Author/s:
McColl-Gausden, EF;Weeks, AR;Coleman, RA;Robinson, KL;Song, S;Raadik, TA;Tingley, R

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MISS EMILY FRANCES MCCOLL-GAUSDEN (Orcid ID : 0000-0001-7200-6725)

DR. REID TINGLEY (Orcid ID : 0000-0002-7630-7434)

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Multi-species models reveal that eDNA metabarcoding is more sensitive than backpack electrofishing for conducting fish surveys in freshwater streams

Running head: eDNA metabarcoding versus electrofishing

McColl-Gausden EF[1]*, Weeks AR[1,2], Coleman RA[3], Robinson KL[1], Song S[2], Raadik TA[4], Tingley R[5]

1. School of BioSciences, University of Melbourne, Parkville, Victoria 3010 Australia
2. cesar Pty Ltd, 293 Royal Pde, Parkville, Victoria 3052 Australia
3. Melbourne Water, 990 La Trobe Street, Docklands, Victoria 3008, Australia
4. Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, 123 Brown Street, Heidelberg, Victoria 3084, Australia
5. School of Biological Sciences, Monash University, Clayton, Victoria 3800 Australia

*Corresponding author: mccolle@student.unimelb.edu.au

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Abstract

Environmental DNA (eDNA) sampling can provide accurate, cost-effective, landscape-level data on species distributions. Previous studies have compared the sensitivity of eDNA sampling to traditional sampling methods for single species, but similar comparative studies on multi-species eDNA metabarcoding are rare. Using hierarchical site occupancy detection models, we examined whether key choices associated with eDNA metabarcoding (primer selection, low-abundance read filtering, and the number of positive water samples used to classify a species as present at a site) affect the sensitivity of metabarcoding, relative to backpack electrofishing for fish in freshwater streams. Under all scenarios (teleostei and vertebrate primers; 0%, 0.1% and 1% read filtering thresholds; 1 or 2 positive samples required to classify species as present), we found that eDNA metabarcoding is, on average, more sensitive than electrofishing. Combining vertebrate and teleostei markers resulted in higher detection probabilities relative to the use of either marker in isolation. Increasing the threshold used to filter low abundance reads decreased species detection probabilities but did not change our overall finding that eDNA metabarcoding was more sensitive than electrofishing. Using a threshold of two positive water samples (out of 5) to classify a species as present typically had negligible effects on detection probabilities compared to using one positive water sample. Our findings demonstrate that eDNA metabarcoding is generally more sensitive than electrofishing for conducting fish surveys in freshwater streams, and that this outcome is not sensitive to methodological decisions associated with metabarcoding.

Introduction

Environmental DNA (eDNA) sampling—detection of environmental cellular or extracellular DNA—has the potential to improve the sensitivity and efficiency of biodiversity surveys, enabling the coverage needed for accurate monitoring at broad spatial scales (Deiner, Fronhofer, Mächler, & Altermatt, 2015; Turner et al., 2014). In aquatic environments, eDNA sampling is safer for operators (Crookes et al., 2020) and less invasive to target animals compared to traditional sampling methods, such as trapping or electrofishing (Valentini et al. 2016). In addition, low detection probabilities associated with some traditional methods mean that biodiversity monitoring programs often use indicator species that are easy to detect (Ji et al., 2013) or only measure a subset of biodiversity (Drummond et al., 2015). By contrast, eDNA sampling can improve the detection of low abundance (Dougherty et al., 2016) and cryptic species (Port et al., 2016; Ransome et al., 2017), providing a broader snapshot of biodiversity (Valentini et al., 2016). Environmental DNA sampling also enables sites to be surveyed that are typically inaccessible, or unsuitable for traditional methods, for example because they are too fast flowing, too deep, or too difficult to access directly.

Environmental DNA sampling in ecology has progressed substantially in recent years. Early ecological studies using eDNA sampling aimed to detect single species in water samples using species-specific primers and approaches such as quantitative PCR (Goldberg, Pilliod, Arkle, & Waits, 2011; Jerde, Mahon, Chadderton, & Lodge, 2011; Thomsen et al., 2012). However, as sequencing technologies have developed, eDNA metabarcoding (also known as environmental barcoding, and (eco)metagenetics (Valentini, Pompanon, & Taberlet, 2009)) has been increasingly adopted in ecological applications. Metabarcoding enables the simultaneous identification of multiple species in environmental samples using high-throughput sequencing and primers that are designed to amplify a target DNA region from a specific group of species or taxa, rather than an individual species (Deiner et al., 2017; Harper et al., 2018; Taberlet & Coissac, 2012).

Widespread adoption of eDNA metabarcoding by researchers and wildlife managers requires field validation with natural communities, and evidence that eDNA metabarcoding is more efficient, accurate, and safer than current sampling methods (e.g., higher detection probabilities and/or higher species richness estimates, without an increase in false positive detections, and safer for both operators and target species). Numerous studies have compared eDNA sampling to traditional sampling methods, but most studies have taken a single-species approach (e.g., Dougherty et al. (2016); Jerde et al. (2011); Smart et al. (2015)). In addition, most studies have not compared the two methods simultaneously. Simultaneous comparisons, in which both methods are used on the same day, at the same sites, remove confounding effects of species movement, environmental changes, or surveyor bias (Lim et al., 2016; Mcdevitt et al., 2019; Olds et al., 2016; Shaw et al., 2016).

Environmental DNA metabarcoding has often been evaluated against traditional sampling methods by comparing raw estimates of species richness and species assemblages (Mcdevitt et al., 2019; Port et al., 2016; Shaw et al., 2016). Here, we use an alternative approach, site occupancy detection models (SODM), which enable simultaneous estimation of occupancy and detection probabilities of individual species in a community. SODM have been used in single-species eDNA studies (Dougherty et al., 2016; Hunter et al., 2015; Lugg, Griffiths, van Rooyen, Weeks, & Tingley, 2017; Schmidt, Kéry, Ursenbacher, Hyman, & Collins, 2013) but have rarely been applied to eDNA metabarcoding. Valentini et al. (2016) illustrated how SODM can be used to estimate the sensitivity of metabarcoding, finding that detection probabilities were, on average, higher using eDNA sampling compared to traditional sampling methods for amphibians. In their analysis, Valentini et al. (2016) treated species identity as a fixed effect. Hierarchical SODM, in which species-level parameters are assumed to be drawn from a common statistical distribution, are a natural extension to this analytical approach, enabling data from the entire community to inform parameter estimates for individual species, including those with low occupancy and detection probabilities (Kéry & Royle, 2016).

Environmental DNA metabarcoding is a rapidly developing technique but there are a variety of methodological decisions that must be made once environmental samples are collected. These methodological decisions are rarely justified, and previous studies comparing eDNA metabarcoding and traditional sampling methods have not considered the potential impacts of these decisions on the sensitivity of eDNA metabarcoding. One such uncertainty is primer selection. *In silico* testing is vital to ensure the selected primers can amplify species of interest. The performance of different primer pairs has been tested using species richness estimates (Alberdi, Aizpurua, Gilbert, & Bohmann, 2018; Hänfling et al., 2016; Olds et al., 2016; Shaw et al., 2016) but the extent to which primer selection impacts comparisons between survey methods and species detectability has not been explored.

Another key methodological decision is the use of a threshold for filtering low abundance reads. Filtering can be used in metabarcoding studies to reduce false positive detections in eDNA data (Ficetola, Taberlet, & Coissac, 2016; Guillera-Arroita, Lahoz-Monfort, van Rooyen, Weeks, & Tingley, 2017; Lahoz-Monfort, Guillera-Arroita, & Tingley, 2016) alongside other data curation steps (Calderón-Sanou, Münkemüller, Boyer, Zinger, & Thuiller, 2020). However, if there are some true positive detections that occur at the same rate as false positives, the filtering approach can incorrectly remove true positive detections (Hänfling et al., 2016). The threshold used for filtering low abundance reads can be selected based on the management question; for example, to minimise the probability of false positive detections, or to ensure that low abundance true positives are retained. Threshold values have differed between eDNA metabarcoding studies (Deiner et al., 2015; Lim et al., 2016; Thomsen et al., 2012; Valentini et al., 2016); this results in uncertainty about the scale of impact that these decisions can have on detection, and therefore the effectiveness of eDNA metabarcoding as a species detection method. Alberdi et al. (2018) investigated how primer sets, PCR replicates, sequencing depth, PCR processing strategy, low abundance read filtering, chimera removal, and thresholds for assignment of Operational Taxonomic Units (OTU) affected the detection of arthropod diversity within scat samples. However, the effects of these types of decisions on the sensitivity of eDNA metabarcoding relative to a traditional sampling method has not been explored.

In this paper, we compare detection probabilities of eDNA metabarcoding and backpack electrofishing for fish in freshwater streams across greater Melbourne, Victoria, Australia. Our comparative study focuses on freshwater fish, an important social and environmental value within these systems (Melbourne Water, 2018), and a taxon that is sensitive to environmental change and therefore a proxy for waterway health (Evans et al., 2016). Backpack electrofishing has been considered the standard for fish biodiversity surveys in Melbourne's freshwater streams. As a result, extensive catch data are also available for this region, to help ground truth results and develop region-specific reference databases for the barcodes used in this study. Through comparing the two sampling methods, we investigate the following questions: (i) is eDNA metabarcoding an effective alternative to a traditional method (backpack electrofishing) for sampling freshwater fish? and (ii) do methodological decisions in the metabarcoding process impact the effectiveness of eDNA metabarcoding compared to electrofishing?

Materials and Methods

Study sites

This study was conducted in freshwater streams across the greater Melbourne region, Victoria, Australia (Figure 1). Melbourne is a temperate city, with average monthly temperatures ranging from 6.0 °C to 26.0 °C and an average rainfall of 648.3 mm/yr (Bureau of Meteorology, 2017). Between October 2016 and January 2017, eDNA samples were collected immediately prior to electrofishing surveys at 25 sites. These sites spanned streams flowing through a range of geographical areas with different total annual rainfall and land use, to maximise the likelihood of detecting most freshwater fish species recorded in the region. Each site consisted of a 100 m stretch of stream netted at both ends. Site characteristics are given in Table 1.

The use of electrofishing imposed several abiotic constraints on site selection to ensure that a comparison with eDNA was indicative of the types of sites at which backpack electrofishing has historically been applied. All sites had an electrical conductivity less than 1000 EC (min = 56 µS/cm, mean = 350 µS/cm, max = 975 µS/cm); a maximum mean wetted width of 15 m (min = 1 m, mean = 5 m, max = 15 m); and an average depth less than 1 m (min = 0.2 m, mean = 0.5 m and max = 1.0 m). Turbidity at the time of surveys ranged from 1.2-196 NTU, with a mean value of 32.9 NTU. In addition, selected sites also had an intermediate level of habitat complexity to avoid sites that were likely to have low species richness (i.e. potentially poor ability to discriminate between the two survey methods) or sites with high instream habitat complexity—dense riparian vegetation or woody debris—that restricted accessibility for backpack electrofishing.

Environmental DNA sampling

Five water samples were taken at roughly 20 m intervals along each 100 m electrofishing reach, working from downstream to upstream at each site. A disposable syringe (60-mL Luer Lock sterile syringes; Hapool medical Technology, Shandong, China) was used to draw water directly from the waterbody and then pass it through a detachable 0.22 µm filter (GP 22µm Filter Unit; Sterivex, EMD Millipore corporation, Billerica MA, United States). Between 300–500 mL of water was filtered per sample. To prevent contamination between samples and sites, disposable gloves, and sterile syringes and filters were used, and field equipment was sterilised with 10% bleach solution and dried between sites. Samples were kept on ice in a dark storage container in the field (< 10 hrs) and then stored at -20 °C until DNA extraction.

DNA extraction and metabarcoding

Environmental DNA was extracted from Sterivex filters using DNeasy Blood & Tissue Kits (Animal Tissue Spin-Column Protocol; Qiagen, Chadstone, Victoria, Australia). We added 540 µL Buffer ATL and 40 µL Proteinase K (Qiagen) to each Sterivex filter and incubated these with constant rotation at 55 °C for 2 hrs. Samples were then processed in 2 mL tubes using the manufacturer's instructions with the following volume adjustments: 500 µL AL buffer, 500 µL ethanol and 100 µL AE buffer (Qiagen).

Two different markers were used in separate assays: one targeting all vertebrates (Riaz et al., 2011; Shehzad et al., 2012) and one specific to bony fish (Valentini et al., 2016). The universal vertebrate assay marker region was approximately 100 bp and the teleostei specific assay marker region approximately 60-65 bp. Both markers were located within the mitochondrial 12S ribosomal RNA (rRNA) gene. Metabarcoding libraries were prepared using a two-step PCR protocol. Primer sequences are listed in Table S1 (Supporting Information).

The first round PCR primers contained a (5'-3') universal adaptor sequence, a 0-6 bp heterogeneity spacer (Fadrosh et al., 2014) and the marker specific primer sequence. Reactions contained 2 µL eDNA, 1x PCR buffer, 3 mM $MgCl_2$, 0.3 µM each primer and 0.2 U KAPA Plant DNA Polymerase (Kapa Biosystems, Massachusetts, USA) in a final volume of 10 µL. Reaction conditions were as follows: one cycle at 95 °C for 3 minutes; 40 cycles at 95 °C for 20 seconds, 57 °C (vertebrate) or 63 °C (teleostei) for 15 seconds, 72 °C for 15 seconds; and one cycle at 72 °C for 1 minute. Four technical replicates were performed for each sample, one for each heterogeneity spacer combination (see Table S1, Supporting Information) with samples randomly allocated across PCR plates. Following qPCR, replicate reactions were pooled together in equal volumes. Therefore, there is no technical replication in our data. PCR products were then purified with Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (GE Healthcare Life Sciences, Massachusetts, USA).

Second round PCR primers contained (5'-3') the Illumina p5 or p7 binding region, an 8 bp index sequence (Fadrosh et al., 2014) and a universal adaptor sequence. Unique forward and reverse index combinations were used for each sample. Reactions contained 2 µL purified first PCR product, 0.5 µM of each primer and 1x Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Massachusetts, USA) in a final volume of 20µL. Reaction conditions were as follows: one cycle at 98 °C for 1 minute; 15 cycles at 98 °C for 10 seconds, 72 °C for 45 seconds; and one cycle at 72 °C for 10 minutes. The concentration of PCR product was normalised for each sample with the SequalPrep Normalization Plate (96) Kit (Invitrogen, California, USA). Normalised samples were pooled and then subject to dual size selection to remove non-specific fragments outside of the desired size range, using Sera-Mag SpeedBead Carboxylate-Modified Magnetic Particles (GE Healthcare Life Sciences, Massachusetts, USA). Sequencing was performed on an Illumina Miseq platform (Illumina, California, USA) using 150 bp PE chemistry, at the Australian Genome Research Facility (Melbourne, Australia). Twelve H_2O (PCR negative) controls and five blank filter (DNA extraction negative) controls were used. One blank control was performed per batch of DNA extractions, with a total of five blank extraction controls. Approximately one out of every 12 PCR wells was an H_20 control and there were 12 H_20 controls in total.

Bioinformatics

Bioinformatic analyses were performed with a custom analysis pipeline that incorporated the software programs VSEARCH v2.9.0 (Rognes, Flouri, Nichols, Quince, & Mahé, 2016) and cutadapt (Martin, 2011). Following preliminary bioinformatic processing and quality filtering, sequencing reads were de-replicated and those with an abundance of less than 10 were excluded from further analyses. Sequences were clustered into OTUs using a pairwise identity of 100% (vsearch --id 1.0). Although using a threshold of 100% at this stage could be considered conservative, this approach was adopted because it allowed for greater transparency in the pipeline (taxonomic assignment for any individual sequence could be traced more easily compared to the scenario where sequences with <100% identity were grouped into the same cluster). Furthermore, a conservative approach was deemed preferable to avoid misassignments or over-assignments in the downstream analyses given the relatively short marker lengths used in this study.

Taxonomy was assigned to OTUs using custom-built databases containing reference sequence data for all fish species known to occur in the greater Melbourne area for which sequence data were available. A separate database was constructed for each marker using *in silico* PCR, performed with the primer sequences listed in Table S1 and the software packages OBITools (Boyer et al., 2016) and ecoPCR (G. Ficetola et al., 2010). The R package rentrez V.1.1.0 (Winter, 2017) was used to retrieve all 12S rRNA sequences present in GenBank, which served as the template 'DNA'. Databases were filtered to contain only locally occurring species. They were then manually supplemented with (i) additional reference sequences generated from tissue DNA collected during this study and (ii) GenBank partial 12S rRNA sequences that spanned the entire barcode. These partial sequences did not contain complete flanking primer sequence data and thus were not detected by *in silico* PCR.

Taxonomy assignment was performed using the Sintax algorithm (Edgar, 2016) built into VSEARCH, with the minimum level of bootstrap support set to 95% (vsearch *--sintax-cutoff 0.95*). For unassigned OTUs, a BLASTN search was conducted and in cases where matches were found at 95% similarity or greater, the best match (highest similarity) was taken to be the species assignment if the species was known to occur in Australia. If two or more matches of equal similarity were found, the lowest common taxonomic rank was used (typically genus). *Percalates sp.* and *Hypseleotris sp.* were dealt with in this way. Statistical analysis was completed using the universal vertebrate marker and teleostei marker separately. Data from both markers were also combined to create a virtual 'combined marker', in which a species was considered detected in a sample if either of the markers detected it.

To reduce the chance that species detections were false positives, we also ground-truthed eDNA data against long term (20-year) electrofishing data from Melbourne Water (unpublished) at the catchment scale.

Electrofishing

Electrofishing surveys were conducted using a 100 m double-pass method (Lieschke, Raadik, & Nicol, 2014) using a battery operated portable backpack electrofishing unit (Smith-Root model LR20B, Vancouver WA, United States). Each electrofishing pass was separated by a 30-minute recovery period, and recorded fish were stored in containers between runs. Total time spent electrofishing at each site, combined between the two passes, ranged from around 34 to 79 minutes (mean = 56 minutes), and total time that the electrofishing unit was activated ranged from 1,020 to 2,409 seconds (mean = 1,684 seconds). Except for *Hypseleotris* sp. complex and a single immature lamprey specimen (that was subsequently identified in the laboratory), all fish were identified to species-level in the field.

Model description

Analysis was restricted to fish species that could be detected using electrofishing and the teleostei-specific metabarcoding primers to ensure a fair comparison between the two survey methods. The observed data took the form of binary detection/non-detection observations of species k $(1,2\ldots n_k)$, at site i $(1,2\ldots n_i)$. Six replicate observations were conducted at each site (five eDNA replicates and one electrofishing event) j $(1,2\ldots n_j)$. The twenty-five streams were spatial replicates.

A hierarchical multi-species site occupancy detection model was used to estimate occupancy and detection probabilities for each species. The model was fit in a Bayesian framework and had two components: a state process describing the latent (unobserved) occurrence of each species, and an observed, replicated detection/non-detection component. Both components were based on Bernoulli distributions (Kéry & Royle, 2016).

$$z_{ik} \sim Bernoulli(\psi_{ki}) \qquad \text{Eq.1}$$

$$y_{ijk} | z_{ik} \sim Bernoulli(z_{ik} p_{ijk}) \qquad \text{Eq.2}$$

$$logit(\psi_{ik}) = \phi_k \qquad \text{Eq.3}$$

$$logit(p_{ijk}) = \alpha_k + \beta_k * M_{ij} \qquad \text{Eq.4}$$

$$\phi_k \sim Normal(\mu_\phi, {\sigma^2}_\phi) \qquad \text{Eq.5}$$

$$\alpha_k \sim Normal(\mu_\alpha, {\sigma^2}_\alpha) \qquad \text{Eq.6}$$

$$\beta_k \sim Normal(\mu_\beta, {\sigma^2}_\beta) \qquad \text{Eq.7}$$

Equation 1 describes the latent occupancy of species k at site i; $z_{ik}=1$ where the species is present, and $z_{ik}=0$ where absent, given the mean probability of occurrence for that species across all sites (ψ_k). Equation 2 describes the observed detection process; $y_{ijk}|z_{ik}=1$ where species k is detected, and $y_{ijk}|z_{ik}=0$ where it is undetected, at site i for replicate j. This is a function of the occurrence (z_{ik}) of species k at site i as well as the probability of detecting an individual (p_{ijk}) at a site with replicate j.

Equations 3 and 4 describe species-level heterogeneity in occupancy (ψ_k) and detection (p_{ijk}) probabilities, respectively. Equation 4, which describes detection probability, includes an overall intercept (α_k) and survey method (M) as a covariate; $M_{ij}=0$ for eDNA sampling, and $M_{ij}=1$ for electrofishing. A positive posterior mean of the β_k parameter for a species therefore indicates that a species was more likely to be detected with electrofishing. Likewise, a negative posterior mean (β_k) indicates that a species was more likely to be detected with eDNA metabarcoding.

Species-level responses are drawn from a prior distribution, which is itself informed by a hyper-prior. Thus, species-level responses are informed by the overall community-level response. Species-specific prior distributions were specified on the logit scale, where ϕ_k, α_k and β_k were normally distributed with mean μ and standard deviation σ^2 (eq. 5, 6 and 7). Hyper-priors for μ_ϕ, μ_α, μ_β were specified as normally distributed with mean 0 and standard deviation = 0.1. Hyper-priors σ^2_α, σ^2_β were specified as uniformly distributed across the range 0-8.

Model fitting

Models were fit using the package rjags v.4-6 in R version 3.2.3 (R Development Core Team, 2015). Three model chains were run for 30,000 iterations each. The first 10,000 samples were discarded, and the remaining samples were thinned by a factor of 10, resulting in 2,000 samples per chain from the posterior distribution. Convergence was measured using traceplots as implemented in the jagsUI package v.1.4.4; chains were well mixed. Gelman-Rubin statistic values $\hat{R}$ were below 1.1, indicating successful convergence of chains.

Primer selection, low abundance read filtering, and the threshold of positive replicate samples

To investigate how different metabarcoding decisions altered modelling outcomes, we ran multiple scenarios. The scenarios included the (i) telostei marker, (ii) the vertebrate marker, and (iii) a ‘combined’ scenario, in which results using each marker were combined, where a species was considered detected in a sample if either of the markers detected it. The filtering scenarios were 0%, 0.1% and 1% of each sample. The 0% scenario acted as a baseline, whereas 0.1% is a more realistic threshold used in metabarcoding when targeting the 12S mitochondrial gene (Hänfling et al., 2016); the 1% filtering acts as our conservative filtering scenario. We also ran these scenarios with two thresholds for the number of positive replicate water samples required to classify a species as present at a site: either 1 or 2 water samples per site. The impact of primer selection, filtering, and positive replicate thresholds on parameter estimates of the sampling method covariate was then investigated using the hierarchical SODM described above.

To explore the potential impact of false positive detections on our results, we also fitted site occupancy detection models using a multiple detection methods approach (Chambert, Miller, & Nichols, 2015) for four species that appeared in our controls (see Results). We could not use this method for *Percalates novemaculeata*, which also appeared in our controls, as it was not detected using electrofishing. We treated eDNA sampling as an ambiguous survey method and electrofishing as an unambiguous method. Using R code in the Supplement of Chambert et al. (2018), we fitted separate models to each of the four 'contaminant' species as an alternative means to compare the detectability achieved with eDNA sampling vs. electrofishing.

Species accumulation

To further compare eDNA metabarcoding and backpack electrofishing, species accumulation curves were developed using the R package vegan (Oksanen et al., 2019). These curves demonstrate the number of species that could be detected using 1, 2, 3, 4 or 5 eDNA samples relative to the number of species detected with electrofishing. Species accumulation plots were produced for data obtained from 0.1% and 1% low abundance read filtering, and with 1 or 2 positive replicate samples for the combined primers scenario.

Results

The sequence length for both teleostei and universal vertebrate primers was relatively well conserved across taxa, except lampreys (*Mordacia mordax*/*Geotria australis*), which had large deletions for the vertebrate marker (marker length=~80bp vertebrate primers) and could not be detected with the teleostei primer pair. One site had very low read numbers (<100 per sample) compared with all other sites across both markers and was therefore discarded from analyses (final number of sites = 24). For the teleostei and vertebrate primers, respectively, 0.2% and 0.05% of reads could not be assigned to any taxonomic group even after conducting BLASTN searches for similarity. Detections/specimens classified as from the genus *Hypseleotris* were included in the analysis, as this species was only identified to the genus-level in both electrofishing and metabarcoding surveys. Other assignments that did not resolve to the species-level were omitted. In addition to fish species, the vertebrate marker detected 69 non-target species that were excluded from analysis, including mammals, birds, and amphibians. For the vertebrate marker, the average number of reads per sample was 28,164 (fish only), whilst for the teleostei marker, the average number of reads per sample was 50,751. Nineteen fish species were detected with backpack electrofishing, while a further eight non-target species, including crustaceans and mussels, were also detected. Species lists across all sites combined are presented in Table S2 (Supporting Information).

In our analysis, 1/5 blank filters and 1/12 H_2O controls using the vertebrate primers had read values within the range of samples collected from field sites and were identified as fish. For the teleostei primers, 2/5 blank filters and no H_2O controls had high read values (>100) for fish.

One species was detected within a catchment using eDNA metabarcoding where that species had not been detected in the last 20 years. That species, *Percalates novemaculeata*, is known to hybridise with the closely related *Percalates colonorum* in the wild (Shaddick, Burridge, Jerry, & Schwartz, 2011). We therefore assigned this species back to the genus-level (*Percalates* sp.).

All species detected using electrofishing were detected using eDNA metabarcoding with the teleostei and vertebrate primers and 0.1% filtering of each sample. Five species were detected using eDNA metabarcoding (teleostei primers and 0.1% filtering) but not electrofishing: *Anguilla reinhardtii, Maccullochella peelii, Percalates sp., Galaxias truttaceus*, and *Galaxiella pusilla*. Six species were detected using eDNA metabarcoding (vertebrate primers, 0.1% filtering) and not electrofishing: *Anguilla reinhardtii, Macquaria novemaculeata, Galaxias truttaceus, Salmo salar, Tandanus tandanus*, and *Geotria australis*. Five species, *Anguilla reinhardtii, Galaxias truttaceus, Galaxiella pusilla, Tandanus tandanus*, and *Percalates sp.* were known in the catchments in which they were detected from the Melbourne Water 20-year dataset. *Maccullochella peelii* was detected in a catchment using eDNA where it has not previously been recorded, and similarly *Salmo salar* was detected for the first time in a catchment using eDNA.

Species detection

The vertebrate primers detected more fish species (25) than the teleostei primers (24). Combining both primers resulted in the detection of 27 species. The sensitivity of eDNA metabarcoding relative to electrofishing ($\mu\beta$) across the community varied depending on the primer(s) used. With the teleostei primers, filtering set at 0.1%, and a threshold of two positive water samples required for species presence, more species were likely to be detected with eDNA metabarcoding compared to electrofishing. In addition, the community-level effect of sampling method on detection probability—that is, the posterior mean of $\mu\beta$, the community hyperparameter—was negative when using the telostei primers (mean [95% CI] = −1.054 [−1.725, −0.324; Figure 2) indicating that eDNA metabarcoding was more sensitive than electrofishing. Using the vertebrate primers, the mean posterior estimate of $\mu\beta$ was also negative (mean[95% CI] = −0.646 [−1.439, 0.212; Figure S1) but the 95% credible intervals overlapped zero. Combining the two primers increased eDNA metabarcoding detection probabilities for many species and led to a stronger negative estimate of $\mu\beta$ (mean[95% CI] = −1.468 [−2.058, −0.887]) (Figure 3a.).

Low read filtering

Species richness did not change with an increase in filtering threshold for any of the scenarios. In addition, read filtering had negligible influence on the effectiveness of eDNA metabarcoding relative to electrofishing in hierarchical models. The combined primers and a threshold of two positive water samples were used for this comparison. In all three scenarios (0%, 0.1% and 1% read filtering), posterior estimates of $\mu\beta$ were strongly negative (0%: mean[95% CI] = −1.488[−2.065, −0.941] (Figure S2);0.1%: mean[95%CI] = -1.468[-2.058,-0.886]; 1%: mean[95% CI] = -1.370[-1.959,0.779] (Figure S3). Detection probabilities for most species were higher with eDNA metabarcoding than with electrofishing across all filtering scenarios (Table S3, Supporting Information).

Water sample threshold

Requiring a species to be detected in two replicate water samples at a site decreased species richness estimates but did not alter the strength or direction of community posterior estimates of $\mu\beta$. For example, when using the combined primers and 0.1% filtering, $\mu\beta$ was still strongly negative and certain (one positive sample required: mean[95% CI] = -1.332[-1.802,-0.887], Figure 3b; two positive samples required: mean[95% CI] = -1.468[-2.057,-0.887], Figure 3a).

Some species that were previously detected in a catchment in the Melbourne Water historical dataset were removed when the two-water-sample threshold was applied. Using the telostei primers, *Anguilla reinhardtii* and *Galaxiella pusilla* were removed. *Anguilla reinhardtii* was, however, detected using the vertebrate primers, and therefore remained in the combined primers dataset. Using the vertebrate primers, *Macquaria sp.* was removed from the one site at which it was detected. Species in this genus, such as *Macquaria novemaculeata,* had been detected previously. Two species were removed that had not been historically detected in the sampled catchments. *Salmo salar* and *Maccullochella peelii* were detected at one site in one sample and were removed when the two-water-sample threshold was applied.

Modelling false positive detections

For three of the four species (*Anguilla australis, Galaxias brevipinnis* and *Salmo trutta*), the mean probability of detection using eDNA metabarcoding (p11) was higher than the mean probability of detection with electrofishing (r11). Mean false positive detection probabilities (p10) of eDNA metabarcoding were generally low for all four species (p10<0.06), with the exception of *Anguilla australis* (p10=0.2) (Table S4, Supporting Information).

Species accumulation

Across all sites, the mean number of species detected with one water sample was greater than the number of species detected with backpack electrofishing (Figure 4). This pattern was consistent across all filtering scenarios.

Discussion

Our results illustrate that eDNA metabarcoding can provide an effective alternative to backpack electrofishing for sampling freshwater fish communities. The sensitivity of eDNA metabarcoding varied among species and scenarios, but the community-level effect of sampling method was always in favour of eDNA. Species richness estimates were also higher using eDNA metabarcoding compared to electrofishing. This is consistent with the literature where methods were conducted concurrently (Liu et al., 2019; Mcdevitt et al., 2019; Sard et al., 2019; Shaw et al., 2016). However, using some markers and during some studies, species richness estimates have been lower compared to a traditional sampling method (Fujii et al., 2019). One plausible explanation for why eDNA metabarcoding was more sensitive than electrofishing is that the latter was only able to detect species within the 100 m reach traversed by electrofishers, while the former could potentially detect species outside of the netted area, particularly upstream. For example, *Salmo salar* was detected at a site using eDNA where it had not been detected before in that catchment. We could have detected the DNA of this species without it occupying the reach being surveyed, through discharge from stocked dams or food waste discharge. A second, non-mutually exclusive explanation is that using electrofishing results in higher false negative probabilities – that the technique simply fails to detect a greater proportion of species that are present compared to eDNA metabarcoding. Importantly, our results were largely insensitive to sources of metabarcoding uncertainty – across all scenarios considered here, eDNA metabarcoding was more sensitive than electrofishing.

Marker (primer) selection

We found that primer selection impacted the sensitivity of eDNA metabarcoding, a finding supported by previous studies (Alberdi et al., 2018; Hänfling et al., 2016; Olds et al., 2016; Shaw et al., 2016; Valentini et al., 2016). Individually, both the universal vertebrate primers and the teleostei primers had similar (or greater) efficiency compared to backpack electrofishing for most species. The two primers performed similarly to one another, although the vertebrate primers detected more fish species overall (with the additional benefit of detecting an additional 69 non-target species). While in the study area the species-level resolution of the reference sequences was similar for both vertebrate and teleostei markers, this may not always be the case in other areas and may influence the ultimate performance of these markers. Compared to each marker in isolation, combining data from both markers resulted in more species being detected, and increased detection probabilities for individual species. Thus, combining markers encompassed the generality of the vertebrate primers and the specificity of the teleostei primers, highlighting how combinations of markers in eDNA metabarcoding can increase species detection probabilities.

Whilst these empirical results relate to a specific study system, if *in silico* testing of primers in a study area is successful, our results suggest that combining different primers should always be considered. Clearly, primer choice will depend on the target taxa and reference library. The use of multiple primers will also increase costs, creating a trade-off between cost-efficiency and the number of species detected, an aspect that should be explored in future studies. It is also important to note that this study was conducted in a well-characterised system in which reference sequences for both markers were available for all species from the study region. The performance of the vertebrate and teleostei primer pairs may be reduced in a system without an extensive local reference library such as that used in this study, and there is the potential for increased misidentification if the broader, universal databases such as GenBank (Clark, Karsch-mizrachi, Lipman, Ostell, & Sayers, 2016) are used in isolation. Custom local databases try to reduce this issue by eliminating taxa that aren't found within the study area, but if closely related species co-occur within the same area then there is the potential for ambiguity.

A final consideration regarding primer selection is the targeted gene region. Both markers used in this study are located within the mitochondrial 12S ribosomal RNA (rRNA) gene, which is relatively well conserved across species (Valentini et al., 2016). This provides the opportunity for a single primer pair to target all fish species but could result in an inability to distinguish between certain fish species. Use of a different mitochondrial DNA region (e.g. cytochrome oxidase subunit I or cytochrome *b*) may give greater resolution at the species-level due to the region being less conserved (Deagle, Jarman, Coissac, Pompanon, & Taberlet, 2014) but this may come at the cost of the ability to target the entire group of interest. Combining specific and general markers will address this issue but, as noted above, will likely also increase costs.

Dealing with false positives

Given sufficient taxonomic expertise, false positive detections are likely rare with electrofishing. However, false positive detections are always a concern with eDNA sampling, particularly eDNA metabarcoding. As with any survey technique, the potential for, and sources of, false positive detections should be investigated. Contamination can occur at various stages of the metabarcoding process, including field sampling, DNA extractions, PCR, library preparation, and sequencing (Lim et al., 2016). Negative controls are routinely used in eDNA studies to help quantify these errors (Deiner et al., 2017; Hänfling et al., 2016; Port et al., 2016), particularly in the laboratory. In our study, there was a low level of contamination in the negative controls, with 2/17 negatives in both the vertebrate and teleostei having significant (>100) read numbers that were identified as fish and not human, mouse, or pig. A possible explanation for the elevated number of reads in these samples could be due to a negligible amount of eDNA being significantly amplified because there was no other template DNA in the control. This is possible due to the two-step PCR process commonly used in eDNA studies (Carew, Metzeling, St Clair, & Hoffmann, 2017; Evans et al., 2017; Kelly et al., 2017; Shaw, Weyrich, & Cooper, 2017; Zizka, Elbrecht, Macher, & Leese, 2019), in which any slight contamination in the first steps (DNA extraction or the first round of PCR) can get amplified in the second (Quinn, Erb, Richardson, & Crowley, 2018). If that negligible amount of eDNA was present in a sample (instead of a control), it would not produce such a high number of reads. Thus, controls likely overestimate the amount of eDNA contamination. Another possible explanation is index switching, which can lead to cross contamination within a pool (Costello et al., 2018).

Several modifications could potentially help rectify, or at least shed light on the above issues. For example, a one-step PCR approach can be used to reduce the chance of contamination events, although this increases costs substantially (Valentini et al., 2016) and could introduce potential biases (Zizka et al., 2019). A positive control could also be used, in which known quantities of species DNA from the study area is amplified and processed. This could identify any false positive and false negative detections and help inform filtering thresholds (Hänfling et al., 2016). A contamination control can also be prepared using the DNA of a species that doesn't occur in the study area. A bioinformatic solution could be to filter field samples at the proportion of reads that clears the controls (Olds et al., 2016); however, this solution is not applicable here, nor is it an ideal solution more generally. Filtering out controls in which there is a high number of reads that are assigned to a species (as opposed to 'unassigned') would result in a high number of false negatives, as many species would be discarded (Deiner et al., 2017). There are other data curation steps that can be important for ecological inferences, such as removing cross-sample contamination and PCR errors, as well as the method used to cluster molecular operational taxonomic units (Calderón-Sanou et al., 2020).

Here we considered three possible ad-hoc solutions for reducing the likelihood of contamination and to understand its potential influence: filtering out low abundance reads, requiring a species to be detected in at least two replicate water samples at a site, and modelling false positive detections using multiple detection method site occupancy detection occupancy models. We found that the threshold used to discard low abundance reads can influence the effectiveness of eDNA metabarcoding as a sampling method, but only to a small degree. Discarding species that comprise a small proportion of overall reads during the filtering process is common practice in the eDNA metabarcoding literature (Deiner et al., 2017; Hänfling et al., 2016; Murray, Coghlan, & Bunce, 2015; Port et al., 2016; Stoeckle, Soboleva, & Charlop-Powers, 2017; Valentini et al., 2016). This process can potentially reduce false positive detections, but risks introducing false negative errors (Hänfling et al., 2016; Lahoz-Monfort et al., 2016). Filtering thresholds used in some previous studies vary, with 0.02% (Port et al., 2016), 0.1% (Hänfling et al., 2016; Stoeckle et al., 2017; Valentini et al., 2016), 0.2% (Hänfling et al., 2016), 0.3% (Valentini et al., 2016) and 1% (Schnell et al., 2018) all being used. The thresholds used also vary depending on the target region or primers (Hänfling et al., 2016; Valentini et al., 2016). We found that when low abundance reads were not discarded, there was no change in the total number of species detected relative to the 0.1% and 1% filtering scenarios, but the mean estimate of the community-level effect of sampling method μ_β became more negative, indicating greater certainty in our finding that species were more likely to be detected using eDNA metabarcoding than electrofishing. This could potentially be due to some low abundance reads being false positive detections. The 1% filtering scenario explored here was quite conservative relative to the literature but could help alleviate false positive detections by removing 'contaminant' species that often have low read abundances. However, this strategy introduces the potential for rare species to be discarded, as they too typically represent a low proportion of reads. Nonetheless, even when 1% filtering was used, there was no change in overall species richness or composition, and the estimate of the community-level effect of sampling method μ_β was similar to the 0% and 0.1% filtering scenarios.

Requiring a species to be detected in at least two replicate water samples was another solution considered to reduce the occurrence of false positive detections. When we used a threshold of two positive samples per site, two species were removed that were not known in the catchment area in which they were detected, potentially representing false positive detections. However, some species were removed which have been detected in the catchment historically, for example, *Galaxiella pusilla*. This species was only detected at one site, in one sample. This solution again introduces the possibility of removing rare species that could occur at a similar rate to false positive detections. The number of water samples used to declare species presence depends on the research question or management objective. If rare species are the target, the occurrence of false positive detections may be less of a concern than failing to detect a rare species. Conversely, if invasive species are the target of the study, a false positive detection may trigger a costly management response.

The final approach we considered to explore the impacts of false positive detections – using multiple detection method models – suggested that false positive detections are unlikely to have qualitatively influenced our results. Indeed, the relative sensitivity of eDNA metabarcoding vs electrofishing was similar for all four species using models that did and did not account for false positive detections. This suggests that our main finding – that eDNA metabarcoding was more sensitive than backpack electrofishing – was largely unaffected by this methodological decision. We used separate multiple detection method models to investigate this issue (Chambert et al., 2015) but future studies could usefully develop multi-species models that account for false positive detections at multiple levels, akin to the single-species approach of Guillera-Arroita et al. (2017).

Multi-species models for eDNA metabarcoding

The modelling approach used in this study, SODM, has only recently been adopted in eDNA metabarcoding studies (Doi, Fukaya, & Miya, 2019; Valentini et al., 2016). Previous studies have used species richness or assemblage estimates to compare eDNA metabarcoding to other sampling methods (Hänfling et al., 2016; Lim et al., 2016; Olds et al., 2016; Shaw et al., 2016) but this information can be inadequate for management decisions. For example, when certain species or functional groups are of greater conservation concern than others, it is important to consider which sampling technique(s) result in a higher detection probability for those species (Hunter et al., 2015). Using a hierarchical SODM allowed us to consider effects of sampling method on detection probabilities of each species, as well as on the community overall. Future studies should use similar hierarchical SODMs to compare sampling methods, to ensure species-specific and community-level inferences.

Our analyses revealed variation between species with respect to the sensitivity of the two sampling methods (β_k). Some species, such as those from the genus *Galaxias*, are very closely related phylogenetically, and will present challenges for eDNA metabarcoding. These challenges, such as local polymorphisms and unresolved taxonomy across a range, have been experienced in other studies (Wilcox, Carim, Mckelvey, & Young, 2015).Variation between species in detection probabilities could also be due to species ecology. Water samples were taken in different locations along the 100 m stretch to maximise habitat heterogeneity; however, certain species may not have been present in the sampled microhabitats (Strickler, Fremier, & Goldberg, 2015), and thus their eDNA concentrations may have been lower than those of other species whose microhabitats were adequately sampled. The biology or life stage of a species could also impact detectability. For example, certain species or life stages may produce more eDNA than others (Maruyama, Nakamura, Yamanaka, Kondoh, & Minamoto, 2014). Species may be more detectable at different times of year, such as reproductive periods or periods of higher feeding activity, and location could interact with other life history traits as some species may be migratory (Souza, Godwin, Renshaw, & Larson, 2016). Finally, primer bias, where some species may be more readily detected than others due to the generality of the primers used, may also impact the detectability of some species (Elbrecht, Vamos, Meissner, Aroviita, & Leese, 2017). The modelling approach used here could be used to explore correlates of variation in detection probability by including ecological and phylogenetic traits of sampled species.

Strengths and limitations of eDNA metabarcoding vs electrofishing

Environmental DNA metabarcoding has several advantages over many traditional sampling methods, providing significant potential for widespread implementation. For example, eDNA metabarcoding can provide additional data that cannot be collected with electrofishing, as is evident from the results of using the vertebrate primers, in which 25 fish species and 69 non-target species were detected. Electrofishing detected 19 target species and eight non-target species. Logistical constraints were also experienced when using electrofishing. For example, some sites could not be surveyed with electrofishing due to high salinity levels, but eDNA samples could be taken at these sites. Very complex waterways with abundant large debris are also more difficult to accurately electrofish (Shepard, Nelson, Taper, & Zale, 2014) as well as sites that are deep, fast flowing, or turbid. The invasive nature of electrofishing can also be an animal welfare concern, especially where rare or threatened species are involved. Skilled operators are required to conduct electrofishing safely, and there are limited operators to complete surveys. In contrast, the expertise required to collect and process eDNA samples is more widespread (Ji et al., 2013), contributing to the potential ease with which this type of sampling could be incorporated into monitoring or surveillance programs, including potential use in citizen science programs. Successful citizen science programs have already been developed for platypuses (Melbourne Water, 2019), invasive mussels (Miralles, Dopico, Devlo-Delva, & Garcia-Vazquez, 2016) and a newt species (Biggs et al., 2015). Environmental DNA metabarcoding also has advantages from a human health and safety point-of-view; unlike electrofishing, researchers do not have to enter the water to collect water samples for eDNA analysis.

Whilst not necessarily a strength or a weakness, the transport of eDNA in lotic systems is something that separates the two methods considered here. Electrofishing is site-specific and gives estimates of fish occupancy only within the traversed reach. In contrast, eDNA sampling can lead to detections from fish upstream of the sampling site. Estimates of eDNA transport vary, seeming to depend on river size (Pont et al., 2018). Environmental DNA can be transported in the water column, retained by the substrate, resuspended and degraded or removed from the system (Shogren et al., 2017). The extent to which this is a concern depends on the specific aims of the study. If data on relationships between fish occurrence or diversity and specific habitat or reach conditions is required, eDNA could potentially confound conclusions. However, if undertaking a broader catchment-scale assessment, then eDNA transport may be considered an advantage.

Whilst eDNA metabarcoding has many advantages, it also has limitations, some of which will require further technological advances to overcome. For instance, eDNA metabarcoding can produce data on genetic diversity (Sigsgaard et al., 2016), but it is not currently possible to obtain demographic data, such as sex ratio, size, health, and age, from eDNA metabarcoding (Olds et al., 2016; Shaw et al., 2016; Valentini et al., 2016). In contrast, demographic and individual-level data can be gained from traditional methods that enable organisms to be captured, such as electrofishing.

Uncertainty surrounding the ability of eDNA metabarcoding to monitor trends in abundance and recruitment also limits the breadth of potential applications (Deiner et al., 2017). Some studies have identified correlations between organismal abundance and DNA concentration estimated via qPCR, particularly in a controlled laboratory environment (Yates, Fraser, & Derry, 2019). Metabarcoding, however, provides different challenges such as primer bias, which can affect read numbers, and therefore may place a limit on the ability to extract abundance data from eDNA metabarcoding (Deiner et al., 2017).

A final consideration is the cost-efficiency of eDNA metabarcoding – a topic that has not been properly addressed in the literature to date. Previous eDNA metabarcoding studies have considered cost-efficiency in terms of person-hours (Ji et al., 2013) but financial costs, including field equipment, marker development and validation, sequencing, reference libraries, and bioinformatic analyses, should also be considered. Optimisation methods, such as those used by Smart et al., (2015), could provide a way forward in this regard. A related topic is the cost-efficiency of different eDNA primers. In our study, we showed that the combination of two different primers markedly improved the sensitivity of eDNA metabarcoding, but we did not consider the cost-efficiency of this approach. Trade-offs between cost and improved detection probability from the use of combined primers should be further investigated to fully understand the need to use multiple primers in eDNA metabarcoding.

Conclusion

For new techniques and technologies such as eDNA sampling and metabarcoding to be widely adopted, they need to be tested against, and found to be at least as effective as standard techniques (Lahoz-Monfort

& Tingley, 2018). While our results demonstrate that methodological decisions made in the eDNA metabarcoding process can affect sensitivity, we show that, overall, eDNA metabarcoding can be more effective than backpack electrofishing for detecting freshwater fish communities.

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Data Accessibility

Field data, laboratory results and R code are available in the online Supporting Information. Sequencing data and sample metadata for demultiplexing FASTQ files are available at the Dryad Digital Repository: https://doi.org/10.5061/dryad.wm37pvmkj

Author Contributions

A.W, R.C and R.T conceived the study. Fieldwork was planned by A.W, R.T, R.C, T.R and E.M. R.C and T.R. conducted the fieldwork. K.R, S.S and A.W performed laboratory procedures, genetic and bioinformatic analysis. E.M and R.T designed the statistical models and modelling analysis. E.M wrote the manuscript with inputs from all other authors.

References

Alberdi, A., Aizpurua, O., Gilbert, M. T. P., & Bohmann, K. (2018). Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution*, *9*(1), 134–147. doi: 10.1111/2041-210X.12849

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R. A., … Dunn, F. (2015). Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (Triturus cristatus). *Biological Conservation*, *183*, 19–28. doi: 10.1016/j.biocon.2014.11.029

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). obitools: A unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*(1), 176–182. doi: 10.1111/1755-0998.12428

Bureau of Meteorology. (2017). *Climate Statistics*.

Calderón-Sanou, I., Münkemüller, T., Boyer, F., Zinger, L., & Thuiller, W. (2020). From environmental DNA sequences to ecological conclusions : How strong is the influence of methodological choices ? *Journal of Biogeography*, *47*, 193–206. doi: 10.1111/jbi.13681

Carew, M. E., Metzeling, L., St Clair, R., & Hoffmann, A. A. (2017). Detecting invertebrate species in archived collections using next-generation sequencing. *Molecular Ecology Resources*, *17*(5), 915–930. doi: 10.1111/1755-0998.12644

Chambert, T., Miller, D. A. W., & Nichols, J. D. (2015). Modeling false positive detections in species occurrence data under different study designs. *Ecology*, *96*(2), 332–339. doi: https://doi.org/10.1890/14-1507.1

Clark, K., Karsch-mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2016). GenBank. *Nucleic Acids Research*, *44*, 67–72. doi: 10.1093/nar/gkv1276

Costello, M., Fleharty, M., Abreu, J., Farjoun, Y., Ferriera, S., Holmes, L., … Gabriel, S. (2018). Characterization and remediation of sample index swaps by non-redundant dual indexing on massively parallel sequencing platforms. *BMC Genomics*, *19*, 1–10.

Crookes, S., Heer, T., Castañeda, R. A., Mandrak, N. E., Heath, D. D., Weyl, O. L. ., … Foxcroft, L. C. (2020). NeoBiota Monitoring the silver carp invasion in Africa : a case study using environmental DNA (eDNA) in dangerous watersheds. *NeoBiota*, *47*, 31–47. doi: 10.3897/neobiota.56.47475

Deagle, B. E., Jarman, S. N., Coissac, E., Pompanon, F., & Taberlet, P. (2014). DNA metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. *Biology Letters*, *10*(9), 20140562. doi: 10.1098/rsbl.2014.0562

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., … Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, *26*, 5872–5895. doi: 10.1111/mec.14350

Deiner, K., Fronhofer, E. A., Mächler, E., & Altermatt, F. (2015). Environmental DNA reveals that rivers are conveyer belts of biodiversity information. *Nature Communications*, *41*(0), 12544. doi: 10.1101/020800

Doi, H., Fukaya, K., & Miya, M. (2019). Evaluation of detection probabilities at the water-filtering and initial PCR steps in environmental DNA metabarcoding using a multispecies site occupancy model. *Scientific Reports*, *9*. doi: 10.1038/s41598-019-40233-1

Dougherty, M. M., Larson, E. R., Renshaw, M. A., Gantz, C. A., Egan, S. P., Erickson, D. M., & Lodge, D. M. (2016). Environmental DNA (eDNA) detects the invasive rusty crayfish Orconectes rusticus at low abundances. *Journal of Applied Ecology*, *53*(3), 722–732. doi: 10.1111/1365-2664.12621

Drummond, A. J., Newcomb, R. D., Buckley, T. R., Xie, D., Dopheide, A., Potter, B. C., … Nelson, N. (2015). Evaluating a multigene environmental DNA approach for biodiversity assessment. *GigaScience*, *4*(1), 46. doi: 10.1186/s13742-015-0086-1

Edgar, R. C. (2016). SINTAX : a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. *BioRxiv*. doi: 10.1101/074161

Elbrecht, V., Vamos, E. E., Meissner, K., Aroviita, J., & Leese, F. (2017). Assessing strengths and weaknesses of DNA metabarcoding-based macroinvertebrate identification for routine stream monitoring. *Methods in Ecology and Evolution*, *8*, 1265–1275. doi: 10.1111/2041-210X.12789

Evans, N. T., Li, Y., Renshaw, M. A., Olds, B. P., Deiner, K., Turner, C. R., … Pfrender, M. E. (2017). Fish community assessment with eDNA metabarcoding: effects of sampling design and bioinformatic filtering. *Canadian Journal of Fisheries and Aquatic Sciences*, *74*, 1362–1374. doi: 10.1139/cjfas-2016-0306

Fadrosh, D. W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R. M., & Ravel, J. (2014). An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome*, *2*(1), 6. doi: 10.1186/2049-2618-2-6

Ficetola, G. ., Taberlet, P., & Coissac, E. (2016). How to limit false positives in environmental DNA and metabarcoding? *Molecular Ecology Resources*, *16*(3), 604–607. doi: 10.1111/1755-0998.12508

Ficetola, G., Coissac, E., Zundel, S., Riaz, T., Taberlet, P., & Pompanon, F. (2010). An In silico approach for the evaluation of DNA barcodes. *BMC Genomics*, *11*. doi: https://doi.org/10.1186/1471-2164-11-434

Fujii, K., Doi, H., Matsuoka, S., Nagano, M., Sato, H., & Yamanaka, H. (2019). Environmental DNA metabarcoding for fish community analysis in backwater lakes : A comparison of capture methods. *Plos One*, *14*(1), e0210357. doi: https://doi.org/10.1371/journal.pone.0210357

Goldberg, C. S., Pilliod, D. S., Arkle, R. S., & Waits, L. P. (2011). Molecular detection of vertebrates in stream water: A demonstration using rocky mountain tailed frogs and Idaho giant salamanders. *PLoS ONE*, *6*(7). doi: 10.1371/journal.pone.0022746

Guillera-Arroita, G., Lahoz-Monfort, J. J., van Rooyen, A. R., Weeks, A. R., & Tingley, R. (2017). Dealing with false-positive and false-negative errors about species occurrence at multiple levels. *Methods in Ecology and Evolution*, *8*(9), 1081–1091. doi: 10.1111/2041-210X.12743

Hänfling, B., Lawson Handley, L., Read, D. S., Hahn, C., Li, J., Nichols, P., … Winfield, I. J. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology*, *25*, 3101–3119. doi: 10.1111/mec.13660

Harper, L. R., Handley, L. L., Hahn, C., Boonham, N., Rees, H. C., Gough, K. C., … Haenfling, B. (2018). Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (Triturus cristatus). *Ecology and Evolution*, *8*(12), 6330–6341. doi: https://doi.org/10.1002/ece3.4013

Hunter, M. E., Oyler-McCance, S. J., Dorazio, R. M., Fike, J. A., Smith, B. J., Hunter, C. T., … Hart, K. M. (2015). Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive Burmese pythons. *PLoS ONE*, *10*(4), e0121655. doi: 10.1371/journal.pone.0121655

Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). “Sight-unseen” detection of rare aquatic species using environmental DNA. *Conservation Letters*, *4*(2), 150–157. doi: 10.1111/j.1755-263X.2010.00158.x

Ji, Y., Ashton, L., Pedley, S. M., Edwards, D. P., Tang, Y., Nakamura, A., … Yu, D. W. (2013). Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecology Letters*, *16*(10), 1245–1257. doi: 10.1111/ele.12162

Kelly, R. P., Closek, C. J., O’Donnell, J. L., Kralj, J. E., Shelton, A. O., & Samhouri, J. F. (2017). Genetic and Manual Survey Methods Yield Different and Complementary Views of an Ecosystem. *Frontiers in Marine Science*, *3*(283), 1:11. doi: 10.3389/fmars.2016.00283

Kéry, M., & Royle, A. J. (2016). *Applied Hierachical Modeling in Ecology: Analysis of distribution, abundance and species richness in R and BUGS*. London, UK: Elsevier.

Lahoz-Monfort, J. J., Guillera-Arroita, G., & Tingley, R. (2016). Statistical approaches to account for false-positive errors in environmental DNA samples. *Molecular Ecology Resources*, *16*(3), 673–685. doi: 10.1111/1755-0998.12486

Lahoz-Monfort, J. J., & Tingley, R. (2018). The technology revolution: improving species detection and monitoring using new tools and statistical methods. In S. Legge, D. B. Lindenmayer, N. Robinson, B. C. Scheele, D. Southwell, & B. A. Wintle (Eds.), *Monitoring threatened species and ecological communities*. CSIRO PUBLISHING.

Lieschke, J., Raadik, T., & Nicol, M. (2014). *Brief assessment of the status of Orbost Spiny Crayfish post Orbost Fire complex, May 2014*. doi: 10.13140/RG.2.1.4825.3609

Lim, N. K. M., Tay, Y. C., Srivathsan, A., Tan, J. W. T., Kwik, J. T. B., Baloğlu, B., … Yeo, D. C. J. (2016). Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. *Royal Society Open Science*, *3*(11), 160635. doi: 10.1098/rsos.160635

Liu, Y., Wikfors, G. H., Rose, J. M., Mcbride, R. S., Milke, L. M., & Mercaldo-allen, R. (2019). Application of Environmental DNA Metabarcoding to Spatiotemporal Finfish Community Assessment in a Temperate Embayment. *Frontiers in Marine Science*, *6*(674). doi: 10.3389/fmars.2019.00674

Lugg, W. H., Griffiths, J., van Rooyen, A. R., Weeks, A. R., & Tingley, R. (2017). Optimal survey designs for environmental DNA sampling. *Methods in Ecology and Evolution*, *9*, 1049–1059. doi: 10.1111/2041-210X.12951

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10. doi: 10.14806/ej.17.1.200

Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M., & Minamoto, T. (2014). The release rate of environmental DNA from juvenile and adult fish. *PLoS ONE*, *9*(12), 118727. doi: 10.1371/journal.pone.0114639

Mcdevitt, A. D., Benvenuto, C., Guimarães, N., Samuel, S., Sparnenn, A. O., Mariani, S., … Coscia, I. (2019). Environmental DNA metabarcoding as an effective and rapid tool for fish monitoring in canals. *Journal of Fish Biology*, *1*(4). doi: 10.1111/jfb.14053

Melbourne, W. (2018). *Healthy Waterways Strategy 2018*. Melbourne Water Corporation, Victoria, Australia.

Melbourne Water. (2019). Platypus census. Retrieved from https://www.melbournewater.com.au/community-and-education/waterwatch-programs/platypus-census

Miralles, L., Dopico, E., Devlo-Delva, F., & Garcia-Vazquez, E. (2016). Controlling populations of invasive pygmy mussel (Xenostrobus securis) through citizen science and environmental DNA. *Marine Pollution Bulletin*, *10*(1), 127–132. doi: 10.1016/j.marpolbul.2016.06.072

Murray, D. C., Coghlan, M. L., & Bunce, M. (2015). From Benchtop to Desktop : Important Considerations when Designing Amplicon Sequencing Workflows. *PLoS ONE*, *10*(4), e0124671.

doi: 10.5061/dryad.2qf0t

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Pierre, L., McGlinn, D., … Wagner, H. (2019). *vegan: Community Ecology Package*. Retrieved from https://cran.r-project.org/package=vegan

Olds, B. P., Jerde, C. L., Renshaw, M. A., Li, Y., Evans, N. T., Turner, C. R., … Lamberti, G. A. (2016). Estimating species richness using environmental DNA. *Ecology and Evolution*, *6*, 4214–4226. doi: 10.1002/ece3.2186

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., … Dejean, T. (2018). Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Scientific Reports*, *8*(1), 10361. doi: 10.1038/s41598-018-28424-8

Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J., … Kelly, R. P. (2016). Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Molecular Ecology*, *25*(2), 527–541. doi: 10.1111/mec.13481

Quinn, T. P., Erb, I., Richardson, M. F., & Crowley, T. M. (2018). Understanding sequencing data as compositions: an outlook and review. *Bioinformatics*, *34*(16), 2870–2878. doi: 10.1093/bioinformatics/bty175

R Development Core Team. (2015). *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing.

Ransome, E., Geller, J. B., Timmers, M., Leray, M., Mahardini, A., Sembiring, A., … Meyer, C. P. (2017). The importance of standardization for biodiversity comparisons : A case study using autonomous reef monitoring structures (ARMS) and metabarcoding to measure cryptic diversity on Mo 'orea coral reefs, French Polynesia. *PLoS ONE*, *12*(4), e0175066. doi: https://dhttps://doi.org/10.1371/journal.pone.0175066

Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., & Coissac, E. (2011). EcoPrimers: Inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Research*, *39*(21), 1–11. doi: 10.1093/nar/gkr732

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH : a versatile open source tool for metagenomics. *PeerJ*, *4*. doi: 10.7717/peerj.2584

Sard, N. M., Herbst, S. J., Nathan, L., Uhrig, G., Kanefsky, J., Robinson, J. D., & Scribner, K. T. (2019). Comparison of fish detections, community diversity, and relative abundance using environmental DNA metabarcoding and traditional gears. *Environmental DNA*, *1*, 368–384. doi: https://doi.org/10.1002/edn3.38

Schmidt, B. R., Kéry, M., Ursenbacher, S., Hyman, O. J., & Collins, J. P. (2013). Site occupancy models in the analysis of environmental DNA presence/absence surveys: A case study of an emerging amphibian pathogen. *Methods in Ecology and Evolution*, *4*, 646–653. doi: 10.1111/2041-210X.12052

Schnell, I. B., Bohmann, K., Schultze, S. E., Bass, D., Sinding, C. M. M. S., Cadle, J. E., … Gilbert, M. T. P. (2018). Debugging diversity – a pan-continental exploration of the potential of terrestrial blood-feeding leeches as a vertebrate monitoring tool. *Molecular Ecology Resources*, *18*, 1282–1298. doi: 10.1111/1755-0998.12912

Shaddick, K., Burridge, C. P., Jerry, D. R., & Schwartz, T. S. (2011). A hybrid zone and bidirectional introgression between two catadromous species : Australian bass Macquaria novemaculeata and estuary perch Macquaria colonorum. *Journal of Fish Biology*, *79*, 1214–1235. doi: 10.1111/j.1095-8649.2011.03105.x

Shaw, J. L. A., Clarke, L. J., Wedderburn, S. D., Barnes, T. C., Weyrich, L. S., & Cooper, A. (2016). Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. *Biological Conservation*, *197*, 131–138. doi: 10.1016/j.biocon.2016.03.010

Shaw, J. L. A., Weyrich, L., & Cooper, A. (2017). Using environmental (e)DNA sequencing for aquatic biodiversity surveys: a beginner's guide. *Marine and Freshwater Research*, *68*, 20–33. doi: http://dx.doi.org/10.1071/MF15361

Shehzad, W., Mccarthy, T. M., Pompanon, F., Purevjav, L., Riaz, T., & Taberlet, P. (2012). Prey Preference of Snow Leopard (Panthera uncia) in. *PLoS ONE*, *7*(2), e32104. doi: 10.5061/dryad.bj376f61

Shepard, B. B., Nelson, L. M., Taper, M. L., & Zale, A. V. (2014). Factors Influencing Successful Eradication of Nonnative Brook Trout from Four Small Rocky Mountain Streams Using Electrofishing. *North American Journal of Fisheries Management*, *34*(5), 988–997. doi: 10.1080/02755947.2014.942042

Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L., & Bolster, D. (2017). Controls on eDNA movement in streams : Transport, Retention, and Resuspension. *Scientific Reports*, *7*, 5065. doi: 10.1038/s41598-017-05223-1

Sigsgaard, E. E., Nielsen, I. B., Bach, S. S., Lorenzen, E. D., Robinson, D. P., Knudsen, S. W., … Thomsen, P. F. (2016). Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. *Nature Ecology & Evolution*, *1*, 0004. doi: 10.1038/s41559-016-0004

Smart, A. S., Tingley, R., Weeks, A. R., Van Rooyen, A. R., & McCarthy, M. A. (2015). Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecological Applications*, *25*(7), 1944–1952. doi: 10.1890/14-1751.1

Souza, L. S. De, Godwin, J. C., Renshaw, M. A., & Larson, E. (2016). Environmental DNA (eDNA) Detection Probability Is Influenced by Seasonal Activity of Organisms. *PLoS ONE*, 1–15. doi: 10.1371/journal.pone.0165273

Stoeckle, M. Y., Soboleva, L., & Charlop-Powers, Z. (2017). Aquatic environmental DNA detects seasonal fish abundance and habitat preference in an urban estuary. *PLoS ONE*, *12*(4), 1–15. doi: 10.1371/journal.pone.0175186

Strickler, K. M., Fremier, A. K., & Goldberg, C. S. (2015). Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, *183*, 85–92. doi: 10.1016/j.biocon.2014.11.038

Taberlet, P., & Coissac, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*, 2045–2050. doi: https://doi.org/10.1111/j.1365-294X.2012.05470.x

Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., … Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology*, *21*(11), 2565–2573. doi: 10.1111/j.1365-294X.2011.05418.x

Turner, C. R., Barnes, M. A., Xu, C. C. Y., Jones, S. E., Jerde, C. L., & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution*, *5*(7), 676–684. doi: 10.1111/2041-210X.12206

Valentini, A., Pompanon, F., & Taberlet, P. (2009). DNA barcoding for ecologists. *Trends in Ecology & Evolution*, (24), 110–117. doi: 10.1016/j.tree.2008.09.011

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., … Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*(4), 929–942. doi: 10.1111/mec.13428

Wilcox, T. M., Carim, K. J., Mckelvey, K. S., & Young, M. K. (2015). The Dual Challenges of Generality and Specificity When Developing Environmental DNA Markers for Species and Subspecies of Oncorhynchus. *Plos One*, *10*(11), e0142008. doi: 10.1371/journal.pone.0142008

Winter, D. J. (2017). rentrez : An R package for the NCBI eUtils API. *The R Journal*, *9*(2), 520–526.

Yates, M. C., Fraser, D. J., & Derry, A. M. (2019). Meta - analysis supports further refinement of eDNA for monitoring aquatic species - specific abundance in nature. *Environmental DNA*, (1), 5–13. doi: 10.1002/edn3.7

Zizka, V. M. A., Elbrecht, V., Macher, J. N., & Leese, F. (2019). Assessing the influence of sample tagging and library preparation on DNA metabarcoding. *Molecular Ecology Resources*, *19*, 893–899. doi: 10.1111/1755-0998.13018

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Table 1. Characteristics of sites sampled in the current study.

Site code	Average channel wetted width (m)	Average water depth (m)	Maximum water depth (m)	Catchment area (km^2)
DW1	7	0.7	1.5	91
DW2	5	1	2	44
DW3	1.5	0.3	1	14
DW4	4	0.5	1.2	15
DW5	6	0.7	1.2	145
DW6	4	0.6	1.3	84
DW7	5	0.4	1.8	65
DW9	6.5	0.7	1.5	262
DW10	6	0.6	1.8	232
WM1	1	0.2	1	7
WM3	5	0.3	0.7	343
WM4	5	0.3	0.5	480
WM5	3.5	0.3	1.2	84
WM9	5	0.8	1.1	100
WM10	15	0.3	1.8	230
Y1	4.5	0.6	0.8	35
Y2	4	0.8	1.2	71
Y3	3	0.25	0.9	20
Y4	6	0.5	1.5	89
Y5	4	0.4	1	35
Y6	3	0.7	1.2	21
Y8	2	0.4	1	44
Y10	3	0.5	2	50
Y12	2	0.7	1.2	23
Y13	2.5	0.6	1.3	74

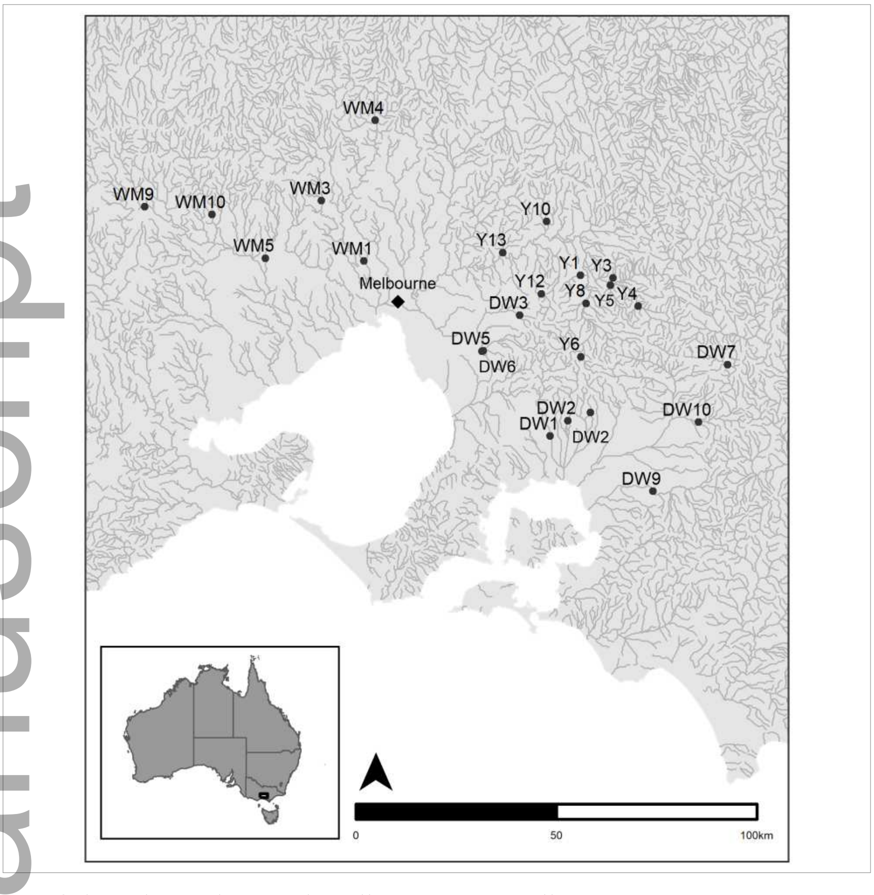


Figure 1: Map of sites situated around Melbourne, Australia.

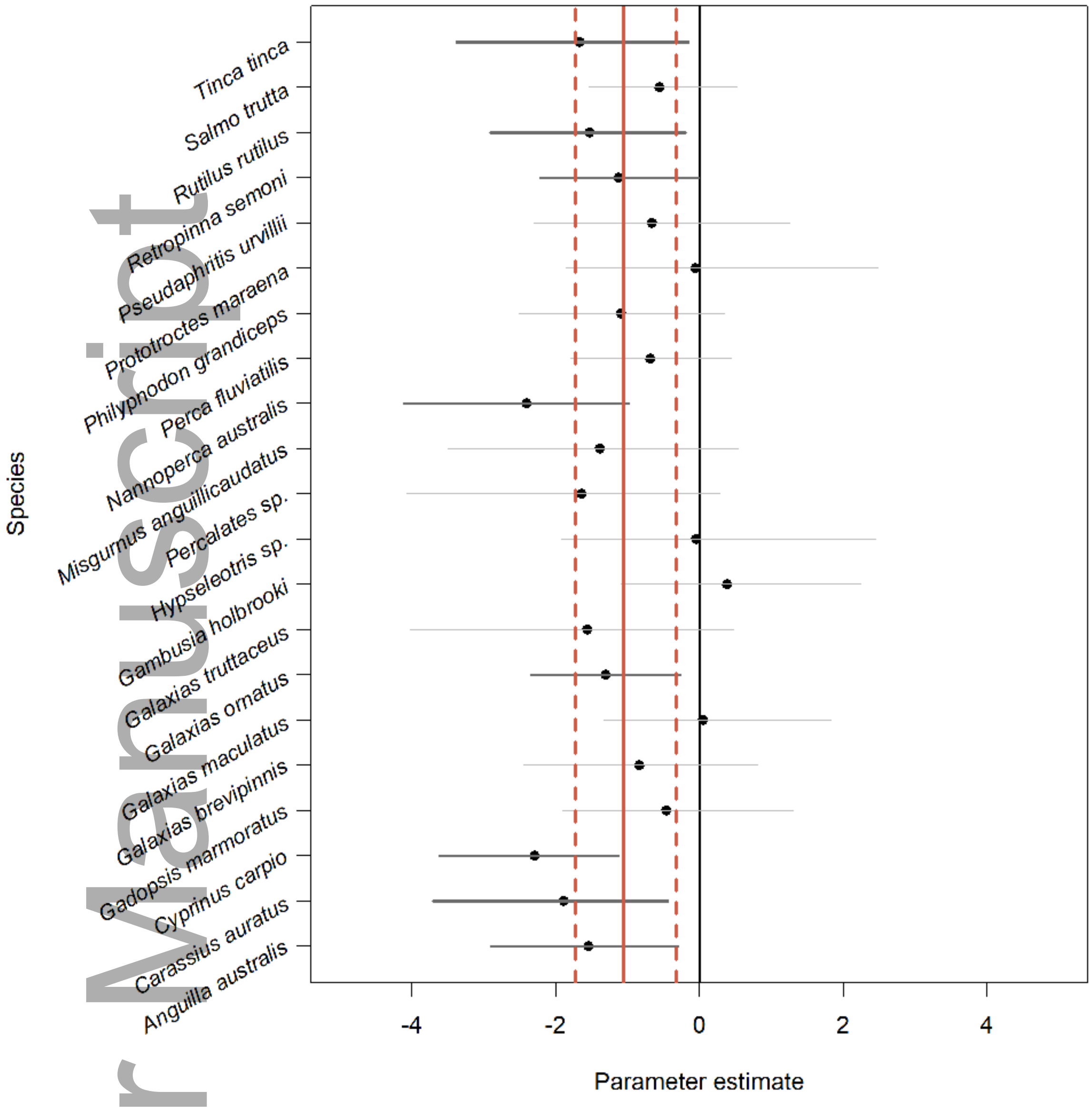


Figure 2: βk parameter estimates for each species using the fish primers, 0.1% filtering and two positive water samples required to classify a species as present. Black dots represent the mean βk parameter estimate for that species. 95% credible intervals are shown in grey. Light grey represents 95% credible intervals that overlap zero whilst dark grey credible intervals do not overlap 0. The red line is the the posterior mean of $\mu\beta$, the community mean hyperparameter and the dashed red lines are the 95% credible intervals for that community mean.

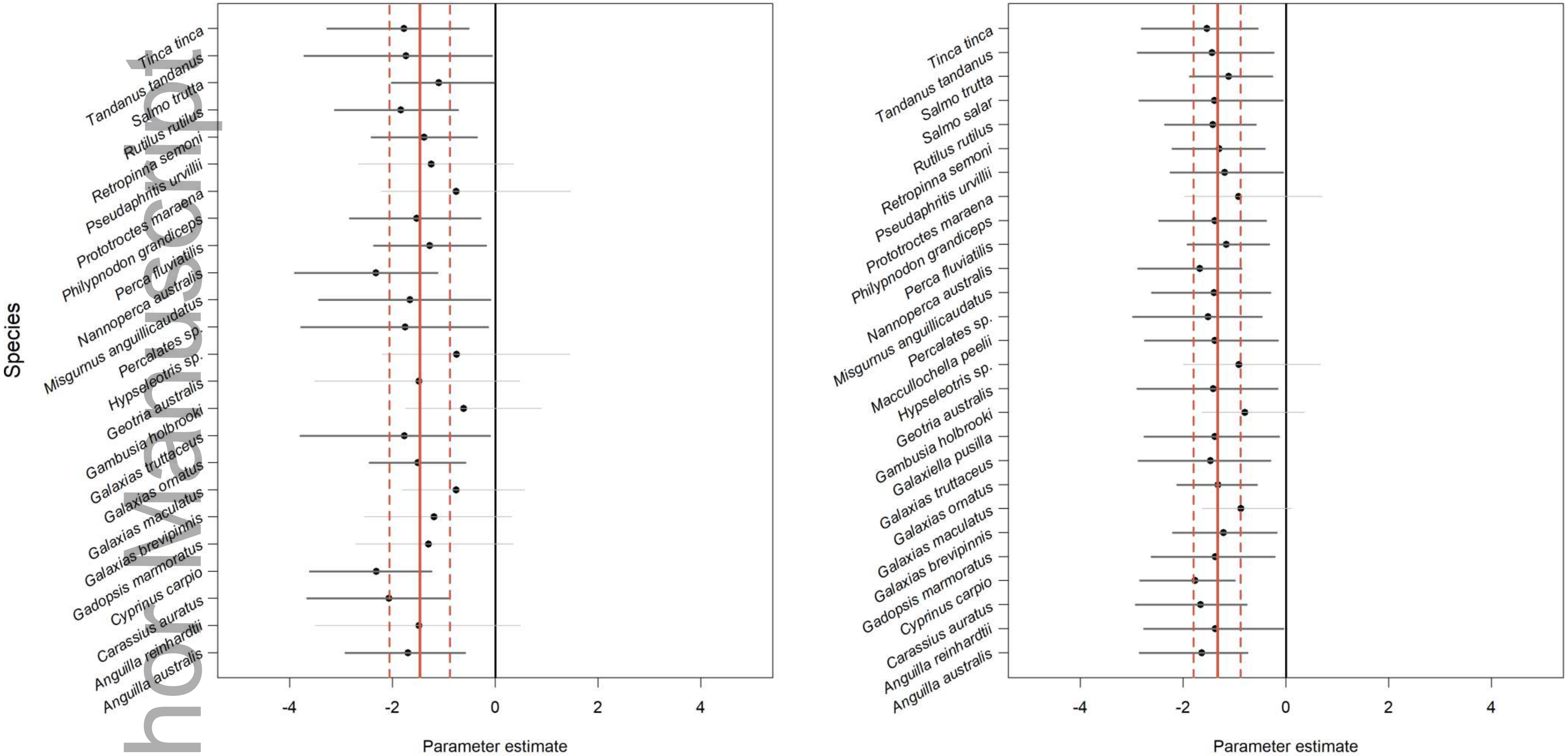


Figure 3a and Figure 3b: βk parameter estimates for each species using the combined primers, 0.1% filtering and two positive water samples required to classify a species as present (a) and one positive water sample required to classify a species as present (b). Black dots represent the mean βk parameter estimate for that species. 95% credible intervals are shown in grey. Light grey represents 95% credible intervals that overlap zero whilst dark grey credible intervals do not overlap 0. The red line is the posterior mean of μβ, the community mean hyperparameter and the dashed red lines are the 95% credible intervals for that community mean.

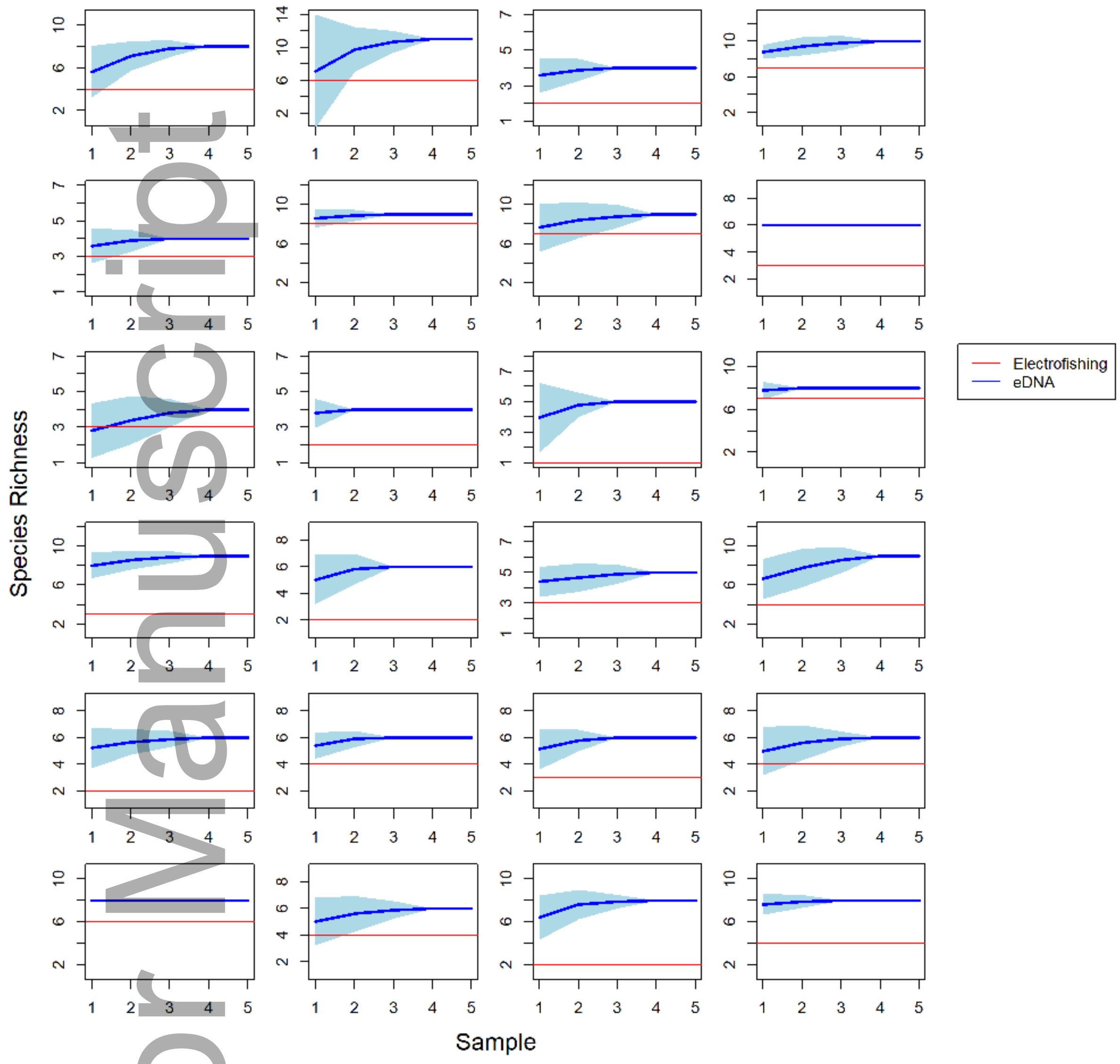
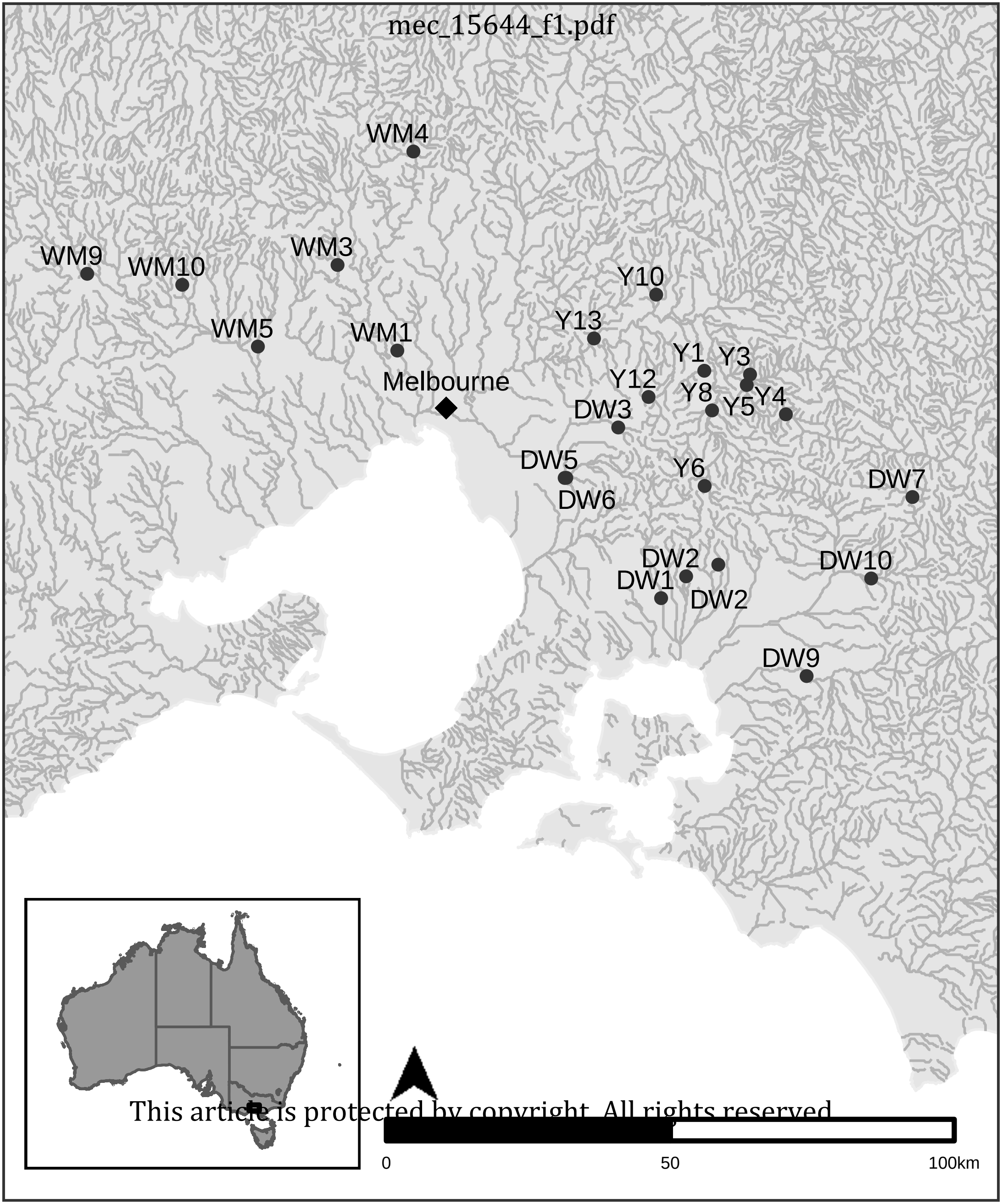
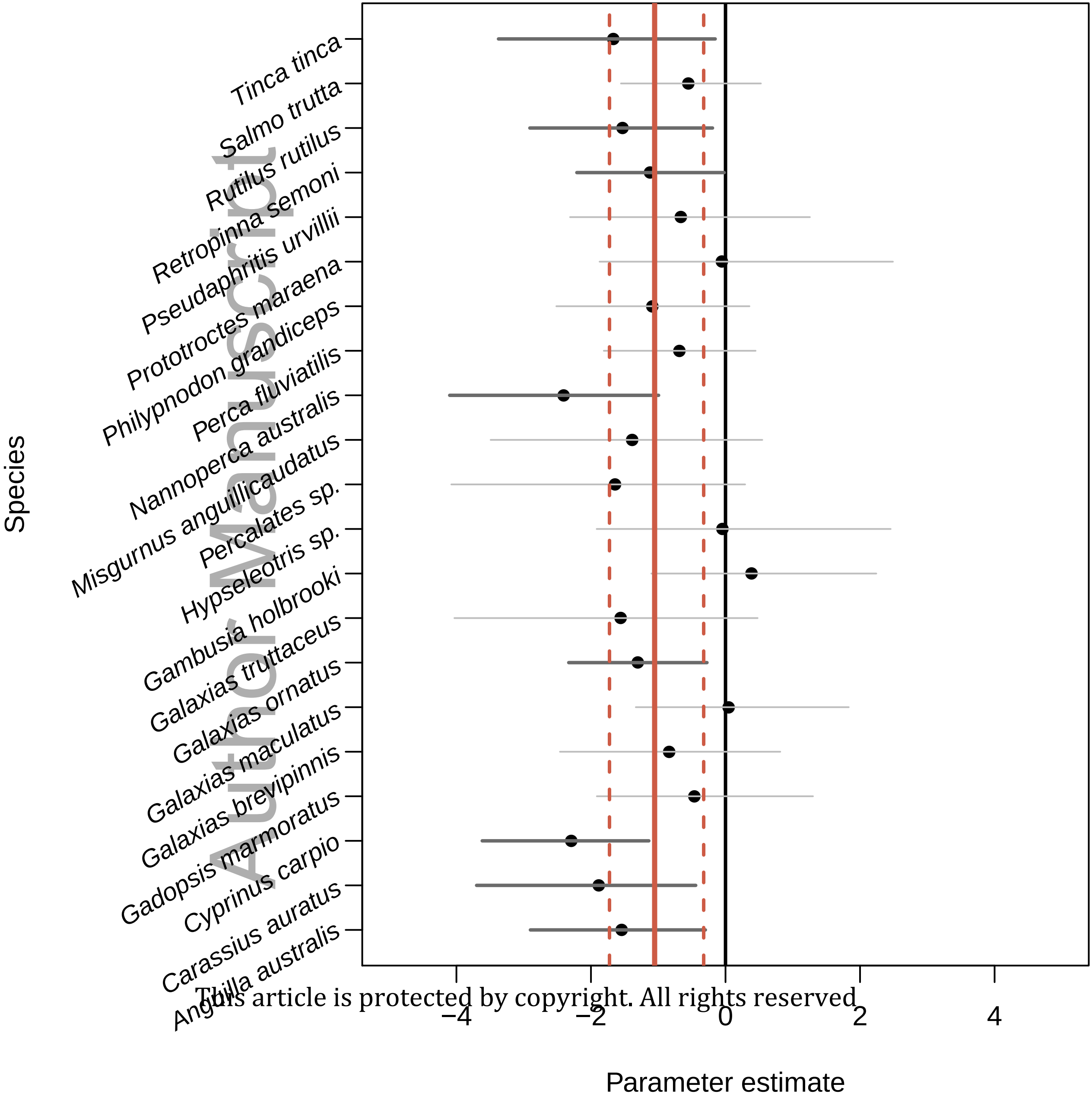
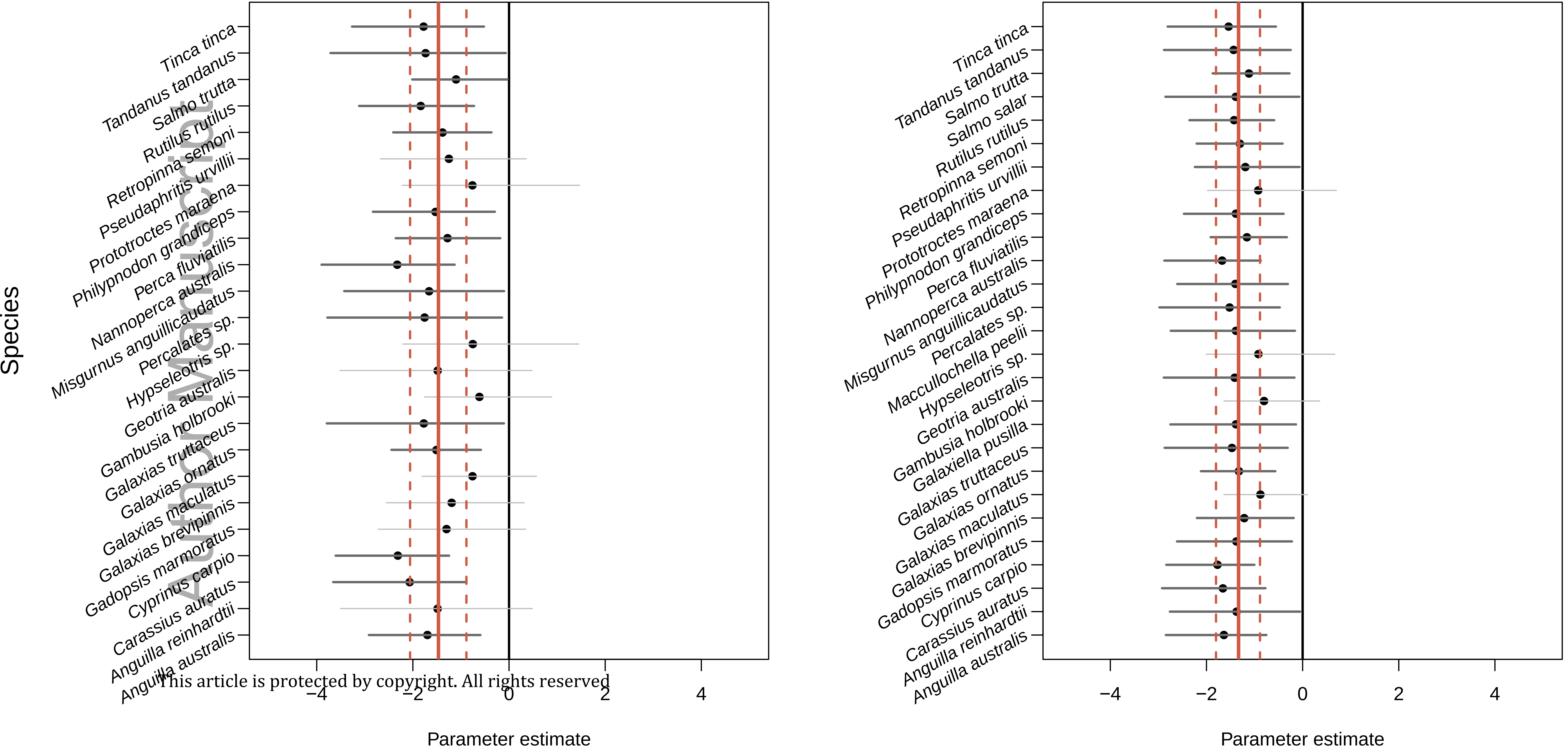
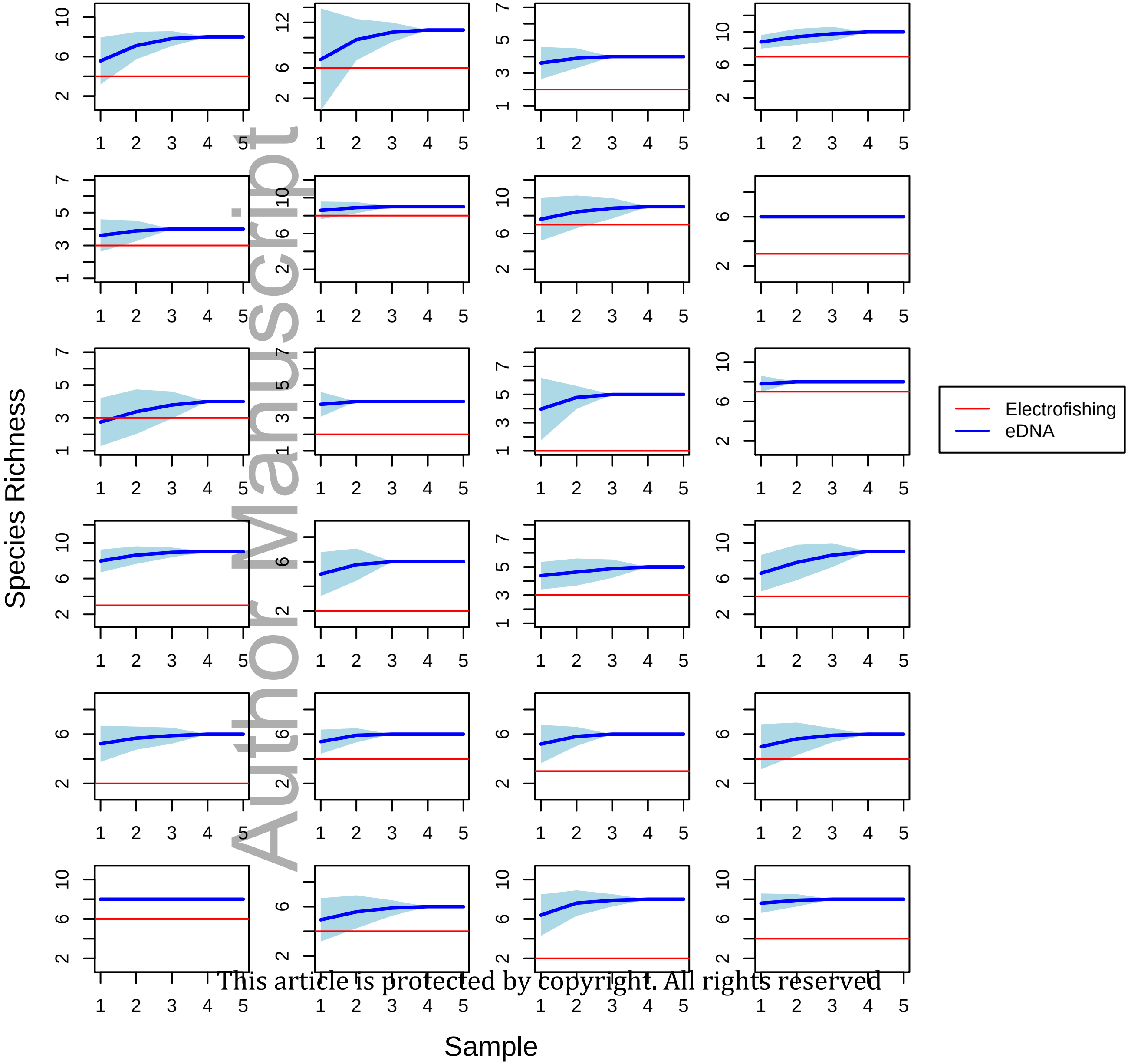


Figure 4: Species accummulation curves for each site. eDNA data from the combined primers, 0.1% filtering and two positive water samples required to classify a species as present. Red line is species detected with electrofishing. Blue line is the number of species that could be detected using 1, 2, 3, 4 or 5 eDNA samples.


WM4
WM9
WM10
WM3
Y10
WM5
WM1
Y13
Y1
Y3
Y12
Y8
Y4
Melbourne
Y5
DW3
DW5
Y6
DW7
DW6
DW2
DW10
DW1
DW2
DW9
0
50
100km


Species
Tinca tinca
Salmo trutta
Rutilus rutilus
Retropinna semoni
Pseudaphritis urvillii
Prototroctes maraena
Philypnodon grandiceps
Perca fluviatilis
Nannoperca australis
Misgurnus anguillicaudatus
Percalates sp.
Hypseleotris sp.
Gambusia holbrooki
Galaxias truttaceus
Galaxias ornatus
Galaxias maculatus
Galaxias brevipinnis
Gadopsis marmoratus
Cyprinus carpio
Carassius auratus
Anguilla australis
−4
−2
0
2
4
Parameter estimate


Species
Tinca tinca
Tandanus tandanus
Salmo trutta
Rutilus rutilus
Retropinna semoni
Pseudaphritis urvillii
Prototroctes maraena
Philypnodon grandiceps
Perca fluviatilis
Nannoperca australis
Misgurnus anguillicaudatus
Percalates sp.
Hypseleotris sp.
Geotria australis
Gambusia holbrooki
Galaxias truttaceus
Galaxias ornatus
Galaxias maculatus
Galaxias brevipinnis
Gadopsis marmoratus
Cyprinus carpio
Carassius auratus
Anguilla reinhardtii
Anguilla australis
−4
−2
0
2
4
Parameter estimate
Tinca tinca
Tandanus tandanus
Salmo trutta
Salmo salar
Rutilus rutilus
Retropinna semoni
Pseudaphritis urvillii
Prototroctes maraena
Philypnodon grandiceps
Perca fluviatilis
Nannoperca australis
Misgurnus anguillicaudatus
Percalates sp.
Maccullochella peelii
Hypseleotris sp.
Geotria australis
Gambusia holbrooki
Galaxiella pusilla
Galaxias truttaceus
Galaxias ornatus
Galaxias maculatus
Galaxias brevipinnis
Gadopsis marmoratus
Cyprinus carpio
Carassius auratus
Anguilla reinhardtii
Anguilla australis
−4
−2
0
2
4
Parameter estimate


Species Richness
Sample
Electrofishing
eDNA