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ORIGINAL ARTICLE

Combining sampling gear to optimally inventory species highlights the efficiency of eDNA metabarcoding

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Abstract

Biodiversity surveys may require the use of multiple types of sampling gear to maximize the efficiency of species detections, yet few studies have investigated how to optimally distribute effort among gear. In this study, we conducted eDNA metabarcoding and capture-based sampling surveys (electrofishing, fyke netting, gillnetting, and seining) to sample fish species richness in a large northern temperate lake. We evaluated the success of the sampling methods individually and in combination to determine the allocation of effort and cost across sampling gear that provides the optimal approach for lake-wide species inventories. We found that eDNA metabarcoding detected more species than any other sampling method, including 11 species that were not detected with any capture-based approach. Optimal gear combination analyses revealed that detected species richness is maximized when most of the effort or budget is allocated to eDNA metabarcoding, with smaller allocations to seining and fyke netting. eDNA metabarcoding and capture sampling gear showed similar patterns of spatial heterogeneity in the fish community across habitat types, with pelagic samples forming a group that was distinct from nearshore samples. Our results indicate that eDNA metabarcoding is a rapid and cost-efficient tool for biodiversity monitoring and that assessing the complementarity of multiple sampling types can inform the development of optimal approaches for measuring fish species richness.

KEYWORDS
biodiversity, cost efficiency, environmental DNA, fisheries, optimal gear combination, sampling effort, species richness

1 | INTRODUCTION

Comprehensively documenting the status and trends of biodiversity is foundational to ecological research and achieving conservation goals (Margules & Pressey, 2000). However, biodiversity assessments may not fully capture the species richness of an ecosystem, particularly in aquatic ecosystems where heterogeneous and complex habitats can be challenging to sample (Gotelli & Colwell, 2001; Vaux et al., 2000). Such surveys may be limited by time or cost, resulting in incomplete species inventories that are spatially, temporally, or taxonomically biased. Inadequate assessments of the complete species assemblage may hinder efforts to conserve species, particularly

those that are rare, elusive, or cryptic (Fleishman et al., 2006; Gotelli & Colwell, 2011). To efficiently obtain unbiased species richness estimates, sampling survey designs should maximize the number of species detected per unit of sampling effort or cost.

The sampling methods and protocols used to measure species presence and richness are typically chosen based on a compromise between sampling logistics, efficiency, precision, and accuracy (Hughes & Peck, 2008). In aquatic ecosystems, fish species may be sampled using a variety of capture methods in which individuals are caught and identified, most commonly through electrofishing, netting, and trapping (Bonar et al., 2009). Non-capture survey methods, including underwater bioacoustics, visual observations, and environmental DNA (eDNA), have also emerged as effective approaches for detecting species in aquatic environments (Deiner et al., 2017; Ellender et al., 2012; Jerde et al., 2019; Linke et al., 2018; McElroy et al., 2020; Olds et al., 2016; Smart et al., 2016; Thomsen & Willerslev, 2015). eDNA metabarcoding—which uses high-throughput sequencing to detect the genetic material shed by species—is a highly sensitive approach for surveying species richness that can result in higher detection probabilities (Valentini et al., 2016) and greater species richness estimates (Afzali et al., 2021; Miya et al., 2015; Pont et al., 2018) compared with capture-based survey methods. Given the breadth of aquatic species survey approaches, understanding the limitations and complementarity of each sampling type is an essential component in the development of a comprehensive plan to estimate species richness.

Sampling biases exist for all types of sampling gear with varying degrees of selectivity and specificity, both of which may depend on the biological and physical characteristics of the sampled environment (Pierce et al., 2001). For instance, passive capture sampling gear such as gillnets may overestimate the abundance of large-bodied, fast-swimming species while large mesh sizes may systematically underestimate or exclude small-bodied species (Prchalová et al., 2008; Rudstam et al., 1984). On the contrary, active capture gear such as electrofishing and beach seining do not efficiently sample benthic species or fast-swimming species that may avoid or escape capture (Mahon, 1980). eDNA metabarcoding is similarly subject to biases in species detections, including those arising from incomplete reference databases favoring certain taxonomic groups (Beng & Corlett, 2020) and primer amplification bias (i.e., the preferential amplification of the sequences of some taxa over others) (Kelly et al., 2019). Because the nature and severity of sampling biases differ among sampling approaches, species inventories in aquatic systems are often completed with survey effort allocated across multiple types of gear to account for different habitat types, species, size selectivity, and efficiency of sampling (Fischer & Quist, 2014). However, it remains unclear how available funds and effort should be distributed among survey approaches to maximize species detections.

In this study, we compare estimates of fish species richness and community composition between multiple capture sampling methods and eDNA metabarcoding. Our objective is to examine the efficiency of the survey approaches independently and in combination, and to use this information to determine how to optimally allocate sampling effort. Following intensive sampling of a fish community with eDNA metabarcoding, electrofishing, fyke netting, gillnetting, and seining, we compare species inventories among gear types and estimate the optimal combination of sampling gear that will maximize the number of species detected across a range of sampling budgets and effort constraints. We also investigate biases in species detections for all sampling types at the lake-wide, habitat-specific (nearshore vs. pelagic), and site scales to help inform the spatial resolution achieved by different biodiversity survey approaches.

2 | MATERIALS AND METHODS

2.1 | Study area

Oneida Lake (New York, USA) is a large, shallow mesotrophic lake with a total surface area of 206.7 km^2 and a maximum depth of 16.8 m (mean depth = 6.8 m; Figure 1). As the largest lake within the borders of New York State, Oneida Lake supports several water-oriented recreational activities as well as an important sport fishery. Research at the Cornell Biological Field Station (CBFS), supported by Cornell University and the New York Department of Environmental Conservation, has focused intensively on the fisheries and limnology of Oneida Lake since the station was established in 1956. The resulting datasets are recognized as some of the best long-term data series in aquatic ecology. As of 2016, historical records documented 85 species of fish in Oneida Lake and its tributaries, comprising 52 genera and 22 families (Jackson, 2016). Of these, 77 species have been documented in the lake or its tributaries since 1990.

2.2 | Sampling surveys

Standardized monitoring surveys of Oneida Lake fish populations are conducted annually at the CBFS using a combination of capture methods including electrofishing, gillnetting, fyke netting, and seining. Although all captured species are identified, each capture survey method is focused on sampling either the nearshore fish community (electrofishing, fyke netting, and seining) or the mid-lake, or pelagic, fish community (gillnetting). Electrofishing surveys were conducted from June 12 to 28, 2017 at each of eight transects distributed around the perimeter of the lake and representing typical shoreline habitats (Figure 1). Each electrofishing transect survey involved two 15-min runs targeting all species for a total of 30 min per site. Fyke nets were deployed between September 19 and 27, 2017 at 18 nearshore sites that were selected to be representative of the proportions of common substrates in nearshore habitats around the entire perimeter of the lake. Each site was sampled for 24 h with 2 fyke nets, where each net was comprised of a 0.9 m × 1.5 m frame fitted with either 12.7 or 5 mm delta knotless mesh. Seine sampling was performed at nine nearshore sites in the southern lake near the CBFS harbor. Each site was sampled monthly from July–September

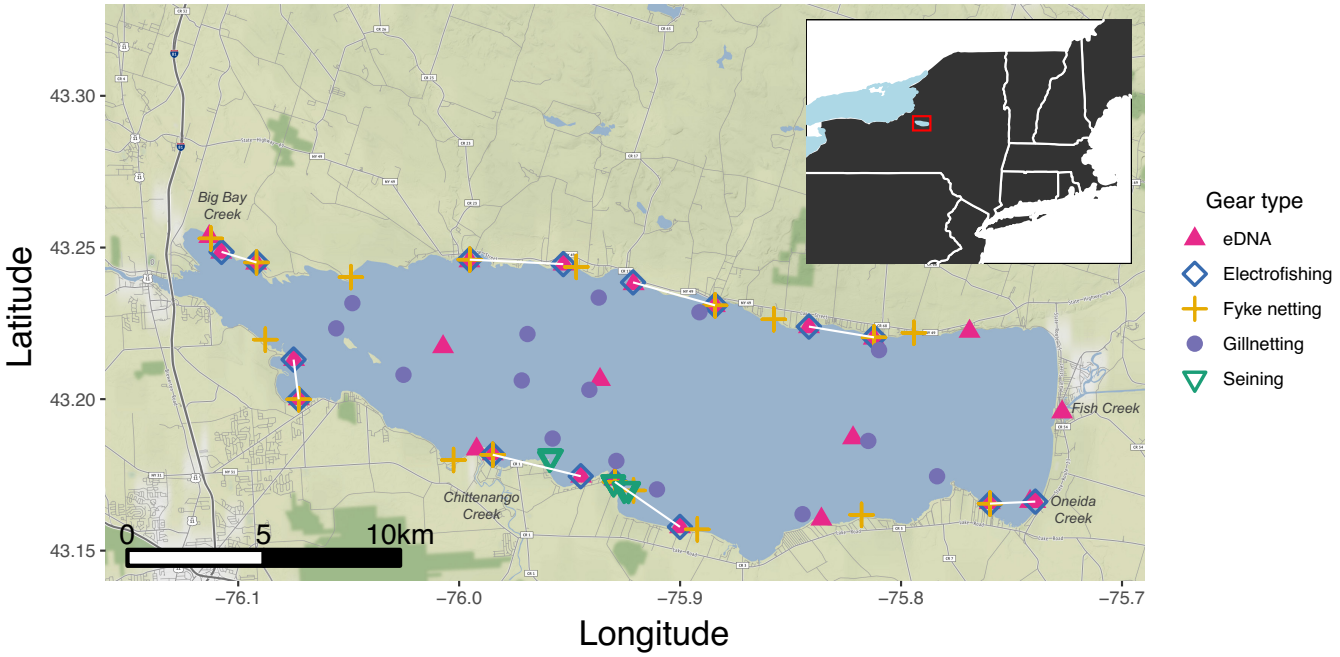

FIGURE 1 Map of eDNA sampling and capture gear survey locations in Oneida Lake, NY, USA. Colors and symbols represent the sampling locations for different gear types. White lines connect the approximate start and end points of each electrofishing transect. Sampled tributary inlets are labeled.

2017 using a 23 m beach seine with 6.35 mm mesh. Species lists were compiled at the site level for each survey type by combining species identifications for the two survey runs (electrofishing), two nets (fyke netting), and three sampled months (seining).

Pelagic gillnet surveys were used to sample the pelagic fish community at a different standard site each week for 15 consecutive weeks from June 8 to September 12, 2017. A multifilament gillnet was deployed on the lake bottom overnight and consisted of four 45.75 m × 1.83 m variable mesh nets sewn together to form a gillnet 183 m in total length. Each of the four nets contained six panels with graded mesh sizes of 38, 51, 64, 76, 89, and 102 mm.

We sampled eDNA in both nearshore and pelagic habitats on June 13, 2017. Nearshore eDNA samples were collected at the start and end of each electrofishing transect (16 locations), as well as at the inlets of Oneida Creek, Fish Creek, Big Bay Creek, and Chittenango Creek (Figure 1). Two additional nearshore samples and three pelagic samples were collected to total 25 eDNA samples. At each sampling site, we collected a water sample from a boat by submerging a sterilized 2-L wide-mouth Nalgene container (Thermo Fisher Scientific) 0.3 m beneath the water's surface. Midway through sampling, a field blank was taken by filling a Nalgene container with distilled water. All samples were stored on ice for up to 12 h until filtration on the main Cornell University campus (Ithaca, NY) in a laboratory built specifically for eDNA sample analysis consistent with best practices (Goldberg et al., 2016).

Each water sample (1.75 L) was filtered onto 1 μm cellulose-nitrate filters (47 mm diameter; Whatman, GE Healthcare) using a vacuum filtration system. A filtration blank of 1.75 L of distilled water was also processed. Membranes from each sample were immersed in 700 μl Longmire's buffer and stored at −80°C until DNA extraction using DNeasy Blood and Tissue extraction kits (Qiagen Inc.) with an optimized protocol for eDNA (Spens et al., 2017). If multiple filters were required to filter the full volume of water for a sample, filters were combined in the same microtube prior to extraction.

2.3 | Library preparation

A NGS library targeting a variable region of the 12S rRNA gene (163–185 bp) was prepared in a two-step process using universal fish primers (MiFish-U-F: GTCGGTAAAACTCGTGCCAGC; MiFish-U-R: CATAGTGGGGTATCTAATCCCAGTTTG; Miya et al., 2015) with either two or three degenerate bases (N) added to increase amplicon heterogeneity. First-stage DNA amplification was conducted in triplicate for each sample and negative control (i.e., field, filtration, extraction, and PCR blanks) in PCR volumes of 20 μl containing 4 μl x5 GoTaq flexi buffer (Promega), 0.5 μM of each MiFish-U primer (forward and reverse), 0.2 mM of each dNTP, 2 mM $MgCl_2$, 0.15 μl GoTaq HotStart polymerase, 0.4 μg/μl bovine serum albumin, and 4 μl of eDNA extract as template. The PCR protocol included the following cycling conditions: initial denaturation at 95°C for 2 min, 40 cycles of 95°C for 30 s, 65°C for 60 s, 72°C for 45 s, and a final extension at 72°C for 5 min. PCR products were then pooled by sample and tagged in a second-stage PCR using Illumina Nextera XT tags. The resulting PCR products were purified and size-selected using Agencourt AMPure XP beads (reaction ratio AMPure beads 0.9: PCR product 1; Beckman Coulter Genomics), and the concentration of each library was estimated using the Qubit dsDNA Broad Range Kit and Qubit 2.0 fluorometer (Life

TABLE 1 Estimated effort (person-hours of work) and cost ($USD) required to obtain one sample for each sampling gear type.

	eDNA (in-house)	eDNA (external)	Electrofishing	Fyke netting	Gill-netting	Seining
Effort (work hours)	2.0	–	4	3.6	13	2.5
Cost ($USD)	152	284	280	92	320	70

Technologies). The libraries were diluted to 18 nM and paired-end sequenced on an Illumina MiSeq sequencing platform with the NextSeq 500 Mid output kit (paired-end 2×150 bp) by Cornell University's Institute of Biotechnology Genomics Facility. Illumina raw sequence data are available from the Dryad Digital Repository.

Sequence data for the 12S gene region are available in public sequence databases (e.g., National Center for Biotechnology Information; NCBI) for most fish species documented in the Oneida Lake historical datasets. We supplemented the reference database by sequencing tissues from six species collected from Oneida Lake: American eel (*Anguilla rostrata*), brook silverside (*Labidesthes sicculus*), longnose gar (*Lepisosteus osseus*), greater redhorse (*Moxostoma valenciennesi*), round goby (*Neogobius melanostomus*), and logperch (*Percina caprodes*). DNA was extracted from fin clips sampled from 2 to 3 individuals per species using manufacturer instructions from a Qiagen DNeasy Blood & Tissue kit and amplified using PCR conditions described above, with DNA template reduced to 2 μl per reaction. PCR products were purified, quantified, diluted to 34.2 ng per sample, and sequenced at Cornell University's Genomics Facility's Full-Service Sanger Sequencing.

2.4 | Bioinformatics

Degenerate bases and adaptors were trimmed from demultiplexed paired reads and sequences <35 bp were removed using Trimmomatic 0.33 (Bolger et al., 2014). Sequences were analyzed using DADA2 1.16 (Callahan et al., 2016), which involved removing forward and reverse primers, trimming reads to 126 bp, and discarding sequences with expected number of errors >2 (EE, calculated based on Phred scores). Sequences were then denoised using the DADA2 error filtering algorithm, which identifies and discards erroneous sequences based on error models learned from each FASTQ file (i.e., sample). Forward and reverse reads were merged with a minimum overlap of 20 bp and a maximum of one accepted error in the overlap region, and putative chimeras were removed. The resulting dataset contains 12S amplicon sequence variants (ASVs) and the associated read counts for each sample.

Taxonomic assignments for each ASV were obtained using the BLASTn algorithm (Camacho et al., 2009) and the nucleotide and taxonomy (nt/taxdb) databases from NCBI (Benson et al., 2005; Federhen, 2012). We retained the top five target sequence matches for each ASV and assigned species-level taxonomy to ASVs that matched a single species with a sequence identity of ≥98%. If multiple species matched the query sequence equally well, ASVs were assigned the lowest common taxonomic rank (genus or family) among the target sequences with equal percent identity. ASVs that could not be classified to the family level were excluded from further analysis.

ASVs with taxonomic assignments were filtered by removing ASVs with read counts <0.1% of all reads in a sample or with fewer than the average number of non-zero reads summed across all negative controls (23 reads). Non-target species including non-fish and marine species were removed from the dataset.

2.5 | Optimal gear combinations

Species accumulation curves and optimal gear combinations were derived using information about the sampling effort (work person-hours) and cost ($USD) required to sample a single site for each gear type (Table 1, Appendix S1). To provide results that would be applicable beyond our particular study, we made several simplifying choices. We assumed that all sampling took place within fully equipped facilities with streamlined protocols; therefore, we did not allocate effort or cost to capitalization, other startup costs, or methods development. Per-sample effort estimates were based on estimated effort for the field and laboratory work conducted for this study and includes time spent on sample collection, filtration, and laboratory and sequence processing (eDNA), as well as deploying nets and identifying species (capture gear). Because eDNA cost estimates can be highly variable depending on the protocol and number of samples processed, we calculated costs using two approaches: (1) based on a published set of laboratory cost estimates in which all sampling, laboratory, and sequencing is completed by the research institution (Bálint et al., 2018); and (2) based on personal communication with a commercial eDNA metabarcoding service (Smith-Root, Inc.) in which sampling is completed by the research institution and samples are processed by an external company. These cost scenarios are hereafter referred to as "in-house" and "external" costs, respectively. Cost estimates for capture methods included rates charged by the CBFS for boat usage, which covers boat maintenance, fuel, and depreciation. Both eDNA and capture gear sampling cost estimates also included personnel costs (Table 1). A detailed breakdown of estimated sampling effort and costs can be found in Appendix S1.

To determine the combination of gear that will maximize species detections at a given amount of effort or cost, we conducted sample-based rarefaction for single- and combined-method surveys. For a dataset with A samples that detects a total of S_{obs} species, the expected number of species observed in a samples is:

$$E\left[S(a)\right] = \sum_{i=1}^{S_{obs}} \left(1 - \frac{\binom{A-n_i}{a}}{\binom{A}{a}}\right) \quad (1)$$

TABLE 2 Lake-wide and habitat-specific species richness for the historical Oneida dataset (since 1990), eDNA metabarcoding, and capture sampling gear (electrofishing, gillnetting, fyke netting, and seining).

Habitat	Historical	eDNA	Capture gear (combined)	Capture gear			
				Electro-fishing	Gill-netting	Fyke netting	Seining
Lake-wide	77	41	36	31	15	27	27
Nearshore	–	40	34	31	–	27	27
Pelagic	–	15	15	–	15	–	–

Note: Types of capture gear are displayed both separately and combined (i.e., the number of unique species identified among all capture gear).

where n_i is the number of samples in which the *i*th species is observed (Colwell et al., 2012; Ugland et al., 2003). For each species, Equation 1 calculates the probability of detection as the complement of non-detection. This sample-based rarefaction interpolates the species accumulation curve between $a = 0$and $a = A$ by assuming samples of a single type are chosen randomly without replacement. We generalize this model to combined-method surveys. Consider a dataset with $k = 1, 2, \ldots, m$ different methods, each containing A_k samples. The expected number of species observed in a survey containing a_1 samples of method 1, a_2 samples of method 2, ..., and a_m samples of method m is:

$$E\left[S(\boldsymbol{a})\right] = \sum_{i=1}^{S_{obs}} \left(1 - \prod_{k=1}^{m} \frac{\binom{A_k - n_{ik}}{a_k}}{\binom{A_k}{a_k}} \right) \quad (2)$$

where $\boldsymbol{a} = (a_1, a_2, \ldots, a_m)$ defines how the survey allocates samples among the available methods and n_{ik} is the number of samples of method k in which the *i*th species is observed. In this instance, non-detection requires that a species is not detected in any of the m independent sampling methods.

To find a species accumulation curve for optimal combined-method surveys, we first found the expected number of species observed for all possible combinations of sampling methods up to some maximum total effort or cost, C_{max}. That is, if we let C_k be the cost per sample of method k and $C(\boldsymbol{a}) = \sum_{k=1}^{m} a_k C_k$ be the cost of implementing a survey with sample allocation $\boldsymbol{a}$, we evaluated Equation 2 on the set G of all possible values of $\boldsymbol{a}$ such that $C(\boldsymbol{a}) \leq C_{max}$. We then found all non-dominated sample combinations, that is, all $\boldsymbol{a}$ in G such that there is no $\boldsymbol{x}$ in G where both $C(\boldsymbol{x}) \leq C(\boldsymbol{a})$ and $E\left[S(\boldsymbol{x})\right] \geq E\left[S(\boldsymbol{a})\right]$, with at least one of the inequalities being strict. These combinations comprise the Pareto frontier, which describes an effort-based species accumulation curve for surveys that use optimal combinations of sampling methods. Optimal gear combinations are defined as those on the Pareto frontier of all possible combinations of samples from the selected gear, up to a total effort of 100 work hours or total cost of $4000.

2.6 | Data analysis

All statistical analyses were performed using R.4.0.4 (R Core Team, 2021) using package *vegan* 2.4-6 (Oksanen et al., 2013). Because sampling approaches may vary in their efficiency in different habitat types, we compared species inventories at lake-wide, habitat-specific, and site scales. For the lake-wide species inventory, we compared species richness observed with eDNA metabarcoding to richness observed by each capture sampling gear alone as well as all types of capture sampling gear combined. We examined the overlap in lake-wide species detections for all combinations of sampling gear and the Oneida Lake historical species records using the *UpSetR* package (Gehlenborg, 2019).

Species inventories at the scale of habitat type involved grouping all sampled sites into nearshore and pelagic habitats and calculating species richness for each sampling method. eDNA samples covered both habitat types, whereas gillnets sampled only pelagic habitats and electrofishing, fyke nets, and seine nets sampled only nearshore habitats. Nonmetric multidimensional scaling (nMDS) based on species presence-absence (*Sørensen* similarity *index*) was used to visualize differences in species composition among habitat types using either eDNA metabarcoding or a combination of all capture gear. For nearshore and pelagic habitats, we calculated species occupancy (i.e., the proportion of sampling sites in which a species is detected) and tested the ordinal association between species occurrence (i.e., presence in a sample) in eDNA samples and capture gear using Kendall's rank correlation coefficient.

Because eDNA samples were collected at the start and end of each of eight electrofishing transects, site-level comparisons are only conducted between eDNA and electrofishing datasets. For this analysis, we combined species identifications from the two eDNA samples taken per electrofishing transect. We tested for correlations in species occupancy using Spearman's rank-order tests.

3 | RESULTS

3.1 | Species inventory comparisons

The eDNA sequencing library contained 6,666,951 raw reads, of which 5,735,219 remained after demultiplexing and adaptor trimming. Filtering, merging, and chimera removal in the DADA2 pipeline resulted in further read removal, leaving a total of 3,814,019 reads. Of the 2982 unique ASVs identified in the eDNA metabarcoding data, 2310 were assigned taxonomy at the species level, 651 at the genus level, and 20 at the family level. We were able to resolve all but one sequence to at least the family level.

Across all eDNA samples, a total of 41 unique species and 7 unique genus- or family-level taxonomic assignments were identified. Total species richness detected in eDNA samples was higher than electrofishing (total of 31 species), gillnetting (15), fyke netting (27), and seining (27), as well as all capture sampling gear combined (36; Table 2; Table S1). Eleven species known to be present in Oneida Lake based on the historical dataset were detected with eDNA but none of the capture sampling gear (Figure 2; Figure S1; Table S1). All species detections in capture sampling gear were redundant with at least one other capture sampling gear apart from a single species detected only by seining: *Hybognathus hankinsoni* (brassy minnow). Six species were detected in capture sampling gear but not in eDNA metabarcoding: *Cyprinella analostana* (satinfin shiner), *H. hankinsoni* (brassy minnow), *L. osseus* (longnose gar), *Pimephales notatus* (bluntnose minnow), *Pimephales promelas* (fathead minnow), and *Pomoxis nigromaculatus* (black crappie; Figure 2; Figure S1; Table S1). Of these, four could be linked to ASVs that were present in at least one eDNA sample but were either filtered out during data processing (*P. notatus*, *P. promelas*, and *P. nigromaculatus*) or were ambiguous at the species level during taxonomic assignment (*L. osseus*).

3.2 | Optimal gear combinations

For any given sampling effort, eDNA metabarcoding detected more taxa than capture sampling gear (Figure 3a). At low levels of sampling effort (<20 work hours), seining accumulated species more quickly than electrofishing, yet the total number of species detected using electrofishing exceeded all other types of capture sampling gear when sampling effort increased. Considering optimal combinations of sampling gear, eDNA alone detected nearly as many taxa as all types of gear combined across all sampling efforts (gray line; Figure 3a). The stacked bar plot denoting the optimal distributions of sampling effort among gear types shows that a combination of seining and eDNA metabarcoding maximized species detections up to 40 work hours; above this level of total effort, it was beneficial to supplement with small amounts of fyke netting (Figure 3d). At 100 h, the highest total effort that we considered, eDNA is allocated 51% of the effort, while 23% and 26% of the total effort is allocated to seining and fyke netting, respectively. This distribution of effort is equivalent to 25 sites sampled with eDNA, 9 sites with seining, and 7 sites with fyke netting. Electrofishing and gillnetting never detected

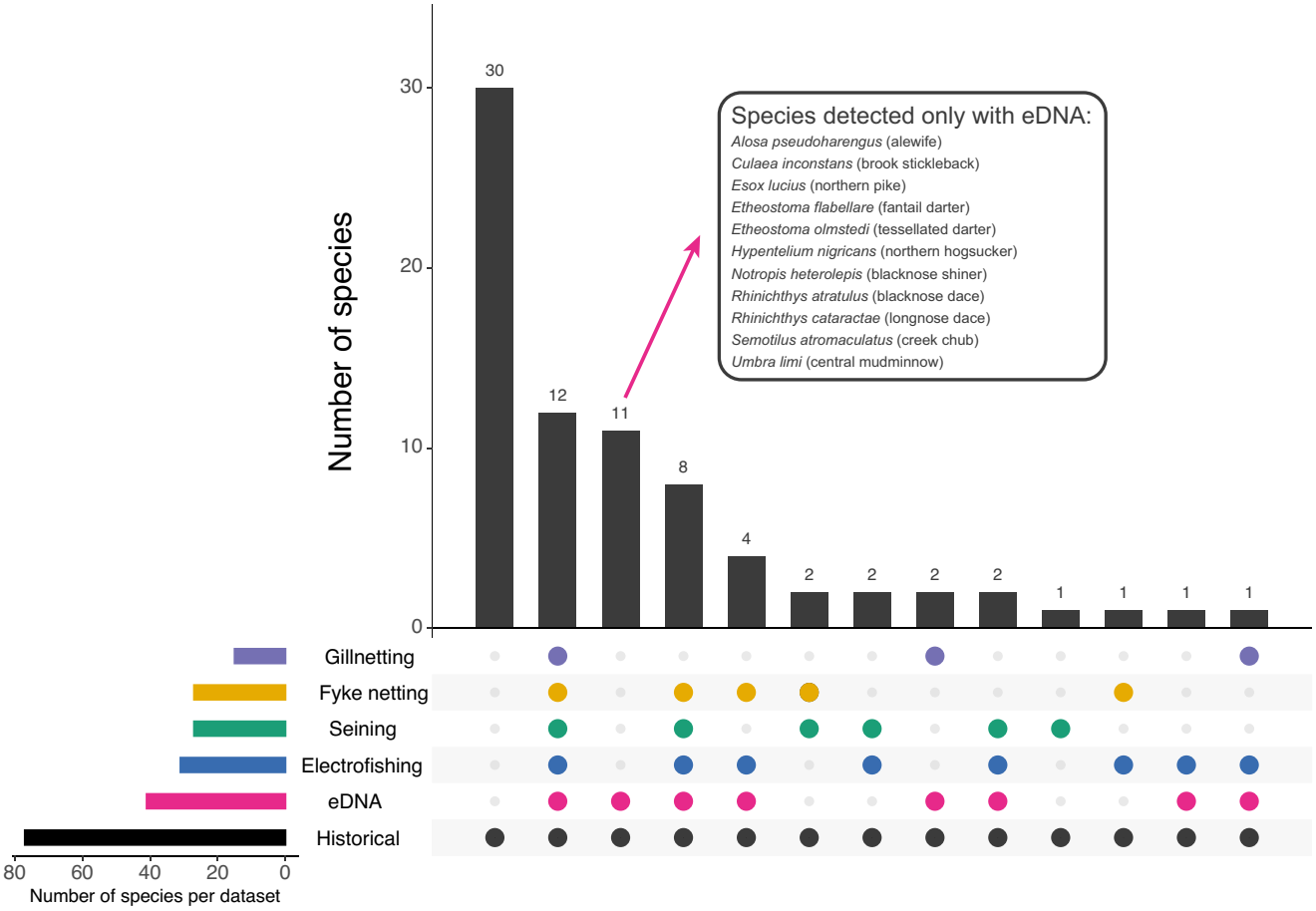

FIGURE 2 Number of detected species uniquely shared by particular combinations of sampling approaches (eDNA metabarcoding, electrofishing, seining, fyke netting, and gillnetting) and the comprehensive historical species list for Oneida Lake. Dots denote combinations of gear types, and the number of taxa detected by all gear types in a combination (and not by any other gear) is represented by the height of the bar. Combinations of gear types that did not share unique species are not represented in the figure.

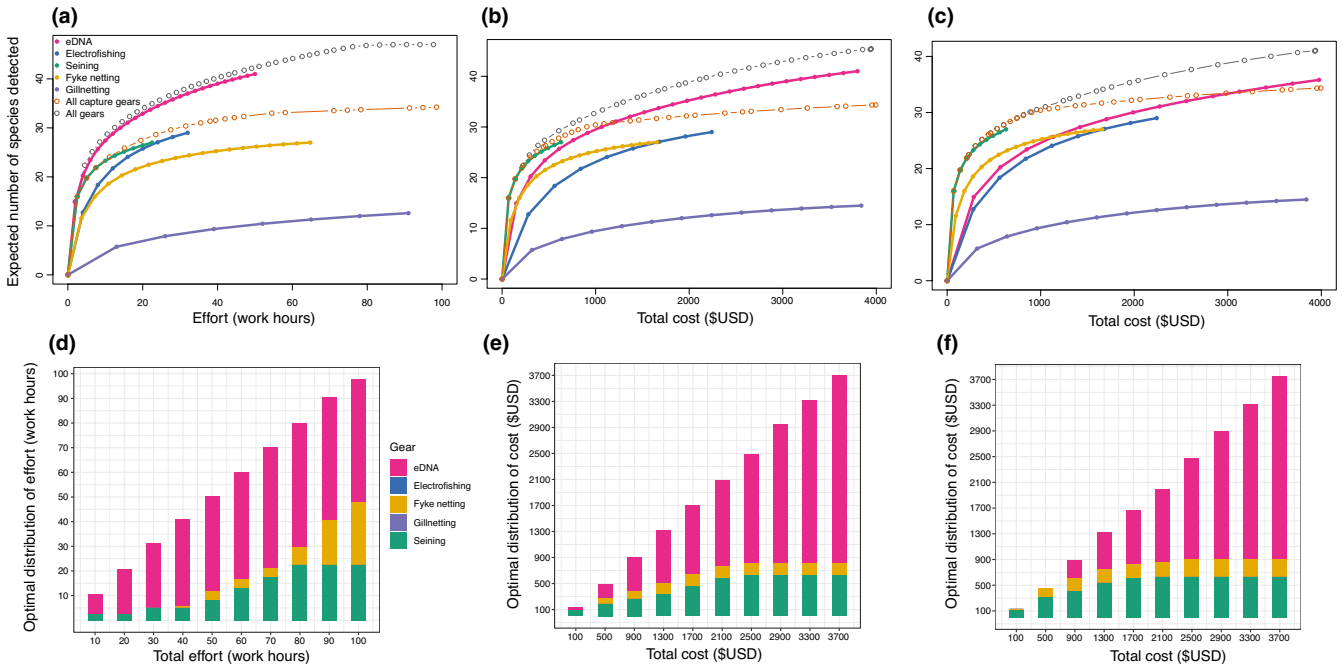

FIGURE 3 Species accumulation curves based on (a) effort (work hours); (b) cost (in-house eDNA); and (c) cost (external eDNA) for each sampling method alone (solid circles) and for optimal combinations of all capture sampling gear (excluding eDNA; hollow orange) and all methods (including eDNA; hollow gray). Stacked bar plots based on (d) effort; (e) cost (in-house eDNA); and (f) cost (external eDNA) indicating the optimal allocation of effort among gear types. For a given total effort, the different colors in each bar indicate how to allocate samples among the five possible types of gear (eDNA, electrofishing, fyke netting, gillnetting, and seining) in a way that maximizes the expected number of species detected.

new species at a rate faster than other gear types could, so they were not allocated work hours at any level of total effort.

In the cost-based analyses of gear optimization, seining accumulates species more quickly than eDNA metabarcoding, with eDNA surpassing seining in the total number of species detected if sampling costs exceed $760 (in-house; Figure 3b) or $1420 (external; Figure 3c). If eDNA sampling costs are calculated based on in-house estimates, the optimal distribution of cost is similar to the sampling effort allocations, with most of the budget allocated to eDNA metabarcoding (78%, equivalent to 19 sites) and a smaller proportion allocated to seining (17%, 9 sites) and fyke netting (5%, 2 sites; Figure 3e). However, if eDNA sampling costs are calculated based on external estimates, eDNA metabarcoding is not part of the optimal allocation of costs until the budget reaches $900 (Figure 3f). Under this scenario, seining and fyke netting dominate the optimal budget allocation when the budget is small, while eDNA consumes 76% (equivalent to 10 sites) of the cost when the maximum budget is reached. The total cost allocated to seining and fyke netting remains low across all sampling budgets, with 17% of the maximum budget allocated to seining (9 sites) and 7% allocated to fyke netting (3 sites). Although species detected with eDNA metabarcoding accumulate more slowly when eDNA is more costly (external scenario; Figure 3c) than when eDNA costs are lower (in-house scenario, Figure 3b), the sampling effort allocated to eDNA is similar in both scenarios if the maximum budget is consumed (Figure 3e–f). Thus, as the sampling budget increases, the relative cost of eDNA does not have as strong of an influence on the optimal distribution of sampling costs among gear types. Neither electrofishing nor gillnetting appeared in the optimal gear combinations for either cost-based optimization.

In all three optimization scenarios, the amount of effort or cost allocated to seining was limited by the number of sampled sites; the amount of seining in a given gear combination cannot exceed the available number of sites sampled by seining in the study. The maximum number of sites sampled by eDNA was also reached in the effort optimization scenario, but not in either cost scenario. Fyke netting did not reach the maximum number of sampled sites at any level of effort or cost.

3.3 | Habitat-scale and site-level species detections

At the habitat scale, nMDS reveals clear differences in community composition between the species sampled in nearshore versus pelagic habitats in both eDNA metabarcoding (stress = 0.11; Figure 4a) and capture-based sampling approaches (stress = 0.16, Figure 4b), with no overlap in the convex hulls surrounding the pelagic and nearshore eDNA samples. When comparing species occurrences between eDNA metabarcoding and capture methods, there was a significant correlation in nearshore habitats (Kendall's tau = 0.48, $p < 0.001$) but not in pelagic habitats (Kendall's tau = 0.27, $p = 0.15$; Figure S2).

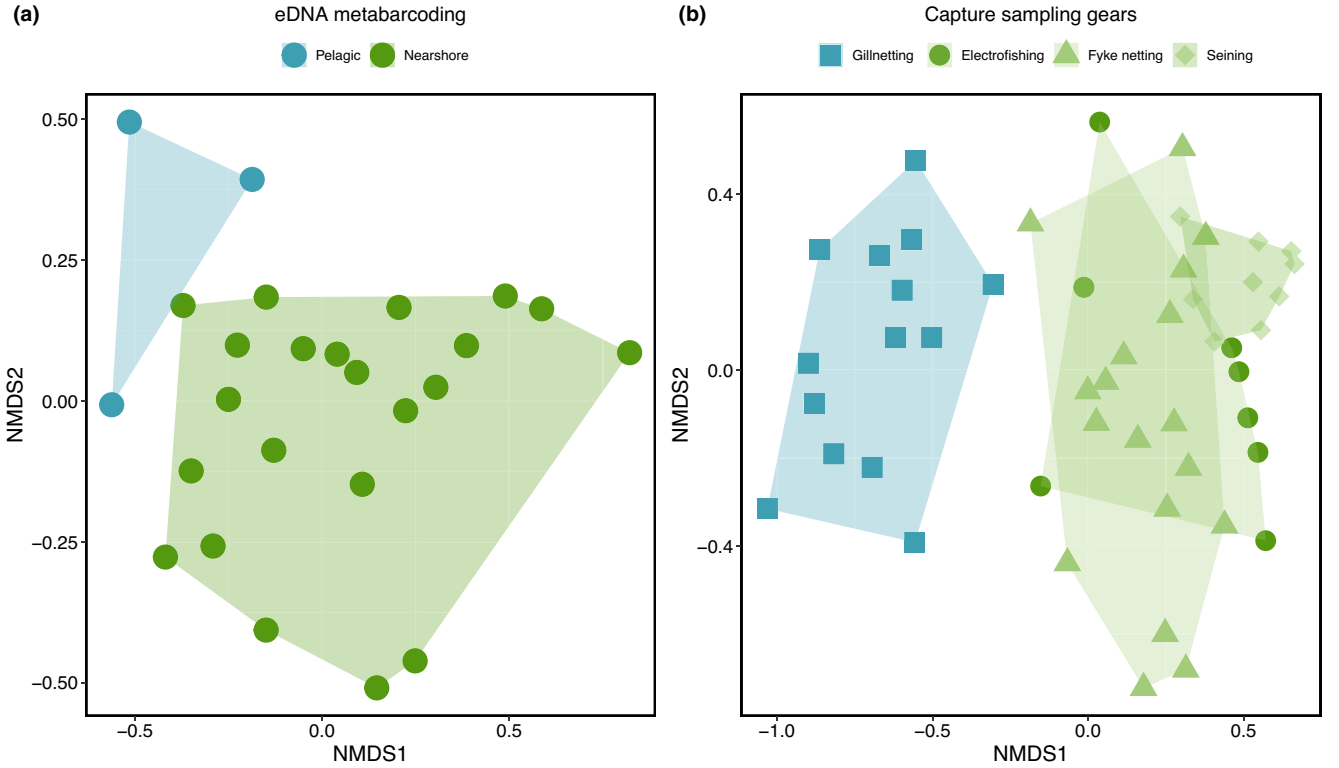

FIGURE 4 Nonmetric multidimensional scaling (nMDS) ordination plots constructed using Sørensen indices of species presence-absence to illustrate habitat differences for eDNA metabarcoding and capture sampling gear. Colors represent habitat types, with (a) eDNA samples taken in both habitats, and (b) capture sampling methods conducted in pelagic (blue; gillnetting) and nearshore habitats (green; electrofishing, fyke netting, and seining). Convex hulls were constructed to encapsulate all points for each habitat and sampling type.

For the site-level comparisons between eDNA and electrofishing, eDNA detected more species than electrofishing at six of eight transects (Figure S3), with higher mean species richness (mean = 19.25, SD = 4.80) than electrofishing (mean = 14.75, SD = 3.84) across all sites, although this difference was not significant (Wilcoxon test, $p = 0.102$). The number of species detected per site from eDNA metabarcoding and electrofishing was not significantly correlated ($r = -0.11$, $p = 0.79$).

4 | DISCUSSION

Freshwater ecosystems face a multitude of ecological stressors and conservation challenges that are contributing to a global reduction in freshwater biodiversity (Reid et al., 2019). Monitoring and preventing further species losses in an era of global change requires strategies to efficiently and rigorously obtain information on the status of species and communities. Over the course of an intensive field sampling effort in a large temperate lake, we assessed the congruency and complementarity of eDNA metabarcoding to capture-based survey methods. We found that eDNA metabarcoding detected more species than any other sampling method, including 11 species historically known to occur in the lake that were not detected with any other survey approach. Species accumulation curves rose more rapidly and saturated at a higher number of species for eDNA metabarcoding than any other sampling method, exceeding the number of species detected with all four types of capture sampling gear combined. However, a combination of eDNA metabarcoding and capture gear is expected to detect more species than eDNA metabarcoding alone, suggesting that combined-method approaches are required to maximize species detections.

Although many studies suggest that eDNA metabarcoding can be a powerful addition to common capture-based biodiversity monitoring approaches, the complementarity of survey methods is rarely addressed (Beng & Corlett, 2020). When the goal is to maximize detected species richness, our approach finds the optimal allocation of time or cost among sampling gear, accounting for the efficiency of each sampling gear as well as the distinctiveness of the species detected. We found that combined-method species inventories were most efficient when most of the sampling effort or budget was allocated to eDNA metabarcoding and smaller amounts of effort or budget allocated to seining and fyke netting. This general result held regardless of whether eDNA metabarcoding costs were estimated for in-house laboratory work (lower costs) or with the use of a commercial service (higher costs). Thus, while eDNA metabarcoding is an efficient approach for assessing aquatic species richness in terms of time and cost, the addition of seining and fyke netting is required to optimize species detections. However, other capture-based survey approaches including

electrofishing and gillnetting are less efficient or are redundant with other sampling gear and are therefore not included in the optimal method combinations for any scenario.

While eDNA is a promising tool with which to perform lake-wide species inventories, the spatiotemporal resolution of eDNA-based inferences remains less well characterized. Oneida Lake is a shallow, well-mixed lake, and surface currents and seiches may transport eDNA long distances, resulting in species detections at sites where they may not be physically present (Harrison et al., 2019). Furthermore, because DNA fragments may persist for longer periods of time in sediments than in water (Sakata et al., 2020; Turner et al., 2015), remnant DNA from resuspended sediments in the water column may result in the detection of species historically more abundant in the water body. Although we conducted eDNA sampling during a calm weather period to minimize the influence of DNA from resuspended sediments on species detections, the dynamics of eDNA transport, retention, and resuspension in a water body should be considered when assessing species richness using eDNA approaches.

DNA may also be introduced by external sources, including lake tributaries. While all eDNA samples were collected within Oneida Lake, we collected four eDNA samples near tributary inputs (Figure 1). However, all but two of the 11 species that we detected only with eDNA sampling have been historically documented within Oneida Lake, with only *Notropis heterolepis* (blacknose shiner) and *Rhinichthys atratulus* (blacknose dace) historically found exclusively in Oneida Lake tributaries (Jackson, 2016). Furthermore, both of these species were detected in a single nearshore eDNA sample that collected along the northern shore of the lake and not at a tributary inlet. Therefore, although DNA inputs from tributaries may be possible, we did not detect strong evidence of species detections originating from tributaries in this study.

While the exact physical location of species detected with eDNA is challenging to determine, we nevertheless detected habitat-level differences in species composition that were congruent between capture-based surveys and eDNA metabarcoding. For both eDNA and capture survey methods, sites sampled in the pelagic zone were distinct from those sampled in nearshore habitats (Figure 4a,b). These results support the growing evidence that eDNA metabarcoding can discriminate habitat-specific eDNA signals in a variety of environments and sampling approaches (Gehri et al., 2021; Jeunen et al., 2019; Lawson Handley et al., 2019). However, at the site level, there was little association between eDNA-based and electrofishing-based measures of species richness, possibly due to limitations in the ability of eDNA metabarcoding to detect fine-scale heterogeneity in species richness. Thus, while eDNA metabarcoding may be the most efficient approach for performing lake-wide species inventories and detecting broad community differences among habitat types, capture surveys may provide more information about fine-scale (i.e., site-level) species composition and richness. Capture gear may also be beneficial for gathering additional information that may be difficult to assess using eDNA including species abundance, population age structure, and sex ratios, although developments and refinements in eDNA approaches may allow for such metrics to be evaluated in the future (Lacoursière-Roussel et al., 2016; Yates et al., 2021).

In a single eDNA metabarcoding survey, we detected all but six of the species identified with a combination of four capture sampling gear types throughout an entire sampling season. Upon further inspection, some of these species (*P. notatus*, *P. promelas*, and *P. nigromaculatus)* were present in the raw eDNA metabarcoding dataset but were removed due to low read counts that were indistinguishable from sampling error. Although we supplemented the genetic database with *L. osseus* (longnose gar) 12S sequences, we were nonetheless unable to genetically discriminate between *L. osseus* and *Lepisosteus platostomus* (shortnose gar) in the eDNA metabarcoding dataset, indicating that these species may not be distinguishable using the short segment of the 12S gene region targeted in this study. However, because the range of *L. platostomus* does not extend into the sampled region (Fuller et al., 2022), this species could be excluded if the reference database was restricted to only species known to occur in Oneida Lake. The remaining two species were infrequently detected in the capture sampling gear, with single specimens of *C. analostana* detected by fyke net and seine surveys, and *H. hankinsoni* collected in a single seine sample.

Maximizing the precision and accuracy of species detections from eDNA metabarcoding may require technical improvements and additional research in both the field and laboratory (Ruppert et al., 2019). For instance, some of the differences in species detections between eDNA and capture sampling gear may be due to interactions between DNA and environmental conditions that influence the detection of species (Barnes & Turner, 2015). Because the persistence of eDNA depends on several factors that can vary over time (Barnes et al., 2014; Dejean et al., 2011), there may be considerable measurement stochasticity between sampling events. Repeated sampling through time may prove useful in reducing the associated estimation error and potentially providing a more comprehensive understanding of fish communities at the lake-wide and habitat level (Bista et al., 2017). Larger eDNA sampling volumes and multiple sampling replicates can reduce sampling stochasticity and allow for assessments of species detection probabilities (Evans, Li, et al., 2017). Furthermore, incorporating information about transport distance and hydrology into eDNA analysis may improve the accuracy of habitat- and site-level species detections (Carraro et al., 2018; Harrison et al., 2019). In the laboratory, modifications to the experimental protocol including limiting the number of PCR cycles (Kelly et al., 2019), sequencing samples at greater depth (Alberdi et al., 2018), and analyzing longer or multiple genetic markers (McColl-Gausden et al., 2021; McElroy et al., 2020) can increase taxonomic coverage and reduce taxonomic biases that arise during PCR and sequencing steps. Although we targeted a single short fragment in this study, a different mitochondrial gene region may have contained sufficient genetic variation to distinguish between closely related species (i.e., *L. osseus* and *L. platostomus*), and multi-marker eDNA metabarcoding methods may be required to maximize species detections (McElroy et al., 2020).

While sampling stochasticity and taxonomic resolution are important considerations in eDNA research, seasonal variation in species composition and abundance must also be acknowledged (Sigsgaard et al., 2017). Although we detected more species in the single day of eDNA sampling than multiple months (June–September) of capture surveys, the detectability of species using all types of sampling gear may vary throughout the year based on seasonal abundance and life history. Therefore, to fully assess the species richness of an ecosystem, temporal changes in species occurrences should be considered when designing sampling surveys. Importantly, because eDNA sampling detects more species with less effort than capture-based sampling gear, eDNA surveys could be conducted multiple times throughout the year, including winter months when deploying capture gear may not be feasible. As noted above, however, the dynamics of eDNA transport, retention, and resuspension may also vary across seasons, and such factors should be considered when designing sampling surveys to maximize species detections.

The fisheries of Oneida Lake have been well-studied for over 60 years with annual monitoring of the whole fish community, enabling the generation of comprehensive species lists, a priori expectations of the spatial and temporal dynamics of species presence, and an opportunity to supplement the reference molecular database of genetic markers. Other aquatic ecosystems, such as those with higher levels of biodiversity or poorly described fish faunas, may require greater investment in ground truthing research before eDNA can be used to reliably characterize species assemblages and abundance (Cilleros et al., 2019). In such cases, the effort and cost expended to build up reference genetic databases and generate species lists may initially reduce the efficiency of eDNA metabarcoding. Ultimately, the efficiency and accuracy of using eDNA metabarcoding is dependent on several factors including availability of reference genetic data, biodiversity of the target assemblage, and the size, complexity, and physicochemical properties of the ecosystem. Regardless of the specific circumstances, our approach for maximizing species detections offers researchers and managers a way to determine how to optimally allocate finite budgets and time across the sampling methods available to them.

eDNA metabarcoding surveys are a powerful and efficient approach for surveying freshwater fish species richness, yet they have not yet been widely adopted by managers as a standardized tool. Questions and concerns are commonly expressed about the sampling effort and costs associated with eDNA surveillance, as well as uncertainty about how eDNA sampling can fit into established monitoring programs (Evans, Shirey, et al., 2017). We demonstrate that eDNA metabarcoding is more efficient than traditional sampling methods for determining species richness, and that eDNA detects habitat-level differences in community composition, in a large, well-studied temperate lake. Some capture-based survey methods, particularly electrofishing and gillnetting, provided little added benefit over other sampling methods in characterizing species richness. However, species detections were maximized with a combination of eDNA metabarcoding, seining, and fyke netting, demonstrating the complementarity of these survey approaches. When designing sampling programs, we recommend that researchers and managers use optimization methods such as the ones presented in this paper to allocate sampling effort among gear types in accordance with the study objectives, available resources, gear efficiency, and desired outcomes.

AUTHOR CONTRIBUTIONS

Field collections were conducted by JRJ and TDL. Laboratory analyses and data analyses were conducted by KJA, TL, and JA. All authors assisted in conceptualizing and designing the study, drafting the manuscript, and providing feedback and manuscript edits.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All sequencing data and sample metadata have been archived in a Dryad Digital Repository: https://doi.org/10.5061/dryad.1c59zw3zk. Sanger sequencing data have been archived with GenBank (accession numbers MZ827011-MZ827027). All scripts used for bioinformatics, taxonomic assignments, and statistical analyses are available on GitHub: https://github.com/karaandres/Oneida_metabarcoding.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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