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RESOURCE ARTICLE

# Replicate DNA metabarcoding can discriminate seasonal and spatial abundance shifts in river macroinvertebrate assemblages

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## Abstract

The delivery of consistent and accurate fine-resolution data on biodiversity using metabarcoding promises to improve environmental assessment and research. Whilst this approach is a substantial improvement upon traditional techniques, critics note that metabarcoding data are suitable for establishing taxon occurrence, but not abundance. We propose a novel hierarchical approach to recovering abundance information from metabarcoding, and demonstrate this technique using benthic macroinvertebrates. To sample a range of abundance structures without introducing additional changes in composition, we combined seasonal surveys with fish-exclusion experiments at Catamaran Brook in northern New Brunswick, Canada. Five monthly surveys collected 31 benthic samples for DNA metabarcoding divided between caged and control treatments. A further six samples per survey were processed using traditional morphological identification for comparison. By estimating the probability of detecting a single individual, multispecies abundance models infer changes in abundance based on changes in detection frequency. Using replicate detections of 184 genera (and 318 species) from metabarcoding samples, our analysis identified changes in abundance arising from both seasonal dynamics and the exclusion of fish predators. Counts obtained from morphological samples were highly variable, a feature that limited the opportunity for more robust comparison, and emphasizing the difficulty standard methods also face to detect changes in abundance. Our approach is the first to demonstrate how quantitative estimates of abundance can be made using metabarcoding, both among species within sites as well as within species among sites. Many samples are required to capture true abundance patterns, particularly in streams where counts are highly variable, but few studies can afford to process entire samples. Our approach allows study of responses across whole communities, and at fine taxonomic resolution. We discuss how ecological studies can use additional sampling to capture changes in abundance at fine resolution, and how this can complement broad-scale biomonitoring using DNA metabarcoding.

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KEYWORDS
abundance, biomonitoring, detectability, DNA metabarcoding, occupancy, taxonomic resolution

## 1 | INTRODUCTION

Ecology involves qualitative and quantitative measures of biological diversity at different organizational levels. As we seek to anticipate and monitor the impacts of climate change and other anthropogenic stressors on ecosystem states, as well as the outcome of interventions, obtaining high-quality biodiversity data is critical (Pereira et al., 2013). In the past, managers have been particularly ill-equipped to address the complex network of responses associated with biodiversity loss because detecting change at large scales relied on a highly limited selection of ecosystem indicators (Bohan et al., 2017). A crucial bottleneck in many survey designs is the consistent detection and taxonomic identification of organisms, and a lack of capacity in this area, particularly at large spatial and temporal scales, constrains the number of samples and quality of the data that can be collected and processed, limiting statistical power to detect change (Bush et al., 2017). Increasingly, the development of high-throughput sequencing technologies, coupled with the advent of DNA metabarcoding, has addressed this challenge and offers the potential to remove constraints associated with processing ecological samples (Baird & Hajibabaei, 2012). Hundreds of studies have now shown that metabarcoding surveys deliver consistent and accurate fine-resolution data, and offer great promise for improving aspects of environmental assessment and research (Bush et al., 2019).

Despite the substantial advantages metabarcoding already has to offer many biomonitoring applications, ecologists may require further information to gauge ecosystem status and understand mechanisms: in particular, species' abundances. Until now, a key question for DNA-based approaches is whether high-throughput sequence data can provide a quantitative signal of abundance or biomass. DNA metabarcoding uses PCR to amplify marker genes, and because the affinity of sequences to primers varies, the resulting profile of community samples can become biased (Deagle et al., 2014; Elbrecht & Leese, 2015; Hajibabaei et al., 2012). Primer affinities of different taxa are difficult to predict a priori for samples with unknown composition and abundance structure (Piñol et al., 2018). Thus, although the number of sequence reads should correlate with the proportional biomass of a species in a sample, the slope and strength of the relationship is highly variable from species to species, making post hoc interpretation highly uncertain (Bista et al., 2018; Elbrecht et al., 2017). Given such uncertainty, the conservative interpretation has been to treat metabarcoding data as presence/absence until evidence for reliable correction factors can be obtained (Pawlowski et al., 2018). However, correction factors only allow changes in the relative abundance *within* a taxon to be estimated (Luo et al., 2022), and the prospect of calibrating for bias (McLaren et al., 2019) remains a distant prospect for those diverse communities where we gain most from DNA-based identification.

Occurrence and abundance are merely different expressions of the same phenomenon (He & Gaston, 2003). The form of occupancy–abundance relationships depends upon how species ecology relates to sampling scale (i.e., spatial grain: Gaston & Fuller, 2009), and naturally the correlation is highest when sample units match the scales of single individuals (Steenweg et al., 2018). The relationship is nonetheless inherently positive because the proportion of sites occupied (a binomial process) is a direct function of mean abundance (a Poisson process) (Royle et al., 2005). As well as being scale-dependent, estimates of occupancy are highly sensitive to detection error, particularly so for rarer taxa. Hierarchical occupancy models explicitly model the observation process and identify the uncertainty resulting from imperfect detection, which otherwise leads to underestimation of occurrence. Interestingly, because the probability of detecting at least one individual increases with abundance, we can expect more detections in total at sites with greater abundances, and thereby estimate changes in a taxon's abundance solely from repeated detection data (Royle & Nichols, 2003). In addition, because the detection rate derives from comparable estimates of occurrence, we can estimate difference in abundance *between* taxa. Given the challenges of associating sequences with ecological quantities, perhaps replicated sampling can offer an alternative approach for assigning quantitative signal strengths to taxa within observed communities?

Trade-offs between the quantity and quality of data available for environmental management are commonplace in freshwater science, and DNA metabarcoding tools are being adopted rapidly to overcome traditional taxonomic constraints (Baird & Hajibabaei, 2012; Bush et al., 2019; Leese et al., 2018). While studies have repeatedly demonstrated that occurrence-based data are sufficient for monitoring ecological status (e.g., Beentjes et al., 2018; Buchner et al., 2019), abundance data are desirable for studying some ecological processes (e.g., population dynamics within communities, phenology, trophic structure). The need for abundance data is also specified by regulatory authorities such as those governed by the Water Framework Directive, and this has consequently delayed adoption of DNA metabarcoding approaches among some European authorities (Leese et al., 2018). In this study, we applied a multi-species hierarchical model (sensu Yamaura et al., 2011) to explore whether detection frequency could be used to estimate abundances of stream benthic macroinvertebrates, and whether it could identify either seasonal changes or differences between assemblages resulting from predator exclusion. We review these results in the context of survey error and bioassessment needs.

## 2 | MATERIALS AND METHODS

### 2.1 | Site and survey design

This study was designed to evaluate a modelling approach to estimate abundance from metabarcoding data, and we therefore chose to test scenarios where significant differences in abundance structure were already expected. Our study aimed to detect seasonal changes in the abundance of stream benthic invertebrate communities, and the impact of predation by experimentally excluding fish. The distribution of benthic macroinvertebrate abundances is determined by the timing of species' life-history cycles (e.g., egg hatch, individual growth, emergence and mortality; Butler, 1984), and seasonality is widely acknowledged as a source of variation that impacts bioassessment (e.g., Boehme et al., 2016; Linke et al., 1999). Likewise, numerous studies have demonstrated fish can exert top-down control on the abundance of invertebrates and alter community composition (e.g., Dudgeon, 1991; Williams et al., 2003); however, this only shifts a community toward smaller size classes, and over longer timescales total biomass and abundance may be comparable (Winkelmann et al., 2011). This study aimed to test whether these two drivers of abundance could be observed solely from data on species' detection.

Our study site was Catamaran Brook (66.104 W, 46.879 N), a third-order tributary of the Miramichi River in central New Brunswick, eastern Canada. The site has a permanent gauge station from which stream temperature and flow were available, and previous research demonstrated at least 14 species of fish are present (Cunjak et al., 1993). To exclude fish, we placed 37 0.8-m$^2$ 5-mm mesh cages on shallow riffle-run sections of the stream (Figure 1). Cages were secured by rebar driven into the streambed. To be confident differences were the result of fish exclusion and not artefacts resulting from cage-effects (e.g., accumulation of detritus, reduction of flow, shading), half the cages were left open on their downstream side. To prevent smaller fish from slipping under the edges of "closed" cages, the mesh was extended outwards so that it could be secured to the streambed with additional cobbles. Sites along the stream were randomly assigned to be Open or Closed cages, and the area within the cage footprint was sampled using a standard 400-µm kick-net. Cages were installed as soon as the flush from spring snowmelt had dissipated, which in 2018 was not until June 8, and then sampled every 4 weeks until late October. Cages were checked once between each survey, primarily to clear accumulated leaf litter blocking flow through the cages. A sixth survey in November had to be abandoned after storm flows dislodged the cages.

FIGURE 1 Cages at Catamaran Brook designed to exclude fish. Note the open sides of the cage in the foreground providing a suitable control environment.

As covariates to the study, we used two temperature loggers to record water temperature every 15 min (HOBO Pendant temperature logger, P/N UA 001 64). Rather than measure time chronologically, and the separation between surveys equally, seasonality was measured in degree-days (the sum of daily mean water temperatures above 0°C), to reflect the influence of temperature on the rate of benthic invertebrate development and phenology (Benstead & Huryn, 2011). Flow data were also available from a nearby upstream gauge (Water Survey of Canada station 01BP002) and point measurement of water temperature, dissolved oxygen, conductivity, pH and turbidity were made during each site visit using a YSI ProDSS multimeter. Dissolved nutrients and metals were tested four times during the survey season at the Atlantic Laboratory for Environmental Testing. As reported from this site previously, water chemistry fluctuates but did not display a pronounced temporal trend (Cunjak et al., 1993), and we did not observe a significant response among invertebrate assemblages to either chemistry or flow. As a result, the remainder of this study only reports upon the responses to cage treatment and degree-days (hereafter seasonality).

### 2.2 | Sample processing

Benthic invertebrates from 31 of the 37 cages were analysed using DNA metabarcoding (15 Open and 16 Closed). To extract DNA, unsorted invertebrate samples were homogenized using a decontaminated household blender, and after blending at maximum power for 1 min, 50 mL of the homogenate was centrifuged at 2400*g* for 2 min. Excess ethanol was removed, and the residual ethanol was evaporated at 70°C for ~4–8 hr. Once dry, 0.2 g of the homogenate was transferred to a bead tube, and DNA was extracted using Qiagen's DNEasy PowerSoil kit according to the manufacturer's protocol. Extractions were performed in batches of 16–24, with one negative control (no sample material) every two batches. The PCR protocol followed Gibson et al. (2015), targeting the F230R and BR5 COI primer sets. Samples were sequenced over two runs, on an Illumina MiSeq using the v3 kit (2 × 300 bp). A 10% PhiX spike-in was included with each run. Sequences were processed using the SCVUC version 2.3 metabarcoding pipeline (https://github.com/Hajibabaei-Lab/SCVUC_COI_metabarcode_pipeline), using the default settings. For SEQPREP these were a minimum phred score of 13, minimum overlap of forward and reverse reads of 25, maximum fraction of mismatches allowed was 0.02, and a minimum fraction of matching overlap of 0.90. The CUTADAPT parameters were minimum sequence length of 150, error rate of 0.1,

minimum adapter overlap of 3 and a maximum number of N's of 3. A minimum cluster size of 3 reads was required after denoising. Taxonomy was assigned using the RDP classifier within METAWORKS version 1.11.3, with the v2 COI training set (https://github.com/terrimporter/CO1Classifier) (Porter & Hajibabaei, 2018; Wang et al., 2007). The sequences generated were deposited in the NCBI Sequence Read Archive (SRA), under project PRJNA809203, and all supporting data are provided in File S2.

To verify that exclusion of fish and seasonal changes resulted in shifts in macroinvertebrate abundance at Catamaran Brook, a certified taxonomic expert identified all specimens from an additional six cages in each survey (three Open and three Closed), hereafter referred to as the manual count data set. Specimens were identified to the lowest taxonomic level possible using a Leica MZ7.5 dissecting microscope and standard taxonomic keys (Merritt et al., 2008; Peckarsky et al., 1990; Stewart & Stark, 2002; Thorp & Covich, 2010; Wiggins, 1996). Voucher samples were collected for a subset of taxa and stored in 70% EtOH at the Environment and Climate Change Canada laboratory. This type of exhaustive processing is extremely time-consuming (40–80 hr per sample in this case) and hence could not be repeated to the same extent as the DNA sample replication. As a result, we also included a previous macroinvertebrate study at Catamaran Brook that combined results from almost 2 years of surveys (Cunjak et al., 1993) as a separate source of taxon abundance data.

Validating the latent states of hierarchical models (true abundance) is challenging because all observation systems carry a degree of error, and further caution is required where the number of taxa exceeds the number of samples, and when counts contain many zeroes (Warton et al., 2015). Rather than relying on small numbers of counts to validate predicted mean abundances, we estimated their agreement with the multispecies abundance model (MSAM) based on the likelihood of observing those values from the model's posterior probability distribution. We further tested whether predicted abundances were correlated with observed counts both across taxa within a treatment (season × cage), and within a species/genus across treatments, and referred to correlations among replicate manual samples to gauge what consistency could be expected. The community-level correlation in turnover was measured using a Mantel test based on a Jaccard index of compositional similarity (Mantel, 1967). In addition, to test the concordance and mismatch between manual and DNA data sets we fitted a series of multivariate generalized linear models (GLMs). The taxonomic breadth and resolution of the manual and DNA data sets differ and hence the series of models checked that inferences did not change when traditional count data were reduced from all observed taxa to just families or genera, to genera matched by the DNA data set, and finally only occurrence information. The impact of data source was then tested by combining manual and DNA occurrence information, or manual counts and MSAM-predicted abundances, and testing for an interaction with seasonality or the cage–treatment effects. Models were fitted using the *mvabund* package (Wang et al., 2019), with negative binomial link functions for counts and binomial links for detection-only data (Figure S17). Significant effects were determined by likelihood ratio tests (LRTs).

## 2.3 | Royle–Nichols MSAM

Individuals of a given taxon commonly go undetected during surveys of occupied sites (Kéry & Royle, 2015). Such imperfect detection adds false absences to survey data, and if this is not accounted for, it can cause substantial error or bias in parameter estimation and obscure ecological responses (Kéry et al., 2010; Lahoz-Monfort et al., 2014). Hierarchical models control for imperfect detection by explicitly modelling both the detection process (associated with sampling design and methods), and the true underlying ecological mechanisms (i.e., state processes), in parallel (MacKenzie et al., 2002). To partition these two processes, hierarchical models require additional information about the detection process, typically derived from spatially or temporally replicated surveys. In our study, replication was provided by separate benthic samples during each survey $v_j$ ($n = 15$ and 16 from Open and Closed cages respectively).

Occupancy models typically make use of detection/nondetection data (as we interpret metabarcoding observations) to estimate the probability of detecting at least one individual of taxon $i$ at site $j$ ($\pi_{ij}$). However, if the individual-level detection probabilities $r_{ij}$ are independent, then the probability of detecting at least one individual, $\pi_{ij}$, is $\pi_{ij} = 1 - \left(1 - r_{ij}\right)^{Z_{ij}}$, where $Z_{ij}$ is the abundance of taxon $i$ at site j (Royle & Nichols, 2003). Changes in abundance are thereby reflected in overall frequency of detection, modelled by a binomial distribution $y_{ij} \sim Binomial\left(v_j, \pi_{ij}\right)$. Detection probability can also vary among surveys for reasons other than abundance, and we included the dependency on sequencing depth ($x_j$) as a covariate: $logit\left(r_{ij}\right) = \alpha 0_i + x_j \alpha_i$. In parallel to the detection process, a Poisson distribution describes the underlying latent ecological process model: $Z_{ij} = Poisson\left(\lambda_{ij}\right)$. Changes in mean abundance are modelled as a function of taxon-specific responses to survey-scale covariates: $log\left(\lambda_{ij}\right) = \beta 0_i + x_j \beta_i$. In this study, the impact of fish-exclusion was included as a binary (0/1) covariate, and linear and quadratic terms for seasonal differences (quantified by degree-days) were included to identify peaked responses. Based on previous studies, we tested whether separate primers also displayed different detection probabilities (Bush et al., 2020), but on this occasion found insufficient evidence to retain that distinction.

As this study focused on the entire benthic macroinvertebrate community, estimates of abundance from the Royle–Nichols model were combined within a hierarchical framework that included hyperparameters to define the shared collective responses of occupancy and detection probabilities to covariates (Dorazio & Royle, 2005; Yamaura et al., 2011). Shared hyperparameters shrink parameter estimates toward the community mean, potentially biasing valid outlier taxa, but primarily enabling stronger inference for many other, often rarer, taxa that "borrow" strength from the collective community response (Dénes et al., 2015). Nevertheless, because only a small subset of taxa were detected frequently, early models estimated

very low probabilities of detection across the community, that in turn subsequently overestimated the abundance of many taxa. We considered the possibility that in addition to imperfect relationship between samples and a population, low detectability could stem from aggregated distributions of particular taxa within the stream, which would limit the proportion of taxa that were "available" for detection in every sample (Joseph et al., 2009). We included a zero-inflation parameter, $\phi_i$, to describe the degree to which each taxon was evenly distributed within the stream, and subsequently interpret the predicted estimates of abundance $Z_{ij}$ as average densities in this section of the stream (Morán-López et al., 2022). Sensitivity analysis indicated the choice of prior had a negligible effect on the model, and we therefore chose flat normal distributions as they are the uninformative priors used by most hierarchical community models (Kéry & Royle, 2015). MSAMs were fit using the *jagsUI* package in R (Kellner, 2019), running four chains drawing 500,000 samples after a burn-in of 500,000 were thinned by 100 to keep a total of 20,000 samples. Convergence was assessed using the $\hat{R}$ statistic, assuming convergence was achieved when $\hat{R}<1.1$ (Gelman & Rubin, 1992). Code detailing the model and further results are provided in Supplement S1.

## 3 | RESULTS

In total, sequences derived from DNA metabarcoding were assigned with confidence to 184 genera (46 ± 16 [*SD*] per sample), and these contained a total of 318 species (60 ± 23 per sample). Rarefaction suggested sequencing depth was sufficient to detect all genera present (Figures S3 and S4). Individual kick-net samples typically only recorded a small proportion of the total diversity, but the combination of high replication suggests that in most surveys most taxa were observed (Figure 2a and Figure S1). The total richness of genera collected in each month declined over time, but the cumulative total richness continued to increase with the addition of survey at the same sites in different months (Figure S1). Fewer taxa could be identified to the genus level in the manual count samples (29 ± 8 per sample; Figure S2).

### 3.1 | Multispecies abundance models

MSAMs indicated that cages excluding fish led to a detectable increase in predicted abundance (Figure 3; Figures S8–S11), although this was only significant for 26% of the taxa (39 genera and 67 species; Figures S8 and S9). On average, abundance peaked in late summer, but this community-level trend also hides significant variation among taxa (Figures S8 and S10), including those that clearly peak in spring and, to a lesser extent, autumn. As we would expect when we subdivide taxa, the species-level MSAM estimated marginally lower mean abundance and detectability at a community level, although the hyperparameter distributions for each model were similar (Figure S5). Interestingly, differences within genera not only suggested differences in species' prevalence, but also potential differences in species' responses to fish-exclusion and seasonality that were otherwise masked at the genus level (Figures S6 and S7). Nonetheless, for the purposes of this study we primarily refer to results at the genus level because comparisons with data collected via

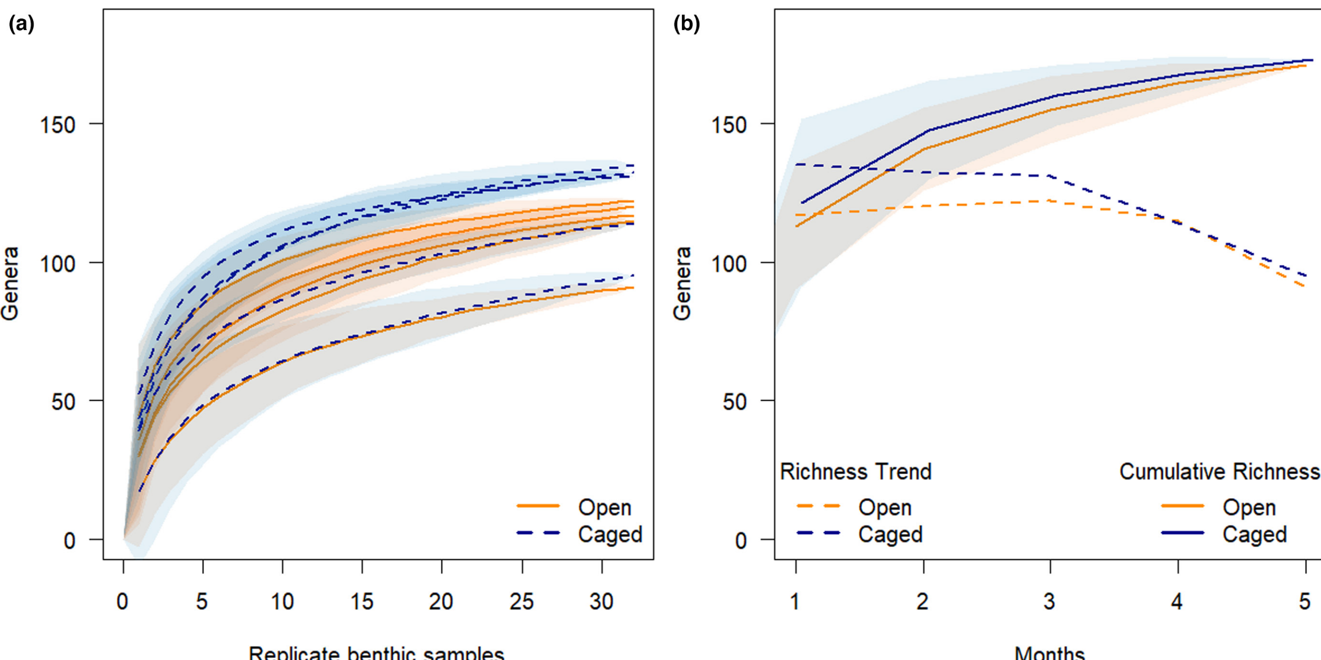


FIGURE 2 Rarefaction curves describing taxonomic richness at the genus level, based on cumulative addition of (a) replicate kick samples (during single monthly survey), and (b) monthly surveys, further subdivided by cage treatment. Shaded areas indicate the 95% confidence intervals. Plot (b) also shows the trend in the total richness observed for each month surveyed. Note the number of months may be nonsequential for Cumulative Richness, but for the Richness Trend refer to a temporal sequence.

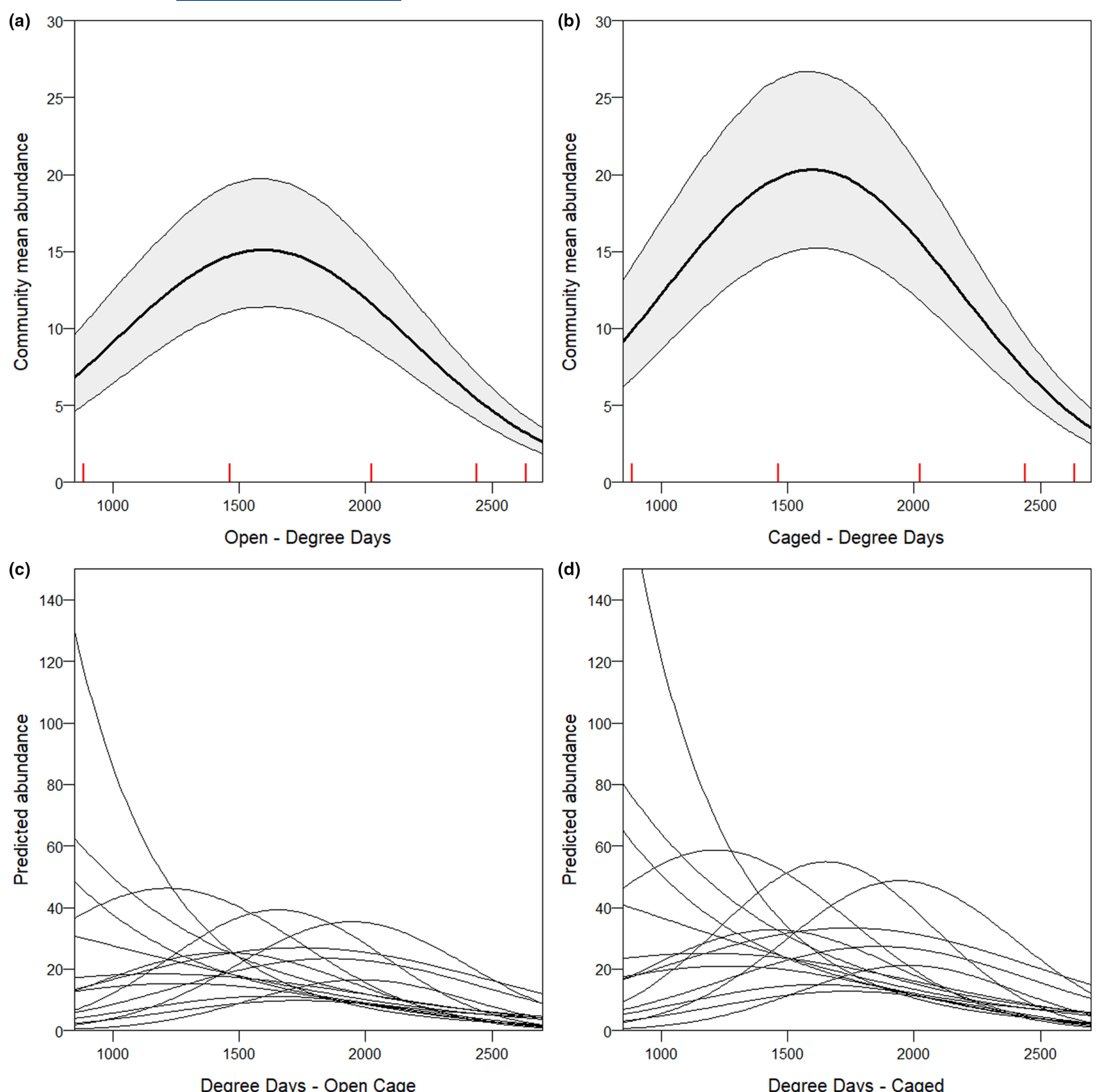


**FIGURE 3** Predicted seasonal changes in mean abundance (and 95th percentiles) of the entire macroinvertebrate community (a, b), and selected individual genera (c, d) in open cages (a, c), and closed cages that excluded fish (b, d). The internal red ticks in (a) and (b) indicate the location of our seasonal surveys. Responses of all genera shown in Figure S10.

microscopy were not possible at the species level, and because the main treatment and environmental responses were similar.

## 3.2 | Parallels between estimated abundance and manual counts

More than 42,000 specimens were manually sorted, 53% of which were Chironomidae. However, comparisons between DNA-derived MSAM and count data for individual taxa were challenging because almost 80% of individuals that were not chironomids could not be identified to a finer resolution than family level and consequently only 66 genera were shared between the two data sets (Table S1, Figure S9). Not only did this exclude 108 genera observed by DNA, but also 34 genera identified according to morphology. Across the overlapping genera, the majority of observed manual counts were within the ranges of the probability distribution predicted by the MSAM, on average within the highest 20th percentile of the posterior mean (SD ±29%, Figure 4 and Figure S18); but not all manual counts were captured within the top 95% of the model's posterior probability

distribution (e.g., the caddisfly larva *Dolophilodes*; Figure 4f). The correlation in abundances between different genera within a survey (same season × cage treatment) and MSAM predictions was generally weak (mean $r = .29 \pm .13$), driven by variation in the count data set, which was consistent with the variation observed in correlations among replicate count samples (mean $r = .50 \pm .28$; Figure S13). Likewise, correlation between the abundances of genera across surveys, where shared by manual and MSAM approaches, were typically weak too, but their rank-level association was stronger (mean $rho = 0.43$, $SD = 0.07$). Further comparisons, including at the family level, are provide in Supplement S1 (Figures S12–S16). The Cunjak et al. (1993) study recorded half as many genera as observed using DNA, both in total and on average per sample. As before, there was a weak association between the abundances predicted by the MSAM and surveys conducted by Cunjak et al. (1993) ($r(39) = .05$, $p = .75$), but a more modest correlation with our manual count data ($r(39) = .41$, $p = .07$).

Despite the volatility in the abundances of individual taxa, the collective responses of the community demonstrated more concordance. Although turnover among manual count samples was high, even when the triplicates from each season and treatment were combined (mean Jaccard similarity = 0.54), the turnover predicted by the MSAM was consistent with those observations (Mantel correlation = .68, $p = .029$). Multivariate GLMs of community composition based on manual counts also identified significant differences between Open and Closed cages (LRT = 5653, $p = .001$), and across the sampling season (LRT = 12,989, $p < .001$), and those effects were typically evident when the taxonomic breadth and resolution were reduced to match the DNA data set. GLMs fit to a combination of detections by both manual and DNA indicated covariate effects were dependent on the data source, but if GLMs are fit to manual counts combined with abundances predicted by the MSAM, then the interactions with data source became nonsignificant for the cage-treatment, and only weakly significant for seasonality (see Supplement S1 section S7).

## 4 | DISCUSSION

This study demonstrates that by analysing changes in the frequency of detection using a Royle–Nichols hierarchical modelling framework we can infer changes in abundance using presence–absence data from DNA metabarcoding. We used the total number of sequences per sample to account for processing effort in detection probability, but the number of reads does not inform our estimates of abundance. Not only were we able to recover changes in patterns of abundance by season (e.g., Linke et al., 1999), but also the

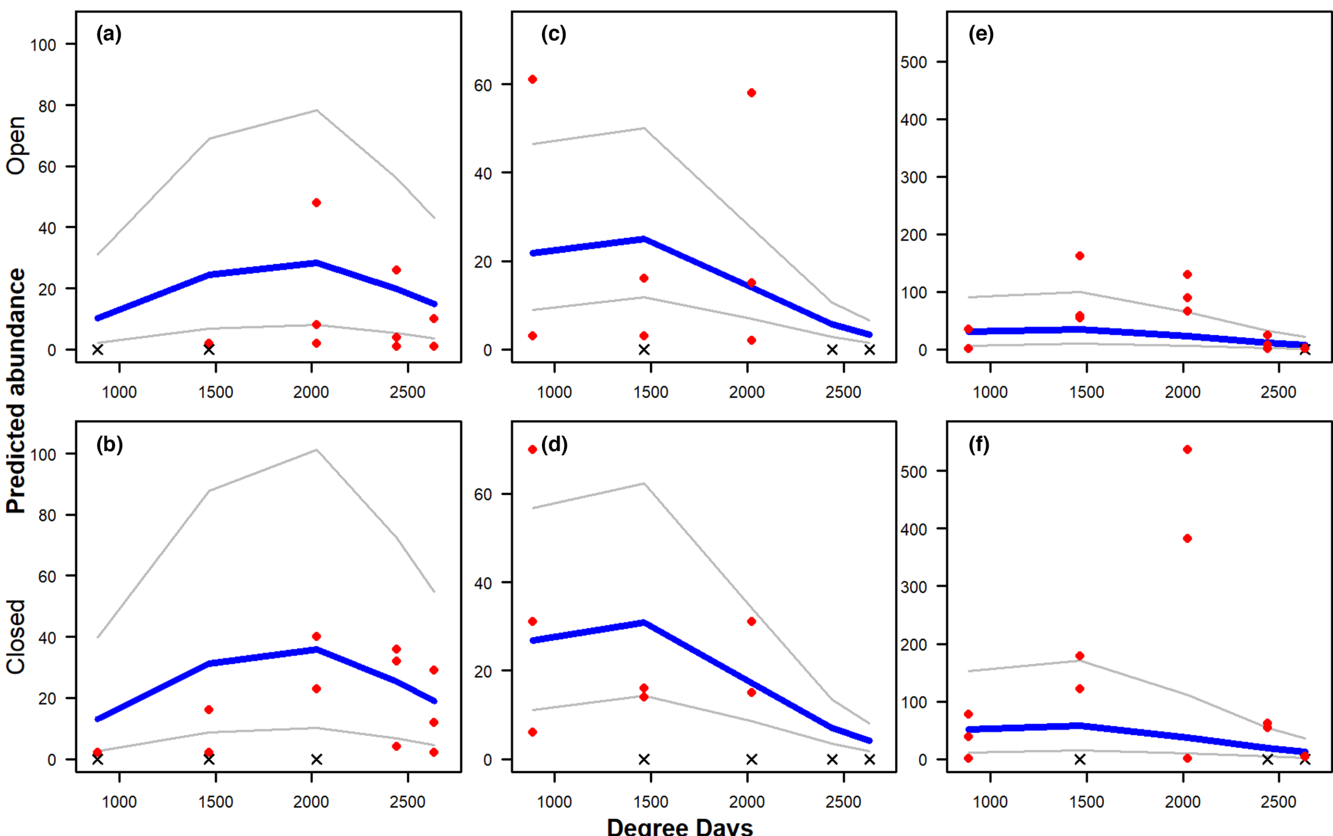


FIGURE 4 Changes in seasonal abundance predicted by the MSAM (mean and 95% highest density interval) for three genera: the mayfly larvae of *Sweltsa* (a, b), *Baetis* (c, d) and the caddisfly larvae of *Dolophilodes* (e, f), in open (a, c, e) and closed (b, d, f) cages. Manual counts are shown in red, including zero counts as crosses.

collective gain in abundance when cages excluded fish (Williams et al., 2003). These inferences were partially corroborated by the parallel analysis of samples sorted via morphological features, although, as shown, providing a robust reference for validation was challenging. Using DNA metabarcoding we can also extend our understanding to a much greater portion of biodiversity, and at fine taxonomic resolution, which may change the balance of diversity gradients, or demonstrate variation within genera that is invisible to traditional monitoring methods. Many applications assume species or genera within higher taxonomic categories share similar environmental responses, and possess similar ecological functions, and ours is the latest in a growing number of DNA-based studies to call this into question (Beermann et al., 2018, 2021).

Invertebrate development and phenology are innately connected to their thermal preferences (Baranov et al., 2020), and as a result seasonality was modelled as a function of degree-days. As expected, we found no evidence that species are seasonally absent from the river: using DNA they can be detected at any time. Instead, changes in the frequency of detection reflect changes in abundance of taxa that are unevenly distributed across the community (Steenweg et al., 2019). A DNA-based MSAM could therefore improve our understanding of phenology for whole communities, temporal partitioning among functional groups, as well as the potential value of abundance information for construction of ecological networks (Freilich et al., 2018). The exclusion treatment represents a dramatic change in ecological interaction strengths and confirmed that direct fish predation can significantly depress average invertebrate abundance in the stream (Bonjour et al., 2020; Dudgeon, 1991; Williams et al., 2003; Winkelmann et al., 2011). Cages inherently alter microhabitat factors, such as flow and shading, but our Open control cages were designed to account for those confounding effects. It is also possible that significant macroinvertebrate responses were not a direct result of reduced fish predation, but an indirect result of interactions with other invertebrate taxa released from predation (Harris, 2016). Unfortunately, we could not collect any concurrent data on the fish fauna, but an interesting avenue of future study would be to connect such observations with those of gut contents (Deagle et al., 2018), because, if derived from DNA metabarcoding, the taxonomic comparisons would be equivalent.

Lastly, an interesting outcome of this study was that a single parameter to describe the likelihood of detection was insufficient to account for the proportion of samples containing absences. As samples were all taken from the same 100-m stretch of river, they were considered as one interacting community. Conversely, the distribution of taxa within the stream bed is known to be highly uneven at a microhabitat scale, with many taxa clustered according to changes in flow, substrate and availability of coarse organic matter (e.g., Burgazzi et al., 2020; Mathers et al., 2017). As a result, not all taxa were expected to be present for detection in every 0.8-$m^2$ sample. Quadrat-scale processes could arguably be viewed as covariates for taxon occurrence or detectability for inference at finer spatial scales, but in the context of this study, the inference for many rare taxa would have been to assume such low probabilities of detection that the underlying state process would be unidentifiable. Instead, introducing a zero-inflation parameter to control for within-stream spatial aggregation helped to resolve the estimation of detectability, and captured the within-habitat heterogeneity that underpins observed patterns such as the rate of species accumulation (see Figure 2a) and past studies of mean–variance relationships (i.e., Taylor's law: Giometto et al., 2015). Indeed, recent simulation-based studies have shown the Royle–Nichols model was more robust to overdispersion when zero-inflation was included, but, as was the case here, to remain identifiable studies should be designed to maximize detectability (Morán-López et al., 2022).

## 4.1 | Abundances within and across species

Currently many DNA metabarcoding studies interpret sequence read information as presence/absence because a chain of factors in study design can influence the relative proportion of the final total in unpredictable ways (Luo et al., 2022; McLaren et al., 2019). The combination of both species-specific biases and sample-specific error distorts the connection between the volume of DNA in a sample and the final sequencing outputs such that neither the sign nor the magnitude of change can be recovered from the number of sequence reads. It is possible to correct for pipeline error if internal standards are supplied in the sequencing pipeline as benchmarks for comparison, and to estimate and correct for species biases using replicate sampling, as we do in this study. Doing so allows users to standardize the number of sequences in different samples and interpret changes in relative abundance or biomass *within* that species (Harrison et al., 2021; Levi et al., 2019). However, corrections to recover estimates of absolute values, and thereby make comparisons *among* species, are not currently possible using metabarcoding (see Williamson et al., 2019). In contrast, using the MSAM approach demonstrated how DNA metabarcoding may be used to compare absolute abundances both within *and* across a community.

As with any modelling approach, the MSAM will be sensitive to violations of its assumptions, which we address below, but it is also worth reflecting initially on current practice. Current standards of practice in aquatic biomonitoring typically process subsets of samples with varying taxonomic accuracy, making errors difficult to define (Cao et al., 2007; Clarke, 2009). Exhaustive manual counts of every individual do minimize detection error, but many individuals, not least juveniles or damaged specimens, cannot be identified consistently (Orlofske & Baird, 2013; Schmidt-Kloiber & Nijboer, 2004). The value of count data in aquatic invertebrate studies is therefore questionable, and is more likely to reduce, rather than improve, statistical power (Bush et al., 2019; Canton & Chadwick, 1988). Although a more robust validation of the MSAM predictions would have been welcome, it was not possible within this study to collect and process dozens of samples typically needed for accurate estimates of abundance (Egglishaw, 1969). By contrast, DNA metabarcoding typically improves taxonomic resolution and detection probability

(Bush et al., 2019, 2020), and allows consistent observations to be made in different surveys, and by different operators (but see Zaiko et al., 2022). Unfortunately, the stochasticity of count data, and the difficulty of manually processing large numbers of count samples meant the accuracy of MSAM estimates was difficult to define. The GLMs indicated significant interaction effects of data source were present, but those effects reduced in combination with the MSAM, implying that while detection-naïve interpretation would result in diverging responses, disagreements may be easier to resolve once errors of both approaches are included. Overall, differences were consistent with variation among replicates, and observed counts were well within the credible range of the MSAM predictions, suggesting this approach could offer a more rigorous method for quantitative studies of whole communities at fine taxonomic resolution.

## 4.2 | Limits to the occupancy–abundance relationship

The density of individuals per unit area is equivalent to occupancy at the scale of an individual, and because individuals within a species are typically clustered, occupancy (and thus frequency of detection) can continue to increase over a broad range of abundances (McGill, 2011). The consistent relationship between occupancy and abundance is one of the most well-documented biodiversity patterns known (Gaston et al., 2000), and the model employed in this study uses the same principle outlined by Royle and Nichols (2003) to adjust the Poisson state process based on frequency of detection in repeated binary observations. The extension of this approach to a multispecies community allows shared parameters to describe distributions across the community, and make model inference stronger and more efficient overall, particularly among rare taxa (Dorazio & Royle, 2005; Yamaura et al., 2011). Nonetheless, ecological populations are highly stochastic, and MSAM predictions are sensitive to minor changes in model fit (Dénes et al., 2015). It is important to understand the assumptions behind such an approach when judging its suitability to taxa that display different types of rarity (Jeliazkov et al., 2022).

The first issue is that when the description of a population is reduced to occurrence, the distribution in a community is bimodal (McGill, 2011); some taxa are detected in most samples, and others only rarely. At either extreme the variation in occurrence relative to changes in abundance is constrained (referred to as "saturated" for high occurrence) and thus the relationship between occupancy and abundance is weaker than at intermediate values of occupancy. Without variation to act upon, the MSAM cannot identify changes in abundance (Dénes et al., 2015). For example, although there was a clear positive shift in the number of the caddisfly *Lepidostoma* in our count data as a result of the cage treatment, it was already observed in >85% of DNA samples from open cages, and consequently a further increase in occurrence was insufficient for the MSAM to infer whether the cage treatment had had a significant impact on their abundance. In principle, study design could be modified using a different sampling area such that $\lambda$ is not saturated (i.e., reduce survey error such that successive occurrences increasingly reflect true abundance; Steenweg et al., 2018), but refining scales to suit one taxon would also result in trade-offs for estimating other taxa in multispecies models (Kéry & Royle, 2015). Alternatively, it may be possible to characterize the abundances of common taxa by combining the insights from an MSAM model with inferences on within-species changes using internal standards in sequencing pipelines (Luo et al., 2022).

A second important feature of the MSAM model is the assumption that individual-level detection probabilities ($p$) are constant. However, the likelihood taxa will be detected via metabarcoding is inevitably lower for the taxa with the smallest proportion of sampled biomass (e.g., Elbrecht et al., 2017; Hajibabaei et al., 2012; Martins et al., 2021). As a result, a concern emerges that detection frequency of some members of the community may be determined by their dilution relative to the broader community, and not as a function of their abundance. Clearly, the relative efficiencies of sequencing protocols for taxa are composition-independent (McLaren et al., 2019) but false absences could occur in diverse community samples where sequencing effort has been insufficient to discover all taxa present, or because, even after homogenization, a subset of a sample may not include the DNA of the least abundant taxa. Indeed, we assume the invertebrates detected in each sample were present in our sampled area, and not incidentally captured as environmental DNA (eDNA), because the DNA of organisms in bulk samples will heavily outweigh other traces (Majaneva et al., 2018). Changes in mean abundance could still be inferred using replicate detections from eDNA samples (e.g., soil), but this may not be appropriate where detectability may be influenced by multiple factors other than abundance (e.g., flow and temperature in aqueous eDNA samples; Cristescu & Hebert, 2018). Rarefaction of taxa with sequencing depth (Supplement S1, Figure S3) may identify low sequencing depth, and turnover among replicate extracts could indicate incomplete detection of DNA within a given field sample (Yang et al., 2021). In principle, hierarchical models can account for detection error at both field sampling and laboratory processing stages (Diana et al., 2022), but replicates of the latter were not available in this study. Therefore, in principle the MSAM could mis-specify parameter estimates for taxa already close to their limit-of-detection due to low biomass, and whose changes in abundance are consistently accompanied by confounding changes in overall community biomass. Regardless of the sensitivity of DNA-based methods, users should remain aware that identifying changes in the abundances of the rarest taxa, which contribute least to the fitting of the model, is inherently difficult (Jeliazkov et al., 2022). Given that metabarcoding provides us with the opportunity to observe whole assemblages simultaneously, we suggest ecologists focus on the collective distribution of responses, and only interpret taxon-specific coefficients where other checks support the model's assumptions.

## 4.3 | Monitoring with DNA

Ecological count data are often highly variable, and stream invertebrates are no exception (Canton & Chadwick, 1988; Downes

et al., 1993). Large sample sizes are required to make statistically robust estimates of abundance and spatial clustering (Egglishaw, 1964, 1969; Elliott, 1971, 2002). To accurately determine what the true abundances of any invertebrate taxa are in streams using direct count data is challenging, and reflects the largely unacknowledged fact that traditional multispecies morphological identification and counting of specimens from area-limited samples is an imprecise quantitative approach (e.g., Doberstein et al., 2000; Hawkins et al., 2000). Stochastic variation among counts may even reduce a user's power to discriminate ecological condition among sites (Bush et al., 2019). Despite this understanding, and a growing body of evidence to show occurrence data are sufficient and potentially more effective at detecting changes in habitat condition (Beentjes et al., 2018; Buchner et al., 2019; Serrana et al., 2022), the application of metabarcoding to support more effective biomonitoring has been hindered by practitioners and legislation demanding authorities collect abundance information (e.g., European Water Framework Directive; Leese et al., 2018). Current biomonitoring practitioners should instead ask themselves if they really need abundance data to answer simple questions based on variations in community composition.

Our study demonstrates how managers and scientists could identify community-wide and taxonomically resolved shifts in abundance. Although the costs of replication could limit application at large scales, as discussed, metabarcoding is likely to improve the needs of most monitoring schemes. Instead, inferences from MSAMs based on detection frequency are likely to be most suited to applications at local scales (e.g., environmental impact assessment monitoring: Curry et al., 2020), situations where quantitative information would accelerate our understanding of ecological responses prior to exclusion (e.g., pollution sensitivity; Liess & Ohe, 2005), or studies to understand how function scales with dominance (Dangles & Malmqvist, 2004). Importantly, consistent identification using DNA metabarcoding will allow quantitative information developed locally to readily inform interpretation of monitoring across all scales.

## AUTHOR CONTRIBUTIONS

A. Bush, Z. Compson and D. J. Baird conceived and designed the study. A. Bush, Z. Compson, N. K. Rideout, B. Levenstein, M. Kattilakoski and W. A. Monk conducted the field and laboratory work. M. Hajibabaei and M. T. G. Wright performed DNA extraction, amplification, sequencing and bioinformatics. A. Bush performed all analyses and wrote the first draft of the manuscript. All authors contributed with subsequent manuscript revisions.

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## CONFLICT OF INTEREST STATEMENT

MH is the founder and CSO of eDNAtec Inc. All authors declare no conflict of interest with this publication.

## DATA AVAILABILITY STATEMENT

Raw sequence reads and sample metadata are deposited in the SRA (BioProject PRJNA809203). The code used to run the multispecies abundance model is provided in Supplement S1.

## BENEFIT-SHARING STATEMENT

Benefits from this research accrue from the sharing of our data and results on public databases as described above and the evidence this provides for statistical inference of ecologically relevant dynamics.

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## SUPPORTING INFORMATION

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