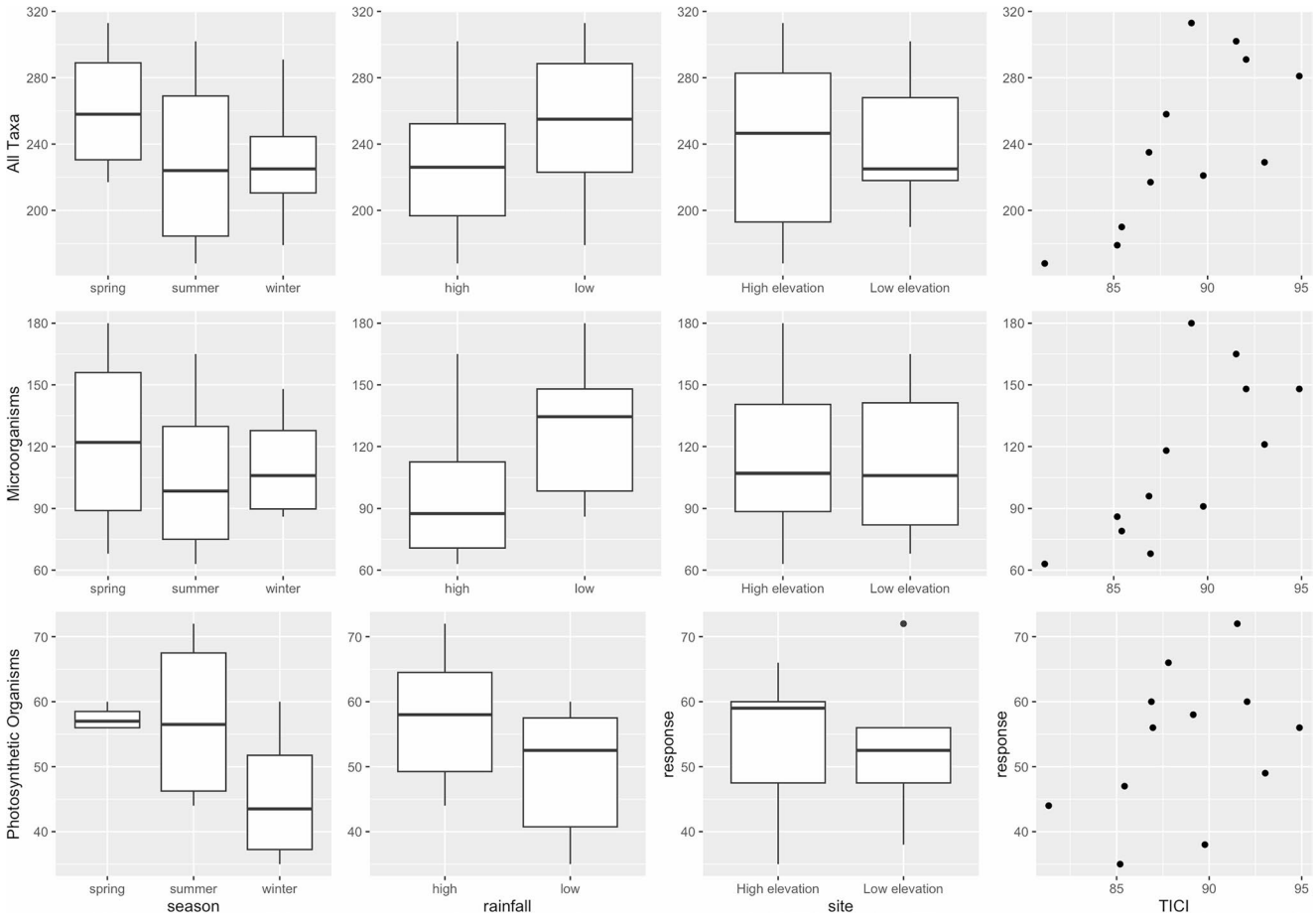

Fig. 8 Boxplots showing taxonomic richness at Parsley Bay Reserve Creek across seasons (spring, summer, and winter), cumulative seven-day rainfall prior to sampling (high > 20 mm versus low < 20 mm), site (high elevation versus low elevation) and TICI values for all taxa (first row), micro-organisms (second row), and photosynthetic organisms (third row)

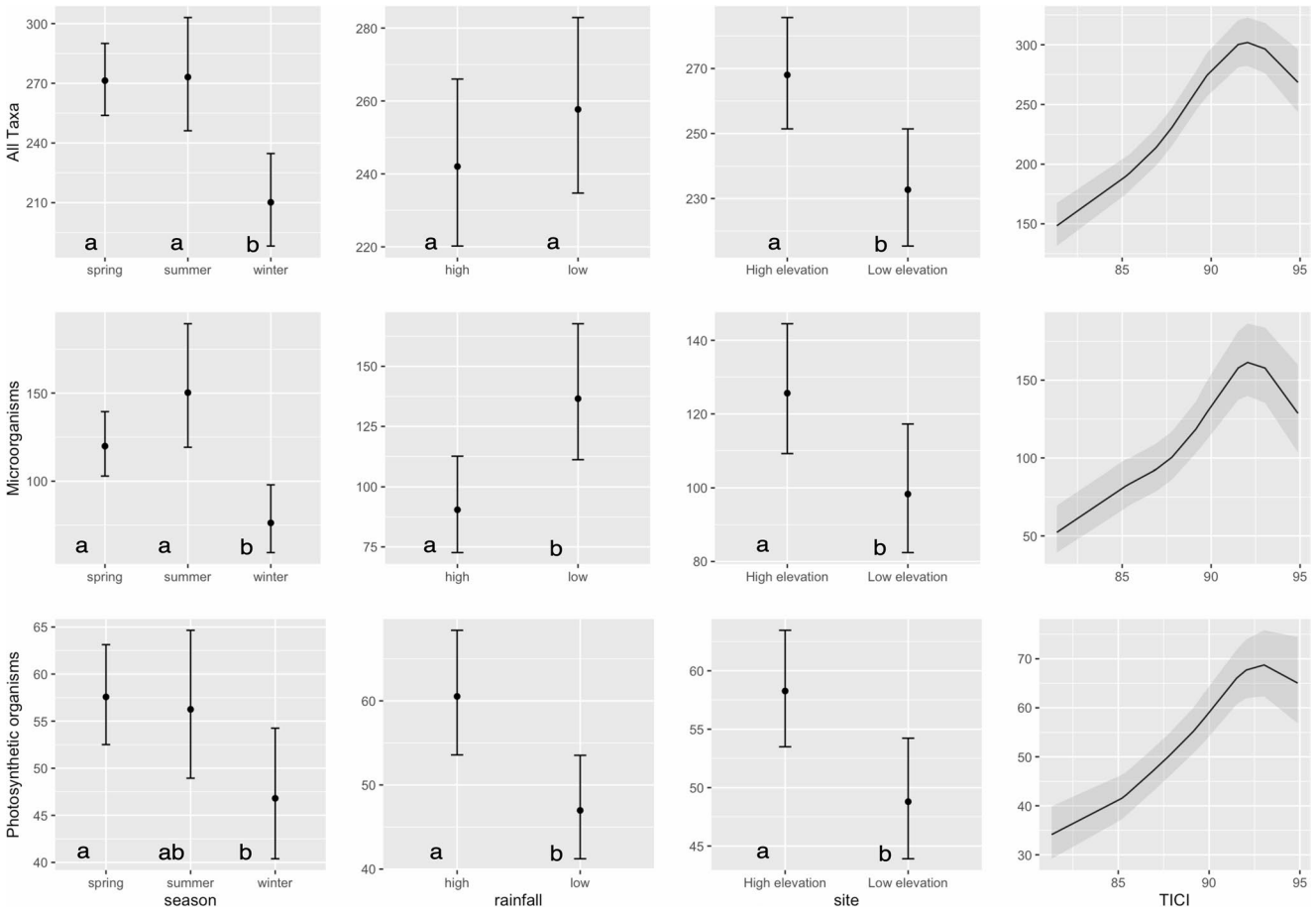

Fig. 9 Point-range plot showing General Additive Model (GAM) adjusted taxonomic richness at Parsley Bay Reserve Creek across seasons (spring, summer, and winter), cumulative seven-day rainfall prior to sampling (high > 20 mm versus low < 20 mm), site (high elevation versus low elevation) and TICI value for all taxa (first row), microorganisms (second row), and photosynthetic organisms (third row). Factors that are significantly different ($p < 0.05$) have different letters

Australian endemic fish, Pinkeye Mullet (*Trachystoma petardi*), which migrates downstream to spawn in estuaries or at sea in summer, was detected at the low elevation site within Parsley Bay Reserve Creek, further demonstrating a connection with the adjacent Parsley Bay and thus Sydney Harbour more broadly. While there are previous records for *Trachystoma petardi* around the Greater Sydney region, there were no previous records on ALA within the Woollahra Municipal Council boundaries, suggesting that our sampling and sequencing has the potential to provide a more exhaustive list of the fish species present in the urban catchments of the Eastern Suburbs of Sydney if sampling is expanded further (e.g., Rushcutters Creek, Double Bay Creek, and Rose Bay Creek). Additionally, our detections included input from terrestrial vertebrate taxa that make use of the surrounding parks and reserves including the grey-headed flying-fox (*Pteropus poliocephalus*) and powerful owl (*Ninox strenua*), both listed as vulnerable species under the NSW Biodiversity Conservation Act (Biodiversity Conservation Act 2016).

Only 24% of the taxa detected using eDNA in our study had previous records on ALA within the Woollahra Municipal Council boundaries, and these records appeared to be skewed towards a few charismatic groups. Indeed, ALA records included 100% of the birds, lizards, snakes, and amphibians, 86% of the fish, 74% of the plants and 64% of the mammal species detected through our water sampling. In contrast, benthic microfauna, suspended microeukaryotes, and prokaryotes, despite their abundance in urban catchments, often go unrecognised in traditional surveys or remain difficult to identify, reflecting limitations of these conventional methods (Gunzburger 2007). Imperfect detectability and taxon bias also contribute to inaccuracy or incompleteness of traditional surveys for these mostly microscopic groups (Katsanevakis et al. 2012; Lardner et al. 2019). For example, bacteria made up most of the detected taxa in our samples, yet only 0.4% of all the bacteria that we detected had previously been recorded on ALA within the Woollahra Municipal Council boundaries. Bacterial detections are important though because these organisms provide

a range of ecosystem services essential to freshwater environments including nutrient cycling, decomposition, and primary production. Given their high metabolic rates and fast growth rates, freshwater microbes are also likely more responsive to pollution and physico-chemical changes, justifying their use as biological indicators for water quality assessments (Sagova-Mareckova et al. 2021; Ma et al. 2022). That said, ASVs that we identified as fungi, suspended micro-eukaryotes (i.e., protists), or benthic microfauna appeared to be the key contributors to TICI condition score, not bacteria.

Temporal trends in taxonomic richness

Repeated sampling of the creek in Parsley Bay Reserve (six samples per site from three sampling events spread across 20 months) indicated a trend for increasing taxonomic richness with time, which may reflect the outcomes of ongoing conservation efforts in the area outlined in the Woollahra Municipal Council Biodiversity Conservation Strategy (Woollahra Municipal Council 2015). There was however a reduction in taxonomic richness immediately following a sewage incursion event into the creek on February 8, 2023 (Fig. 5). While there is not enough data in our study to draw conclusions about the effects of pollution on taxonomic richness and community structure in urban catchments (but see Mushtaq et al. 2020; Tchakonté et al. 2015), ongoing monitoring of Parsley Bay Reserve Creek along with other freshwater sites using ToL-metabarcoding may provide future insight into their cumulative impacts using biotic baselines established with eDNA.

Characterising biological communities and taxa underpinning site condition

As expected, our multivariate statistical analysis indicated that TICI score and thus condition category significantly influenced biological community structure given that presence or absence of diagnostic ASVs are the basis for the calculation of this metric. These biotic differences were most significant for the full community, micro-organisms, and invertebrates when all condition categories were considered, but restricted to invertebrates when only the two most data rich condition categories were compared (i.e., poor vs. average). More than 1967 of the 3000 DNA sequences used for the TICI in New Zealand (Wilkinson et al. 2024) have been previously detected in Australia and thus are available to assign a TICI score to Australian water samples (Wilderlab, unpubl. data). That said, our water samples collected from urban catchments and processed with eDNA workflows generated less than 100 to 367 of these diagnostics ASVs. In fact, one of our samples (Trumper Park Duck Pond) did not pass the threshold of 100 diagnostic ASVs required to calculate and assign a TICI score. Trumper Park Duck Pond also had a lower level of taxonomic richness based on its full community (108 ASVs) compared to the average across all other samples (276 ASVs). Despite the low coverage on the TICI and relatively fewer taxa detected, this sample still made an important contribution to our faunal knowledge of the area based on the invasive Eastern Mosquitofish *Gambusia holbrooki* identified at this site (also described above).

The low number of sequences used to generate a TICI condition score in our study in general might be explained by the taxonomic differences between Australian and New Zealand freshwater ecosystems, for which there is limited comparative research. Winterbourn et al. (2010) suggested that the ecosystem characteristics of New Zealand are most akin to alpine regions in Australia, and therefore largely consist of alpine-like taxa. While Australian taxa in alpine regions may be like those in New Zealand, our current sampling was restricted temperate creeks and ponds, in some cases (i.e., Parsley Bay Reserve Creek) surrounded by littoral rainforest (R. Kooyman, pers. comm.), a Critically Endangered ecological community defined by its proximity to the ocean and influence from salty winds. Adapting the TICI metric with Australian taxa may therefore provide stronger confidence in future evaluations of urban catchment condition and more accurately represent Australian freshwater ecosystem dynamics.

The limited scope of sampling in our study further reduced the power to detect statistical differences among groups with the smallest sample sizes. For example, given that there were fewer detections of BMH ($N=14$) and fish ($N=22$) relative to all other taxonomic subgroups, we had limited ability to explore differences within them. This low detection rate may reflect fewer species in the area, a result of the disturbed condition of most of the urban sites. It may also simply reflect the fact that there are fewer large vertebrates to account for compared to the higher relative diversity of micro-eukaryotes and prokaryotes. Additionally, no significant differences in total diversity were found between TICI condition categories in pairwise comparisons for BMH and fish, likely due to the unbalance among TICI condition categories assigned. Expanding sampling to additional urban catchments in the Eastern Suburbs of Sydney, as well as re-visiting the sites that we sampled at multiple time points, would allow us to confirm the relative contributions of purely statistical versus biological relationships.

Our indicator species analysis was important because it allowed us to confirm the taxonomic identities of ASVs contributing most to the TICI condition score in our samples. Our analysis suggested that micro-organisms (amoebae, free-living protists, and fungus) and invertebrates (freshwater flatworms, mud snails, and hydroids) served as the strongest indicators

of TICI condition category, which as previously outlined is indicative of ecosystem health. This statistical finding makes sense given that similar invertebrate groups are historically used as indicators of freshwater catchment health, including in Australia (Chessman 2003; Davies et al. 2010). The Macroinvertebrate Community Index (MCI) used in New Zealand as a related example (Begum et al. 2023), which forms the motivation for building a TICI (Wilkinson et al. 2024), is also reliant on macroinvertebrate indicators (largely insects, molluscs, snails, and worms). Despite these similarities, our results suggest that micro-organisms are more strongly associated with TICI values in Australian urban catchments, supporting recent research that advocates for the inclusion of micro-organisms in freshwater ecosystem monitoring (Begum et al. 2023). Given the difficulties in physically sampling or morphologically identifying micro-organisms in general, metabarcoding these taxa isolated from water samples provides an excellent opportunity to overcome these challenges.

Several of the taxa that were strongly associated with a TICI category in our study are also known bioindicators of water quality and pollution, including some that appear to pose direct threats to ecosystem health. Indeed, while one of our indicator invertebrate groups was only identified at the family level (Dugesiidae), species nested within this family of freshwater planarians (i.e., flatworms) are often used as bioindicators of aquatic ecosystem health (Tripathi 2022). Another indicator that we identified, *Phytophthora*, is a genus of plant-damaging oomycetes (water mold) that poses a significant threat to the Australian environment. Dieback caused by the root-rot fungus associated with *Phytophthora cinnamomi* is listed as a key threatening processes under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*. Finally, our invertebrate indicator from the genus *Potamopyrgus* likely refers to the New Zealand mud snail, *Potamopyrgus antipodarum* (Gray 1843) (Gastropoda: Hydrobiidae), a highly fecund species endemic to New Zealand but now considered a global freshwater invader, with populations established in Europe, Asia, the Americas and Australia (Schreiber et al. 1998). In its invasive range, which includes Australia, this mud snail is most often found associated with ecosystems subject to human disturbance (Alonso & Castro-Diez 2008). This suggests that despite significant differences in the flora and fauna of Australia and New Zealand, the New Zealand TICI can still provide some insight into freshwater catchment health in Australia in its current form.

Testing the effects of season, rainfall, and site condition on taxonomic richness

Significant differences in taxonomic richness were identified for the full biological community in winter compared to other seasons, which was also true for micro-organisms and photosynthetic organisms in spring compared to other seasons. Statistical inferences for the other taxonomic subgroups were limited by small sample size and poor model fit. While the raw data suggests that taxonomic richness was lowest in summer overall, the GAM results suggest that richness was lowest in winter when all other factors (site, rainfall, TICI value) were controlled. As mentioned above, micro-organisms (defined in this study as amoeba, archaea, bacteria, ciliates, and fungi) are an extremely diverse group, and as such, rarely conform to elevational or seasonal patterns across sites (Jiang et al. 2021; Wang et al. 2017), especially in catchments with the added complexity of urban influences (but see Simon et al. 2015). One explanation for the observed lower micro-organism richness in summer is an increase in anthropogenic activity (Reitschuler et al. 2016). Though Parsley Bay Reserve Creek is not used recreationally for swimming or wading due to its small size and low quality, increased foot traffic during the summer may have an impact, but testing this hypothesis would require measuring the creek's physico chemical properties (i.e., water quality indicators) through time. Photosynthetic organisms followed a different pattern in seasonal richness compared to the full community, as taxonomic richness was significantly lower in winter versus summer. This pattern conforms to known trends in autotrophs due to increased temperatures and less limiting factors such as nutrients and light in summer (Maberly et al. 2022; Velho et al. 2021).

The two other factors that we statically tested (cumulative rainfall and TICI condition category) displayed more consistent patterns across taxonomic subgroups. The pattern of detections suggested that rainfall volume negatively impacted the freshwater biological communities by decreasing taxonomic richness, a possible outcome of increased runoff associated with the surrounding urban environment. Runoff introduces pollutants into waterways and is exacerbated in urban settings by soils with lower infiltration capacity and impervious surfaces such as roads, roofs, and car parks (Alaoui et al. 2018; Yao et al. 2016). In contrast, Staley et al. (2018) found that richness in urban creeks based on eDNA sampling was reportedly higher after extreme rain events due to runoff containing faecal matter from terrestrial organisms. That said, our eDNA detections may not include as much of these exogenous inputs as we did not sample water within three days of a rainfall event. Moreover, while the DNA of terrestrial species such as dogs (*Canis lupus familiaris*), possums (*Trichosurus vulpecula*), and rats (*Rattus rattus*) were present in our samples, these were detected after both high and low rainfall scenarios (Online Resource 1), suggesting consistent activity of these animals in or near the urban catchments that we sampled.

Suitability of ToL-metabarcoding for future monitoring of urban catchments

There are several caveats associated with using ToL-metabarcoding to detect biodiversity in aquatic environments, no matter how big or small the target catchment. One of the technological challenges is related to gaps in the DNA reference libraries used for taxonomic assignment (Duarte et al. 2020; Jackman et al. 2021). These reference gaps may limit the scope of detectable flora and fauna, which is particularly relevant for unique environments with limited archival DNA collections of the local species (Marques et al. 2021). Another caveat is related to incomplete taxonomic coverage of the genetic assays, an inevitable byproduct of the need to increase sensitivity for detections but with limitations on the number of assays that can be realistically run in tandem. For example, in our study, Australian water dragons (*Intellagama lesueurii*) and eastern long-necked turtles (*Chelodina longicollis*) were sighted in the field when collecting the water samples at some locations, and there are signs in each of the parks indicating the presence of the invasive red-eared slider turtle (*Trachemys scripta elegans*), but we did not detect these species. None of our genetic assays targeted reptiles, and so these taxa were likely missed and thus underrepresented in the data. There also remain further challenges associated with reptilian DNA amplification in metabarcoding applications to be resolved in future (West et al. 2021).

Ambiguity or gaps in the reference database used for taxonomic assignment also presents a challenge for delimitation of organisms with high genetic similarity and intragenomic variation (Paloi et al. 2022; Pereira et al. 2020). This was the case for the Eastern Mosquitofish (*Gambusia holbrooki*) detected in our study, which shares high genetic similarity with *Gambusia affinis* (known from the central United States; Pyke 2005). This means that the same ASV might be assigned to one of the two taxonomic names on a regional basis. These challenges can therefore result in under- or over-representation of taxonomic richness, and more critically obfuscate the early detection of invasive species that are closely related to an endemic species.

Another limitation of eDNA is the introduction of contamination resulting in false positives, which includes eDNA pollution introduced by wastewater (Inoue et al. 2023), both in its raw and treated form. This source of contamination is likely amplified when monitoring aquatic ecosystems in urban environments. In fact, it appears that advanced wastewater treatment methods utilising UV, NaClO, and O_3 still retain significant eDNA upon release, which can lead to false positives when that wastewater is discharged into an urban catchment (Xiong et al. 2024), though this form of discharge is more relevant to large versus small catchments.

Conclusions

ToL-metabarcoding provides an ideal opportunity for generating biodiversity data with non-invasive water sampling. In our study, we were able to characterise the biological communities of permanent urban freshwater ecosystems in the Eastern Suburbs of Sydney. Though preliminary in scope, this sampling effort across different sites and time points included the detection of invasive species, previously unrecorded taxa, as well as the identification of variation in taxonomic richness related to season, rainfall, and as expected, site condition category. These biotic baselines can serve as reference points for future sampling efforts that generate long-term, consistent data needed to accurately track biodiversity changes over time, in our case, within small urban catchments. To maximise their utility however, we suggest that ToL-metabarcoding, where possible, should be integrated into a comprehensive monitoring framework that includes calibration against known taxa, comparison with traditional monitoring methods, and periodic validation. Additionally, adapting and refining the indicator DNA sequences used in the TICI for Australian contexts, by incorporating locally relevant reference data, can only enhance the accuracy and reliability of the index and assist stakeholders committed to safeguarding and managing urban freshwater ecosystems in variable states of health.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11252-026-01901-z.

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Author contributions J.D.D. and S.P.W. contributed to conceptualization, funding acquisition, methodology, and project administration; all co-authors contributed to data curation, investigation, formal analysis, and validation, with C.H. contributing to statistical analysis; C.H. and J.D.D. contributed to visualization; C.H. and J.D.D. contributed to writing the original draft manuscript, and all co-authors contributed to writing – review and editing.

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Data availability All raw data needed to replicate the study, and statistical analyses, are available as supplementary material. Raw sequence data are also available as .fastq files on the NCBI Sequence Read Archive (BioProject Accession Number: PRJNA1241474).

Declarations

Statement on inclusion Our study had a regional focus but with much broader applications and therefore included local data collection. Most of the co-authors are from the country where the study was carried out, and several are current members of the local community that actively collected data. Whenever relevant, literature published by scientists from the region was cited.

Competing interests Shaun P. Wilkinson is a director and shareholder of Wilderlab NZ Ltd., a commercial eDNA processing laboratory. All other co-authors declare no known competing financial interests or personal relationships that influenced the work reported in this paper.

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