Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA

Mark Y. Stoeckle [ORCID] [1]*, Jason Adolf[2], Zachary Charlop-Powers[1], Keith J. Dunton[2], Gregory Hinks[3], and Stacy M. VanMorter[3]

[1]*The Rockefeller University, New York, NY, USA*
[2]*Monmouth University, West Long Branch, NJ, USA*
[3]*Bureau of Marine Fisheries, New Jersey Department of Environmental Protection, Port Republic, NJ, USA*

Corresponding author: tel: +1 212 327 8630; fax: +1 212 327 7519; e-mail: mark.stoeckle@rockefeller.edu.

Environmental DNA (eDNA) technology potentially improves the monitoring of marine fish populations. Realizing this promise awaits better understanding of how eDNA relates to fish presence and abundance. Here, we evaluate performance by comparing bottom trawl catches to eDNA from concurrent water samples. In conjunction with New Jersey Ocean Trawl Survey, 1-l water samples were collected at surface and depth prior to tows at about one-fourth of Survey sites in January, June, August, and November 2019. eDNA fish diversity from 1 l was same as or higher than trawl fish diversity from 66 M litres swept by one tow. Most (70–87%) species detected by trawl in a given month were also detected by eDNA, and vice versa, including nearly all (92–100%) abundant species. Trawl and eDNA peak seasonal abundance agreed for ~70% of fish species. In log-scale comparisons by month, eDNA species reads correlated with species biomass, and more strongly with an allometric index calculated from biomass. In this 1-year study, eDNA reporting largely concorded with monthly trawl estimates of marine fish species richness, composition, seasonality, and relative abundance. Piggybacking eDNA onto an existing survey provided a relatively low-cost approach to better understand eDNA for marine fish stock assessment.

Keywords: allometric scaling, bottom trawl surveys, eDNA, environmental DNA, fish stock assessment, marine ecology, marine fisheries, range shifts, seasonal variation

Introduction

Fishing impacts marine fish populations through direct catch removal, by-catch mortality, habitat destruction, noise pollution, and ghost nets and traps (Hollingworth, 2000). Effective management for sustainable fisheries and conservation needs accurate and timely information on fish stocks. Bottom trawl surveys, a mainstay of marine fisheries monitoring, are labour intensive and hindered by costs, environmental damage, and inaccessibility of some sites. Environmental DNA (eDNA) technology largely sidesteps these restrictions. Essential field work is limited to collecting water samples, which can be performed rapidly over large scale by non-experts with modest equipment (Ficetola *et al.*, 2008; Lodge *et al.*, 2012; Deiner *et al.*, 2017). Potential confounding factors include differences by species or habitat in eDNA production, decay, and dispersal, and technical constraints such as primer mismatch (Collins *et al.*, 2018; Hansen *et al.*, 2018; Andruszkiewicz *et al.*, 2019; Kelly *et al.*, 2019). Calibrating this new technology against traditional methods is complicated by fact that all marine censusing techniques have "catchability" biases (Arregúin-Sánchez 1996; Fraser *et al.*, 2007). For example,

bottom trawl captures are influenced by equipment aspects such as net type, mesh size, and towing speed, and by biological factors including avoidance behaviour, patchy distribution, and non-benthic habitat preference. These constraints may operate differently depending on fish species and age. Confidence in eDNA assessment asks for multiple comparisons to established methods in diverse habitats under a variety of weather and hydrographic conditions.

Marine eDNA assessments have been compared to historical data (Andruszkiewicz *et al.*, 2017; Stoeckle *et al.*, 2017; Lafferty *et al.*, 2018; Liu *et al.*, 2019; Jeunen *et al.*, 2020), *ad hoc* surveys (Thomsen *et al.*, 2012; Port *et al.*, 2016; Kelly *et al.*, 2017; Yamamoto *et al.*, 2018), and established ocean monitoring programmes (Berry *et al.*, 2019; Closek *et al.*, 2019). Despite the practical and scientific desirability of improving marine fish stock assessments, to our knowledge so far only three studies directly compare eDNA to bottom trawls (Thomsen *et al.*, 2016; Knudsen *et al.*, 2019; Salter *et al.*, 2019). Here, we take advantage of an ongoing marine survey to evaluate eDNA performance. The New Jersey Bureau of Marine Fisheries Ocean Trawl Survey (NJ OTS), in operation since 1988, samples the neritic zone ocean from New York Harbor south to Delaware Bay, from shoreline out to 30 m depth contour (Figure 1) (Levesque, 2019). In this study, water samples were collected prior to about one-fourth of Survey tows over 1 year and analysed for fish eDNA using a metabarcoding approach with broad-range vertebrate primers and high-throughput sequencing. We compare trawl and eDNA species diversity and seasonal and relative abundance and discuss limitations and possible extensions.

Methods

Trawl survey

NJ OTS field sampling was conducted during ∼1-week periods in January, June, August, and November 2019 (Hinks and Barry, 2019) (Figure 1, Supplementary Table S1). The survey followed a depth-stratified random sampling design with a minimum of ten tows per depth interval (0–10, 10–20, and 20–30 m), for a total of 30 (January) or 39 (other months) tows per month. Sampling strata matched those utilized by Northeast Fisheries Science Center bottom trawl surveys (Politis *et al.*, 2014). The 2019 NJ OTS was conducted with a 30-m "three-in-one" otter trawl net (25-m headrope, 30.5-m footrope) with 12-cm stretch mesh tapering down to 8-cm stretch mesh rear netting, and cod end with 6.4-mm mesh liner. Gear was towed with 1.5-m Thyboron brand type 11 steel trawl doors. Fishing performance was monitored with a combination of Simrad PI and PX net-monitoring sensors, indicating horizontal and vertical openings and footrope bottom-contact to ensure that net was fully operational throughout tows. Trawls were conducted between dawn and dusk with target duration of 20 min at 5.6 km/h. Tows were occasionally as short as 15 min due to obstructions or unforeseen circumstances (e.g. poorly suited bottom structure, fixed commercial fishing gear). For abbreviated tows, catch results were expanded to the expected 20 min goal. For each tow, species identification, number of individuals, total weight per species, and geographic and physicochemical data (YSI EXO-1 Sonde) were recorded. Physiochemical data taken with each tow were summarized by averaging values from the surface (upper 2 m) and bottom (deepest 2 m) of each cast.

### Water collection and eDNA extraction

The target for each month was ten paired samples (one surface and one bottom sample per selected tow site) distributed across strata by depth and north-south location (Figure 1). Prior to tows, a 1.2-l stainless steel, polypropylene-lined Kemmerer water sampler was deployed at surface and bottom, and collected water was transferred to 1-l polypropylene bottles. Between collections, the Kemmerer apparatus and collection bottles were stored in a protected cabin location not exposed to trawl catches. One of us (GH) plus an assistant performed all collections. Before transfer, collection bottle was washed three times with aliquots of sample water. Once filled, collection bottle was placed inside a plastic bag and into a dedicated cooler kept in a protected cabin location.

Samples were maintained on ice and transported to a −20°C freezer within 24 h of collection. Collection bottles were thawed for 24–48 h at 4°C and contents poured into a glass filter manifold attached to wall suction with a 47-mm, 0.45-μM pore size nitrocellulose filter (Millipore). Filters were folded to cover retained material and stored in 15-ml tubes at −80°C. As negative controls for each monthly set, 1 l of laboratory tap water was filtered using the same equipment and procedures, and on the same day as for field samples. Average interval between collection and filtration was 15 d (range 0–35 d). After filtration of contents, collection bottles were decontaminated by washing extensively with tap water, including vigorous shaking of partially filled containers with tops closed, and then air-dried and stored at room temperature. We previously established that this protocol, which relies on mechanical cleansing and dilution, eliminates amplifiable fish DNA from field collection bottles and filtration equipment, while avoiding possible exposure of water samples to residual bleach or other DNA destroying agents (Stoeckle *et al.*, 2018).

DNA was extracted with PowerSoil kit (Qiagen) with modifications from the manufacturer's protocol as previously described (Stoeckle *et al.*, 2020). Average interval between filtration and DNA extraction was 26 d (range 2–56 d). Average yield was ∼600 ng DNA per litre filtered (range 7–2128 ng), with the corresponding average concentration of extracted DNA, 6 ng/μl. DNA recovery averaged about 1.5× higher from bottom vs. surface samples and was lowest in January and highest in August. Tap water negative controls produced on average 10 ng per litre filtered (range 0–19 ng). Collection dates, strata, depths, processing intervals, and DNA yields are provided in Supplementary Table S2. No animals were housed or experimented upon as part of this study. No endangered or protected species were collected during water sampling. Trawls that captured protected species were covered by NOAA incidental take permit number NER2018-14763.

Metabarcoding

DNA processing and bioinformatic analysis were performed as previously described (Stoeckle *et al.*, 2020). Briefly, amplifications were carried out in 25 μl of total volume with TaKaRa High Yield PCR EcoDry™ Premix, 5 μl of DNA sample or 5 μl of molecular biology grade water, and 200 μM Illumina-tailed "ecoPrimers" (IDT) that target an ∼106-bp segment of vertebrate mitochondrial 12S V5 region (Riaz *et al.*, 2011). These primers were selected because binding sites are highly conserved, which is expected to minimize amplification bias among species, and the target segment has good coverage of regional fishes in GenBank (Kelly *et al.*, 2019; Stoeckle *et al.*, 2020). Each sample was amplified once for bony fishes and once for cartilaginous fishes.
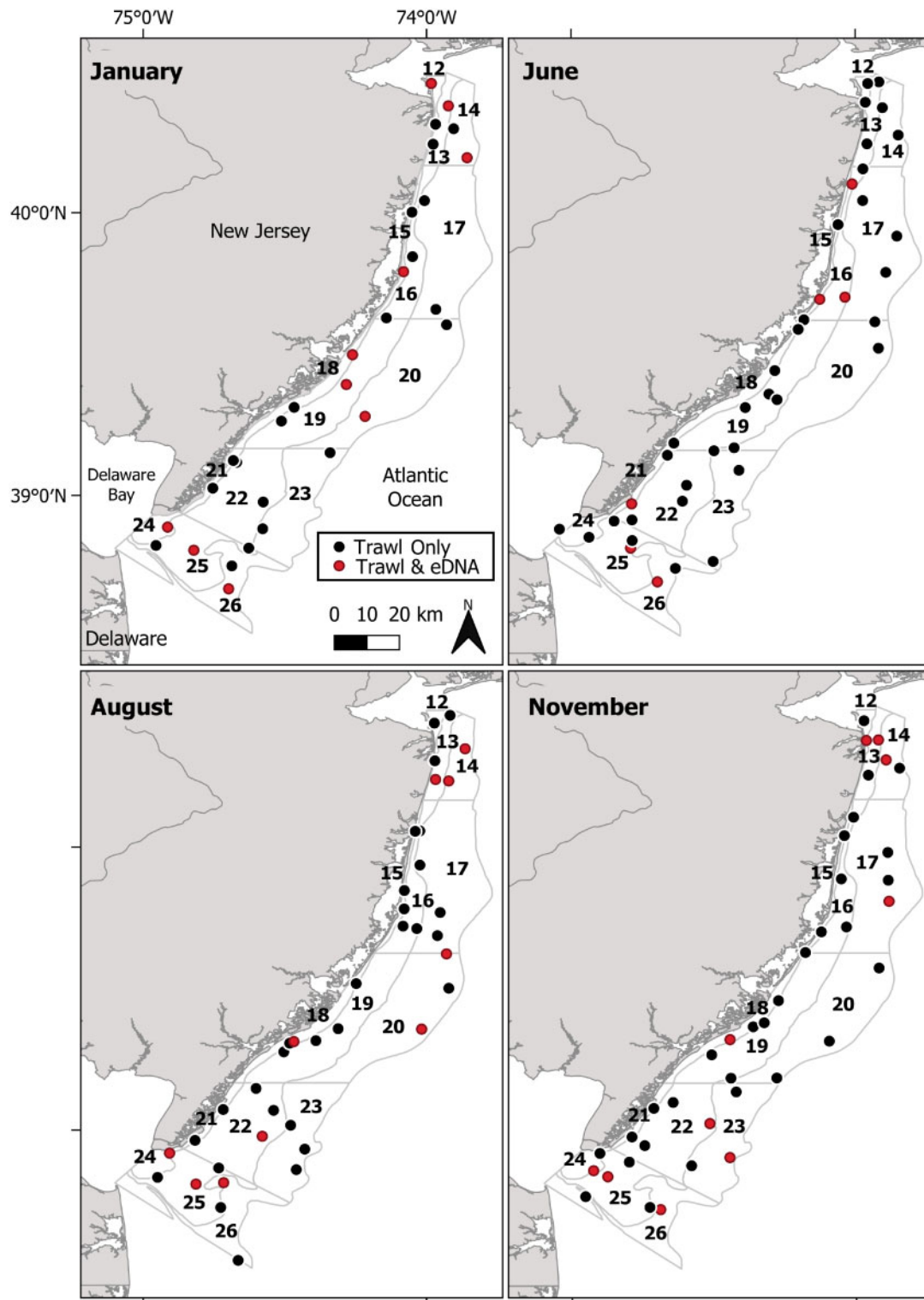
Figure 1. Left: New Jersey Ocean Trawl Survey tow sites during 2019. Strata are numbered 12–26. Isobaths (10, 20, and 30 m) define east-west strata boundaries.

Negative control reactions (tap water eDNA and reagent-grade water) were included in all amplification sets. Bony and cartilaginous fish amplifications were indexed separately. Sequencing was done at GENEWIZ on an Illumina MiSeq (2 × 150 bp). A total of

136 field sample and 79 negative control (tap water eDNA or reagent-grade water) libraries, together with other samples not reported here, were analysed in four MiSeq runs with 96 libraries per run. In addition, to assess reproducibility, 10 of 20 August

2019 eDNA samples were repeat amplified, indexed, and sequenced. PhiX was not routinely employed. To assess possible utility, November 2019 libraries were sequenced with and without 20% PhiX spike-in. Bioinformatic analysis was performed using DADA2, which identifies all amplicon sequence variants (ASVs) (Callahan *et al.*, 2016, 2017). Our DADA2 pipeline (Stoeckle *et al.*, 2017) generated taxon assignments by comparison to an internal 12S reference library of regional fishes and other commonly recovered ASVs (e.g. human, domestic animal, non-fish wildlife) (Supplementary Table S3). All species-level identifications were based on 100% match to a 12S reference sequence. Tap water eDNA and reagent-grade water libraries were negative for fish ASVs after filtering as previously described (Stoeckle *et al.*, 2020).

Trawl and eDNA data curation and statistical analysis

Catch records were sorted to exclude partially identified fishes (e.g. "unclassified skate") and non-fish taxa. Partial identifications comprised <0.1% of fish biomass by month. We extracted data for each tow including date, stratum, stratum depth, species identification, species weight, and number of individuals. To enable comparison across months with differing numbers of tows, monthly catch weights for each species were normalized by converting to per tow values. Original and normalized catch data by month and by individual tow are provided in Supplementary Tables S4–S6. Considering that surface area might be a determinant of eDNA shedding, an approximation was calculated for each species by month as [(monthly biomass per individual)$^{2/3}$ × (monthly number of individuals)]. This formula assumes that volume is proportional to mass and that surface area and volume scale as square and cube of linear size, respectively. Our index is roughly consistent with experimental measurements. For example, O'Shea *et al.* (2006) report mass–surface area scaling exponents of 0.59–0.65 for six commercial marine fish including representative salmonids, pleuronectids, and gadids. It should be noted that other parameters that likely influence eDNA shedding have similar allometric scaling, including metabolic rate, consumption, and excretion (Yates *et al.*, 2020). Thus, in the following, we refer to this more generally as an allometric index. For each eDNA sample, reads from bony and cartilaginous fish amplifications, which were used to generate separate libraries, were combined. To facilitate comparison across months with fewer than 20 samples, read numbers were normalized by converting to per sample values and multiplying by 20. Original and normalized reads by month and water sample are provided in Supplementary Tables S7 and S8. Corresponding results from repeat amplification and sequencing and PhiX spike-in described above are in Supplementary Tables S9 and S10. Seasonal abundance by eDNA and trawl for individual species was examined on linear-scaled plots of absolute values for reads and weight. To assess possible correlations among multiple species, reads, biomass, and allometric index values were expressed as percent total by month and converted to logarithm base 10 form. Linear regression, Fisher's exact test, *t*-test, and box plots were performed in SigmaPlot 14 or Prism 8. In box plots, box spans 25th to 75th percentiles; whiskers mark 10th and 90th percentile, and dots represent outliers.

Diversity index

The Shannon–Wiener diversity index (H') was calculated for each tow and water sample (source data in Supplementary Tables S4 and S8) and then averaged ($\pm$ *SD*) for each month:

$$H' = -\sum_{i=1}^{S} P_i \times \ln P_i,$$

where S is the number of species determined for each station and P_i is the proportion of biomass or reads represented by ith species. For trawl data, P_i was calculated as a species' biomass relative to total trawl biomass. For eDNA data, P_i was calculated as the number of reads for a given species relative to the total reads identified for that sample. Potential differences in H' between trips (January, June, August, November) and technique (trawl, eDNA) were examined in a two-way analysis of variance (ANOVA) (TIBCO Spotfire S-Plus 8.2).

Results

Environmental conditions

Comparisons of surface and bottom temperatures and salinity indicated thermal stratification in June and August and absence in January and November (Supplementary Table S11). On all cruises, average surface dissolved oxygen (DO) levels were close to 100% saturation (7.4–10.4 mg/l). Average bottom water DO during the period of thermal stratification was markedly lower, declining to 6.9 mg/l (79% saturation) and 5.5 mg/l (68% saturation) in June and August, respectively. A high coefficient of variation for DO in June and August reflected large differences among sites, and mapping showed low readings to be mostly localized nearshore in northern strata consistent with prior reports (Glenn *et al.*, 2004). pH values (7.8–8.1) were in the typical range for this coastal region.

Trawling and water collection

Thirty (January) or 39 tows (June, August, November) were carried out per survey month and included two or three tows in each of 15 strata (Figure 1, Supplementary Table S1). A planned survey for April was cancelled due to equipment unavailability, and the October trip was delayed until November due to unfavourable weather conditions. The calculated average volume of water swept in one tow [net opening area (3 m × 12 m) × tow distance (1852 m) × 1000 l/m^3] was 66 M litres. We collected an average of 17 of 20 target water samples per month (range 11–20) (Supplementary Table S1). Three were lost due to bottle breakage during freezer storage in January and nine were not obtained due to collection equipment failure in June. All targeted sites (n=10) were successfully sampled for eDNA in January, August, and November, and six were sampled in June. The analysed set consisted of 33 surface and 35 bottom samples.

Diversity

Species richness

A total of 102 marine fish species were identified in trawl samples (Supplementary Table S12). Most (80%) had unique 12S reference sequences in GenBank, 10% shared target sequences with one or more trawl species, and 10% had no or incomplete reference sequences (Supplementary Tables S12 and S13). Average species per tow increased from 12 in January 2019 to 22 in November 2019 (all comparisons $p < 0.05$ except for June vs.
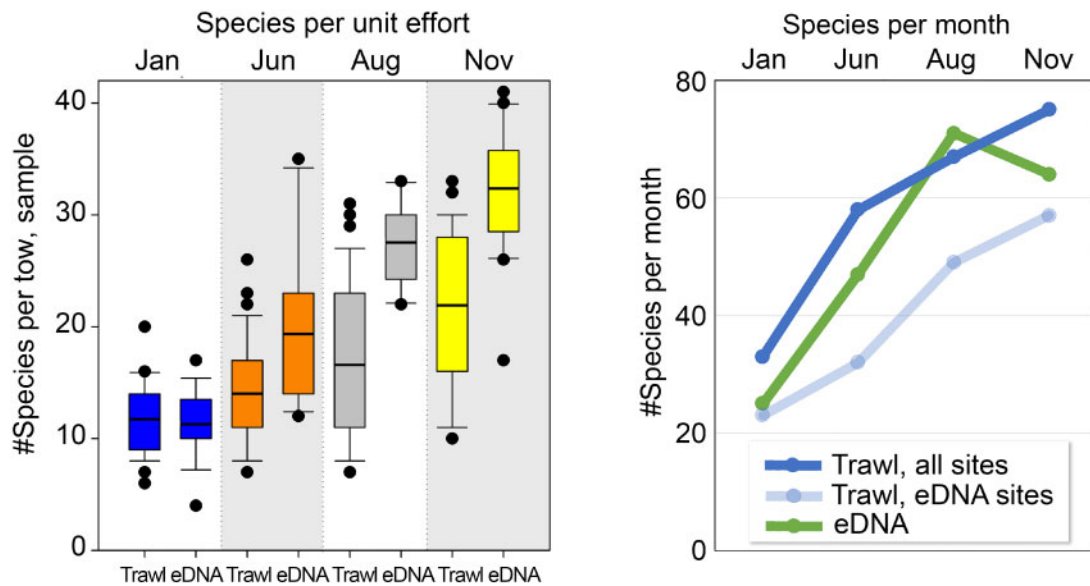
Figure 2. At left, box plots show species per tow and per water sample. Mean value indicated by line inside box. At right, total species per month by method and sites.

August, two-tailed *t*-test), and total species per month ranged from 33 in January to 75 in November (Figure 2). Regarding eDNA results, 99 ASVs representing marine fish species were recovered from water samples. Most (84%) matched a single regional fish species, 9% matched two or more regional fishes, and 7% had no exact matches in GenBank (Supplementary Table S14). Average eDNA species per sample increased from 11 in January to 32 in November (all comparisons $p < 0.01$, two-tailed *t*-test) (Figure 2). Except for January, eDNA recovered more species per unit effort than did tows, i.e. average species per water sample was higher than average species per tow (all comparisons $p < 0.01$, two tailed *t*-test). In all months, Shannon–Wiener diversity indices (H') were significantly higher calculated from eDNA as compared to trawl data, with the greatest difference in November and the least in January (all months, $p < 0.001$, two-way ANOVA) (Supplementary Figure S1).

Despite greater diversity per eDNA sample than per tow, total species per month by trawl and eDNA followed similar trajectories (Figure 2). This may reflect lower sampling intensity—water was collected at about one-fourth of tow sites per month. Considering just the subset of sites where eDNA was collected, eDNA recovered more species per month than trawling did (Figure 2). Average species per tow was greatest in near shore strata and least in deepest strata (10 vs. 30 m, $p < 0.01$ for each month, unpaired *t*-test) and this gradient appeared less steep by eDNA (10 vs. 30 m, $p < 0.01$ in August only) (Figure 3). Average eDNA species per sample did not differ between surface vs. bottom samples ($p = 0.2$–0.8, two-tailed *t*-test). (Figure 3). In fact, even considering only samples from the time of greatest seasonal stratification (June and August, Supplementary Table S11), there was no significant difference between surface and bottom samples for the number of reads ($t = -0.93$, $p = 0.37$), species richness ($t = 0.79$, $p = 0.44$), or biodiversity index (H') ($t = 1.18$, $p = 0.26$) (all paired *t*-tests).

Species composition
We made simplifying assumptions in comparing species composition between trawl and eDNA. First, in compiling totals, we included trawl species lacking GenBank reference sequences, and eDNA species lacking GenBank matches. This potentially lowered the apparent concordance. As noted above, these subsets comprised about 10% of respective lists. Second, for species with shared ASVs, we considered an eDNA detection in a given month as indicating the presence of all matching species detected by trawl in that month, which potentially inflated the apparent agreement. These assumptions were aimed at comparing technologies as they currently operate, rather than under potential future conditions, for instance with better DNA reference libraries. Under these assumptions, overall concordance in species composition was about 75%, and close to 100% for abundant species, i.e. those comprising >1% of monthly biomass or reads (Table 1). Conversely, most species detected only by one technology were relatively rare, i.e. making up <0.1% of monthly biomass or reads.

Seasonal abundance
Individual fish species differed strongly in biomass and eDNA reads across months. For the majority of fishes (70%), trawl and eDNA agreed on the most abundant month ($p < 0.0001$ vs. null hypothesis of 25% agreement by chance, Fisher's exact test) (Figure 4, Supplementary Table S15). High biomass species, here defined as those with yearly trawl biomass >1000 kg, were more likely to show peak month agreement than were low biomass species (yearly total <1000 kg) (81% vs. 57%; $p = 0.042$, Fisher's exact test).

Relative abundance
To assess whether our eDNA protocol captured relative abundance as assessed by trawl, we first examined the monthly distributions of biomass and reads. Biomass per species per month ranged over 5 or 6 orders of magnitude and, when rank-ordered, appeared to approximate a log-linear distribution, a pattern characteristic of animal communities (Fisher *et al.*, 1943; Foster and Dunstan, 2010). If metabarcoding reads reflect fish biomass, then
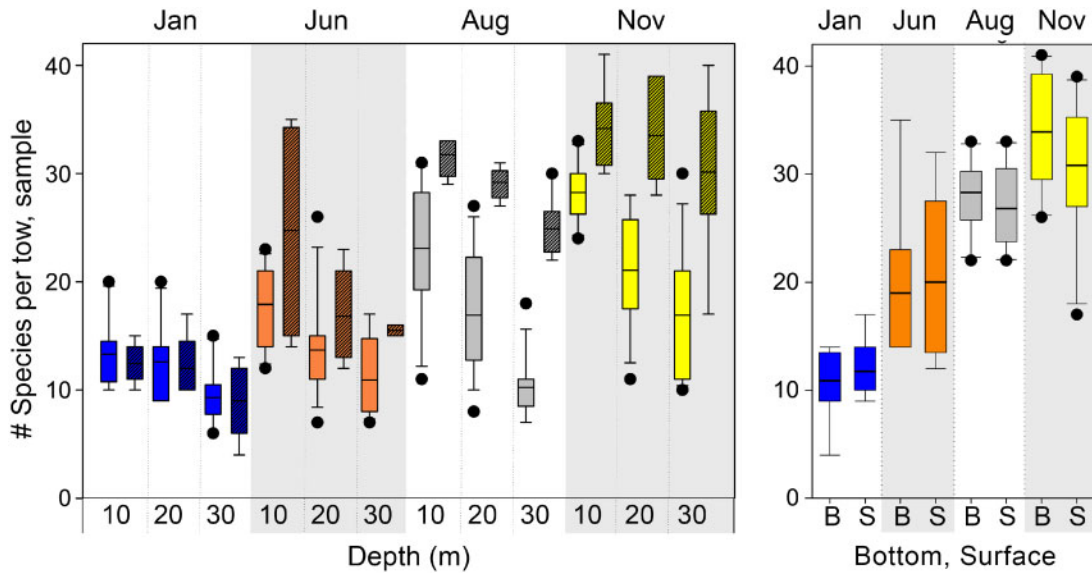
Figure 3. At left, species per tow (open boxes) or water sample (hatched boxes) by month and stratum depth. At right, eDNA species per sample from bottom and surface samples by month. In both plots, line inside box indicates mean.

Table 1. Concordance between trawl and eDNA species detections.

Trawl fishes detected by eDNA

	Total	eDNA+		Abundant	eDNA+	
January	33	23	70%	10	10	100%
June	58	42	72%	13	13	100%
August	67	50	75%	13	13	100%
November	74	55	74%	7	7	100%
Average			73%			100%

eDNA fishes detected by trawl

	Total	Trawl+		Abundant	Trawl+	
January	25	19	76%	13	12	92%
June	46	40	87%	14	13	93%
August	71	48	68%	16	16	100%
November	64	51	80%	16	16	100%
Average			78%			96%

they should follow a similar pattern. In qualitative support of this hypothesis, the rank-ordered sets of eDNA reads per species per month also appeared to approximate log-linear distributions, albeit with a narrower range than for biomass values, spanning 2–5 orders of magnitude depending on month (Figure 5).

We then compared reads and biomass for individual taxa by month on log–log plots scaled as percent monthly values (Figure 6). There was a positive relationship, strongest in August and November. Monthly correlations were higher for reads vs. an allometric index based on biomass (see Methods for details) (Figure 6). We note that converting values to percents, as done here, does not change underlying relationships. Thus, log–log plots of absolute values for monthly reads and biomass gave identical R^2 parameters and slopes (compare text Figure 6, Supplementary Figure S2).

When all survey months were combined, the relationship between reads and biomass remained statistically significant, and, as

with monthly plots, reads correlated more strongly with an allometric index based on biomass (Figure 7). Combined monthly plots of absolute values gave similar results as compared to combined percent-scaled plots (compare text Figure 7, Supplementary Figure S3). The preceding analyses examined fish catches at all monthly tow sites. Correlation was weaker for eDNA reads compared to catches at just the "eDNA sites", both when analysed by month and by individual tow (Supplementary Figure S4).

Other vertebrates
Human and domestic animal ASVs were amplified from multiple field samples and negative controls, as commonly reported (Leonard *et al.*, 2007; Port *et al.*, 2016; Stoeckle *et al.*, 2018); these were considered as of unknown origin. ASVs matching marine and terrestrial wildlife were detected in field samples, notably bottlenose dolphin (*Tursiops truncatus*), present in about one-third. Other marine vertebrates included humpback whale (*Megaptera novaeangliae*) (two samples), fin whale (*Balaenoptera physalus*) (one), harbour seal (*Phoca largha*) or other seal (two), and loggerhead turtle (*Caretta caretta*) (one) (Supplementary Table S16). The accuracy and completeness of eDNA reference library for non-fish vertebrates was not assessed. Trawl catches of other vertebrates were limited to single individuals of loggerhead turtle and green turtle (*Chelonia mydas*).

Discussion

In this study, we took advantage of an ongoing marine fisheries trawl survey to evaluate eDNA performance. Water samples were collected during four survey months—in winter, spring, summer, and fall—and analysed for fish eDNA using metabarcoding with broad-range primers targeting bony and cartilaginous fishes. We found eDNA reporting on marine fish diversity, seasonality, and relative abundance largely agreed with bottom trawl catches. To our knowledge, this is the most detailed comparison of a marine fisheries survey and concurrent eDNA sampling to date. The marginal cost of adding eDNA testing to an existing program was
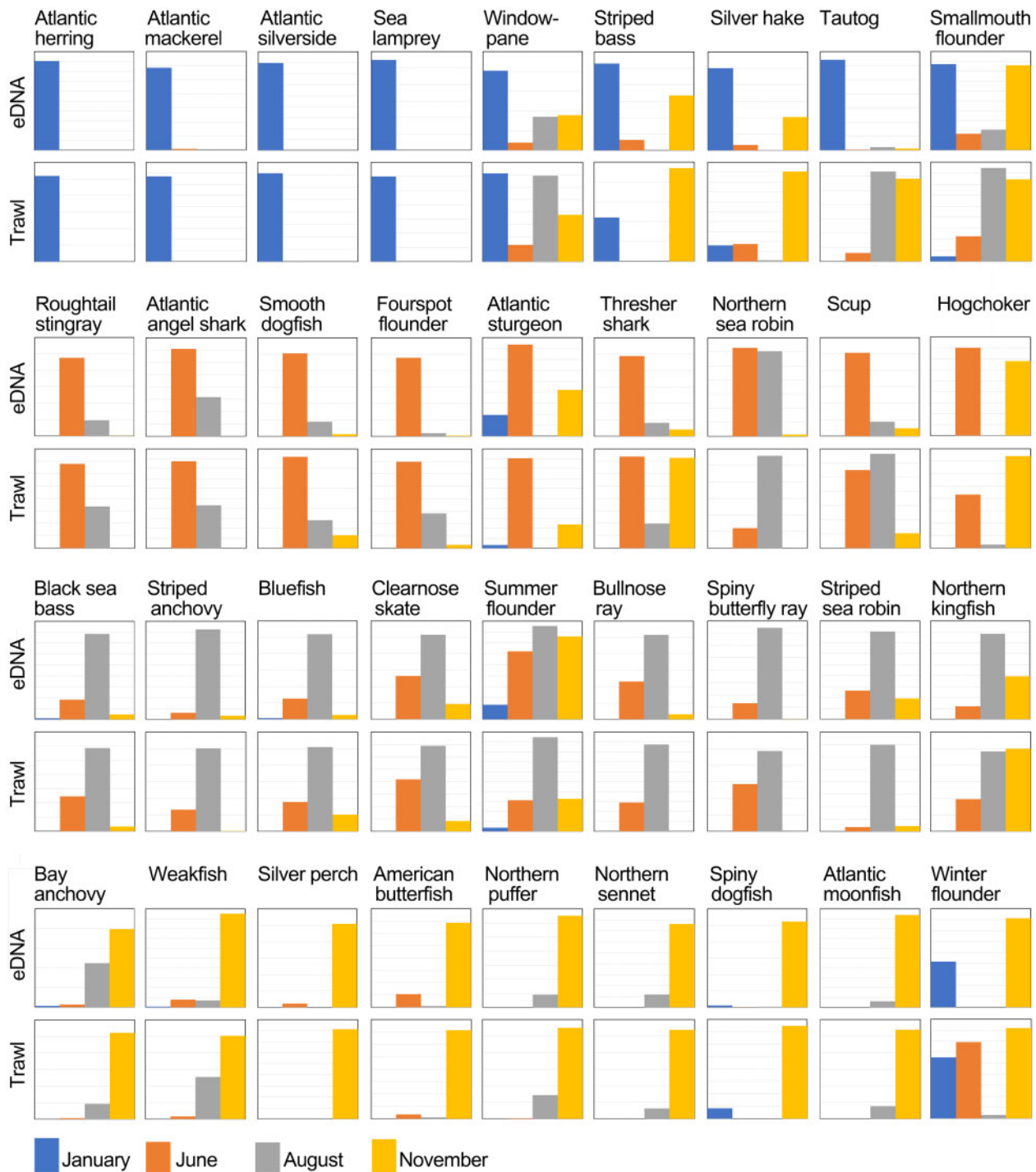
Figure 4. Seasonal abundance by eDNA and trawl for selected marine fishes. Vertical scale is linear and differs between species (source data including all species in Supplementary Table S15).

relatively modest, about $12 000 not including salary support. This report adds backing to efforts incorporating eDNA sampling in fisheries assessments, with goals of augmenting traditional approaches and speeding assessments of diversity and abundance.

Average eDNA species diversity from 1 l of water exceeded average trawl fish diversity from one tow with a calculated swept volume of 66 M litres—seven orders of magnitude improvement in sensitivity. A similar calculation could be made for other

aquatic eDNA surveys (e.g. Thomsen *et al.*, 2016). The extraordinary gain in fish detection per volume exemplifies both the promise of eDNA and the challenge inherent in monitoring marine fish populations. Typical for marine fisheries trawl surveys, NJ OTS conducts multiple tows over a large geographic area to reliably assess fish populations spatially and temporally. The need for extensive trawling arises from patchy fish distribution, capture avoidance, and other factors (Fraser *et al.*, 2007). Looked at the
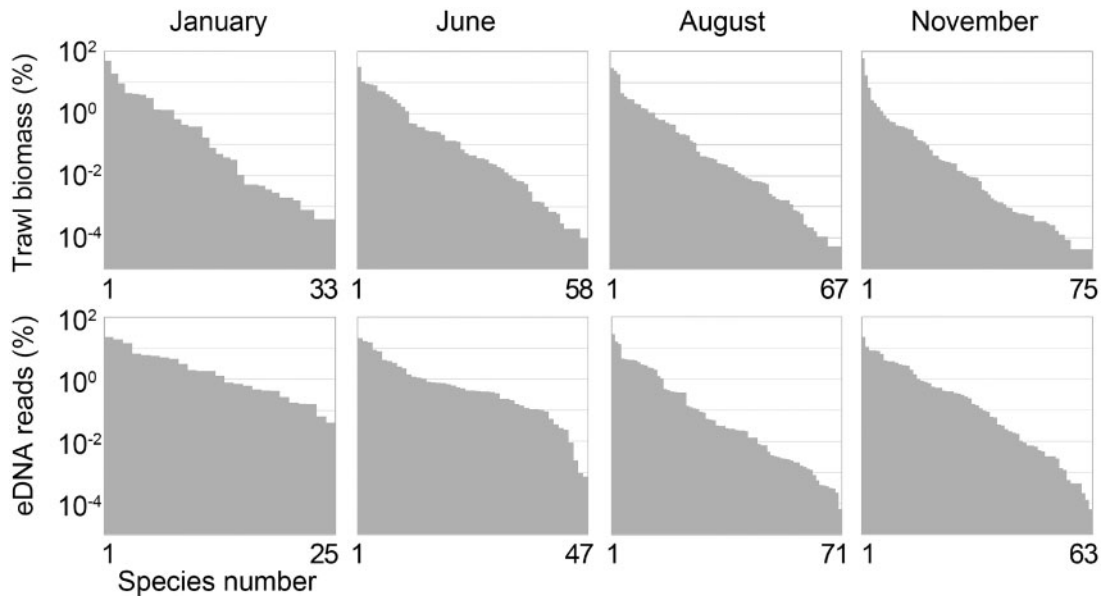
Figure 5. Distribution of biomass and reads by month. Species values for each month are rank ordered and shown as percent total in logarithmic scale, with horizontal dimension normalized to facilitate comparison across months. The order of particular species differs between months and technique.

other way around, eDNA was orders of magnitude less localized than captured fish were, presumably due to eDNA dispersal, movements of fish, or other unknown factors (Barnes and Turner, 2016; Jerde, 2019). eDNA from surface and bottom samples showed no obvious difference in species richness or composition, even during the period of vertical stratification that is typical for the mid-Atlantic Bight (Schofield *et al.*, 2008). This was unexpected, as some regional fish species are strongly benthic or demersal while others are pelagic specialists. Although eDNA was collected only at a fraction of tow sites, eDNA signals best reflected the complete set of monthly trawl catches. Furthermore, although average trawl fish diversity was greatest nearshore and diminished progressively in deeper strata, this gradient was less evident for eDNA. The apparent dispersal of eDNA in this study differs from reports demonstrating localization between sea floor habitats separated by distances as short as 90 m (Port *et al.*, 2016), between surface and depth samples 20–40 m apart (Andruszkiewicz *et al.*, 2017), within 100 m and 1 h of removal of caged striped jack in a marine environment (Murakami *et al.*, 2019), and between samples collected immediately above and below a halocline (Jeunen *et al.*, 2020). In addition, Atlantic cod abundance by trawl and eDNA vary concordantly among kilometre-scale regions defined by sea floor features and local currents (Salter *et al.*, 2019). Regarding limited localization in this study, it may be relevant that our survey area, which covers ~200 km × 50 km, is a relatively uniform sandy bottom habitat that, with a few exceptions (e.g. see Atlantic angel shark findings below), supports similar fish communities throughout. In addition, our research area experiences primarily thermal stratification, which may be more permissive of fish movements across the pycnocline as compared to salinity stratification described in Jeunen *et al.* (2020).

One limitation of this study is that we cannot exclude the distortion of eDNA results by trawl operations. Although multiple precautions were taken, including obtaining water samples prior

to initiating tows, it is possible that fish residues on personnel, on the vessel, or in water immediately surrounding the boat were introduced into collected water specimens. Recent trawl-eDNA studies followed procedures similar to those described here, with the primary safeguard being collecting water before trawls, without obtaining field blanks (Thomsen *et al.*, 2016; Knudsen *et al.*, 2019). Appropriate field blanks for trawl-eDNA work are unclear; one procedure is to fill collection bottles with laboratory-grade water while field sampling is underway (Salter *et al.*, 2019). In addition, it might be useful in future work to collect water from a nearby vessel not engaged in trawling or via an autonomous vehicle. It is also possible that there was carry-over between one sample and the next due to retained water in the Kemmerer bottle, although we think that this is unlikely to be significant given the open cylinder design of the apparatus, which promotes volume exchange during sampling. As an aside, we note that our analyses of seasonal and relative abundance involved summing all detections for given month, and so were likely robust to potential carry-over. More generally, although multiple precautions to prevent contamination are taken in eDNA studies, relatively little work has been done assessing the sources and magnitudes of risks or the benefits of particular precautions. For instance, at what volume threshold does carry-over between field samples result in carry-over of detectable eDNA? In this regard, it may be informative to mix water from different habitats, i.e. containing eDNA from non-overlapping sets of species, in different proportions (e.g. 1:100 000, 1:10 000, 1:1000, 1:100, 1:10), and then filter and process for metabarcoding.

Geographic localization was noted for one species, Atlantic angel shark (*Squatina dumerii*), an elasmobranch that moves seasonally into inshore waters. Both trawl and eDNA indicated summertime presence in southernmost strata (Supplementary Figure S5). Geographical localization of eDNA may become increasingly relevant for this region. The Mid-Atlantic Bight has experienced poleward shifts in species distributions due to
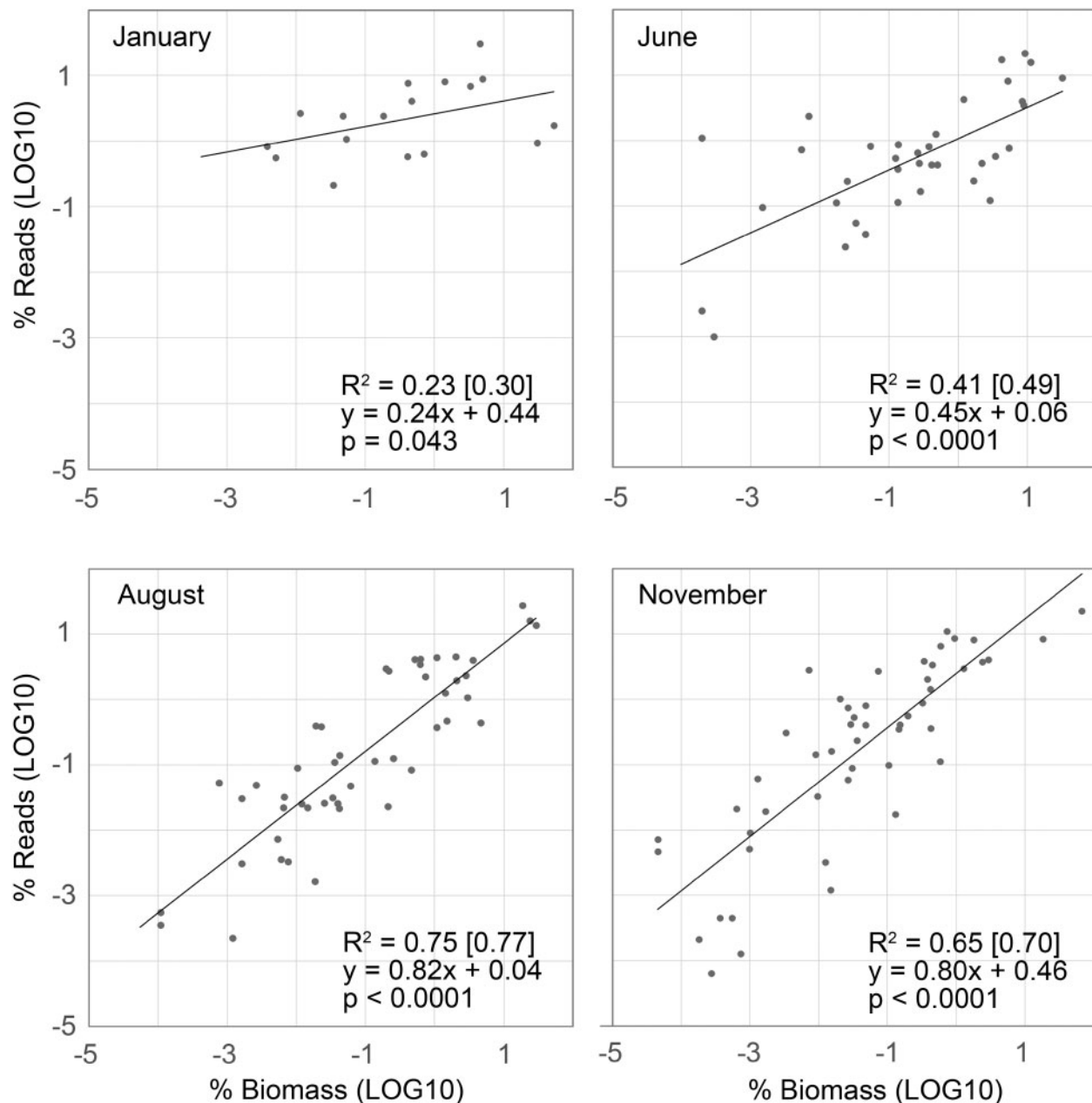
Figure 6. Monthly percent reads compared to monthly percent biomass in log–log scale. Each point corresponds to a species detected by trawl and eDNA during month shown. Biomass of trawl species represented by shared ASVs (Supplementary Table S13) was combined before analysis. Trendline and R^2 parameter show log–log correlation, and *p*-value is probability that trendline slope differs from 0. Value in brackets is R^2 of log–log correlation between percent reads and percent allometric index based on biomass (see Methods for details).

anthropogenic climate change, with an increase in southern taxa and a contraction of northern taxa (Nye *et al.*, 2009; Walsh *et al.*, 2015). A recent New Jersey eDNA time series detected seasonal incursion of southern species in the surf zone that were not found in NJ OTS (Stoeckle *et al.*, 2020), highlighting how eDNA can augment traditional assessments to help document range shifts when species are in low abundance, utilizing habitat not able to be sampled by traditional approaches, or occurring in months not surveyed by trawl.

Species compositions by trawl and eDNA were largely (∼75%) concordant. Most dropouts were rare taxa. Inconsistent detection

of rare species is characteristic of ecological surveys regardless of technique (MacKenzie *et al.*, 2002; Furlan *et al.*, 2016; Sato *et al.*, 2017). Additional tows, water samples, or replicate amplifications might recover some of those missing. Even so, the combined trawl plus eDNA census likely overlooked fish taxa. About one-fifth of species detections by trawl or eDNA were from a single tow or water sample, whereas exhaustive sampling should yield multiple detections for all taxa. Biomass and reads followed a "hollow curve" logarithmic distribution, characteristic of animal communities. This pattern predicts greater effort by trawl or eDNA will be required to detect the remaining rare species. We
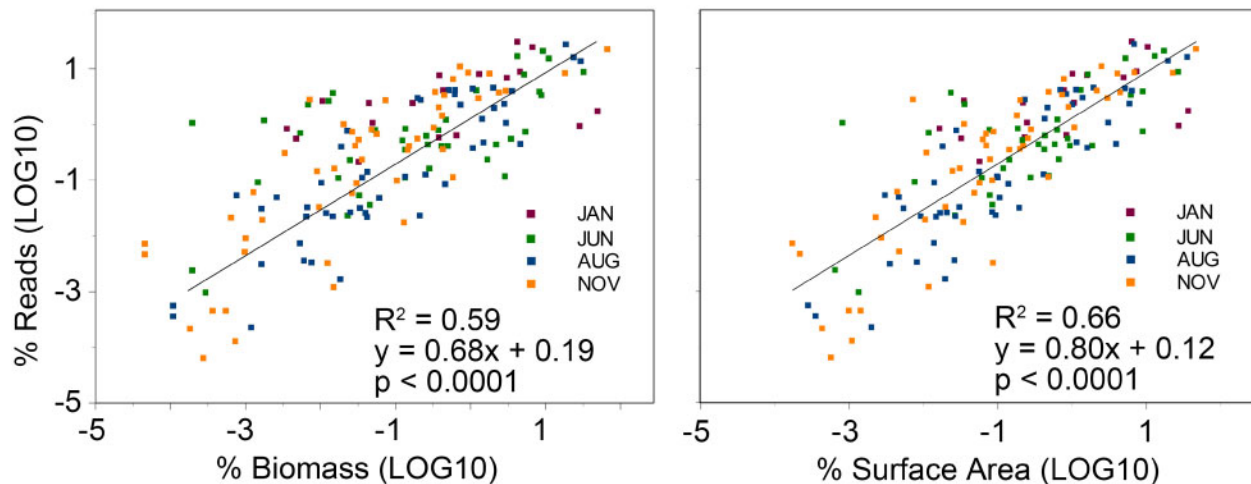
Figure 7. Log–log plots of percent reads vs. percent biomass and vs. percent allometric index for all survey months combined.

did not find obvious bias by technology in types of fish recovered, although differences may become evident with further study. In addition, while eDNA species composition appeared similar between surface and bottom samples, more work is needed regarding the distribution of individual species' eDNA within the water column at different times of year and under different oceanic conditions. Such analyses would be especially important in water deeper than the maximum of 30 m in our study.

It would greatly expand utility if measured eDNA abundance reflected fish abundance. Mesocosm and freshwater studies in lentic environments demonstrate potential for reliable assessment (Takahara *et al.*, 2012; Evans *et al.*, 2016; Spear *et al.*, 2020). However, multiple factors potentially hamper eDNA utility as a proxy for abundance. Rates of production and decay differ between species and life stage and are affected by exogenous factors such as predation and mortality. In marine systems, dispersal by oceanic and tidal currents depends on geography, season, and weather. Technical factors related to PCR can distort eDNA findings, including co-purification of inhibitors along with DNA, primer bias, unrecognized cross-contamination, and non-linear amplification kinetics (Jerde *et al.*, 2011; Roussel *et al.*, 2015; Taberlet *et al.*, 2018; Kelly *et al.*, 2019). Regarding primer bias, although we employed 12S primers that exactly match binding sites in mtDNA of most regional fish species, this does not exclude non-uniform amplification, which can occur due to the effects of flanking regions (Hansen *et al.*, 1998). Quantitative assessment of relative primer efficiency in amplifying DNA isolated from tissue specimens of different species may help shed light on this important parameter. eDNA identifications may be in error due to absent representation of relevant species or unrecognized errors in the reference database. As with other mtDNA genes, 12S identifications can be misled by hybridization, which can result in mitochondrial genomes shared between species. The current study is limited by duration over a single year and inability to distinguish some species due to shared target sequences. In addition, inherent limitations to eDNA include absent information on fish age, weight, or sex, critical parameters in fish stock assessment.

In support of the hypothesis that eDNA indexes marine fish abundance, peak seasonal abundance by eDNA and trawl were largely (~70%) concordant. Phenological variation in eDNA is reported in marine habitats (Sigsgaard *et al.*, 2017; Berry *et al.*,

2019) but to date appears a relatively underutilized axis for calibrating eDNA against established survey methods. Reads per species positively correlated with biomass per species by month (log–log R^2, 0.23–0.75) and when compiled across entire year (R^2, 0.59) (Figures 6 and 7). Of note, eDNA reads were more closely related to an allometric index than with biomass itself (R^2 for year, 0.66) (Figures 6 and 7). We believe that this is the first metabarcoding report providing evidence for an allometric relationship, i.e. one dependent on body size, between eDNA production and biomass. Our findings are consistent with a recent qPCR study demonstrating that brook trout eDNA in natural lakes is best predicted by allometric scaling of fish biomass with the exponent of 0.72 (Yates *et al.*, 2020). In their report, eDNA vs. adjusted biomass yielded R^2 of 0.78, compared to R^2 of 0.63 for eDNA vs. unadjusted biomass. The authors note that allometry presumably arises from differences in eDNA production, rather than decay or dispersal, and that determinants of eDNA production likely include several parameters that are known to scale allometrically, including metabolic rate, external surface area, excretion, as well as other factors. Further investigation of possible allometric scaling of eDNA levels in relation to biomass by different species in natural and mesocosm settings appears warranted.

Our results compare favourably with the single bottom trawl plus metabarcoding report to date (R^2, 0.26) (Thomsen *et al.*, 2016), and with qPCR studies of Atlantic cod (R^2, 0.64–0.79) (Knudsen *et al.*, 2019) and Baltic Sea fish (absent reads-biomass correlation among individual trawls) (Salter *et al.*, 2019). In the present study, the relationship was strongest in summer and fall; continued seasonal monitoring would be needed to establish whether this pattern is valid. If confirmed, a possible contributing factor might be relative dominance of demersal vs. pelagic species at different times of year. Preliminary analyses suggest reads vs. biomass correlations may differ among taxonomic groupings (e.g. Supplementary Figure S6). Regarding reproducibility, repeat amplification, and sequencing gave highly similar results (log–log R^2, 0.99), with a few dropouts at low read numbers (Supplementary Figure S7). Adding PhiX did not yield greater species diversity or eliminate presumptive PCR or sequencing errors (Supplementary Figure S8), supporting our protocol without PhiX.

Fisheries are under stress worldwide. Debates abound about the value and effects of marine protected areas (MPAs). Fisheries and MPAs are the focus of intense monitoring to protect stocks and promote sustainable harvesting and conservation. Our study further establishes aquatic environmental DNA as a relatively inexpensive, low-impact way to census marine life diversity and abundance. Assuming techniques and reliability continue to advance, eDNA has potential to improve the management of fisheries and MPAs and help monitor ecological impacts of marine industrial activities, including offshore wind installations, resource extraction, and maritime shipping.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Data availability

Illumina FASTQ files underlying this article are deposited in NCBI Bioproject ID PRJNA638560. Trawling data are available on reasonable request to the Bureau of Marine Fisheries, New Jersey Department of Environmental Protection, Port Republic, NJ, USA.

Author contributions

Designed study: MYS, JA, KJD, and GH. Designed bioinformatics: ZC-P. Collected samples: GH. Analysed samples: MYS and JA. Analysed data: MYS, JA, KJD, GH, and SMV. Prepared figures: MYS, JA, KJD, and SMV. Wrote first draft of manuscript: MYS. Revised manuscript: MYS, JA, ZC-P, KJD, GH, and SMV.

References

Andruszkiewicz, E. A., Koseff, J. R., Fringer, O. B., Ouellette, N. T., Lowe, A. B., Edwards, C. A., and Boehm, A. B. 2019. Modeling environmental DNA transport in the coastal ocean using Lagrangian particle tracking. Frontiers in Marine Science, 6: 477.

Andruszkiewicz, E. A., Starks, H. A., Chavez, F. P., Sassoubre, L. M., Block, B. A., and Boehm, A. B. 2017. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. PLoS One, 12: e0176343.

Arregúin-Sánchez, F. 1996. Catchability: a key parameter for fish stock assessment. Reviews in Fish Biology and Fisheries, 6: 221–242.

Barnes, M. A., and Turner, C. R. 2016. The ecology of environmental DNA and implications for conservation genetics. Conservation Genetics, 17: 1–17.

Berry, T. E., Saunders, B. J., Coghlan, M. L., Stat, M., Jarman, S., Richardson, A. J., Davies, C. H., *et al.* 2019. Marine environmental DNA biomonitoring reveals seasonal patterns in biodiversity and identifies ecosystem responses to anomalous climatic events. PLoS Genetics, 15: e1007943.

Callahan, B., McMurdie, P. J., and Holmes, S. P. 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME Journal, 11: 2639–2643.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, J. A., and Holmes, S. P. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods, 13: 581–583.

Closek, C. J., Santora, J. A., Starks, H. A., Schroeder, I. D., Andruszkiewicz, E. A., Sakuma, K. M., *et al.* 2019. Marine vertebrate biodiversity and distribution within the central California Current using environmental DNA (eDNA) metabarcoding and ecosystem surveys. Frontiers in Marine Science, 6: 732.

Collins, R. A., Wangensteen, O. S., O'Gorman, E. J., Mariani, S., Sims, D. W., and Genner, M. J. 2018. Persistence of environmental DNA in marine systems. Communications Biology, 1: 185.

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., *et al.* 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. Molecular Ecology, 26: 5872–5895.

Evans, N. T., Olds, B. P., Renshaw, M. A., Turner, C. R., Li, Y., Jerde, C. L., Mahon, A. R., *et al.* 2016. Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. Molecular Ecology Resources, 16: 29–41.

Fraser, H. M., Greenstreet, S. P. R., and Piet, G. J. 2007. Taking account of catchability in groundfish survey trawls: implications for estimating demersal fish biomass. ICES Journal of Marine Science, 64: 1800–1819.

Ficetola, G. F., Miaud, C., Pompanon, F., and Taberlet, P. 2008. Species detection using environmental DNA from water samples. Biology Letters, 4: 423–425.

Fisher, R. A., Corbet, A. S., and Williams, C. B. 1943. The relation between the number of species and the number of individuals in a random sample of an animal population. Journal of Animal Ecology, 12: 42–58.

Furlan, E. M., Gleeson, D., Hardy, C. M., and Duncan, R. P. 2016. A framework for estimating the sensitivity of eDNA surveys. Molecular Ecology Resources, 16: 641–654.

Foster, S. D., and Dunstan, P. K. 2010. The analysis of biodiversity using rank abundance distributions. Biometrics, 66: 186–195.

Glenn, S., Arnone, R., Bergman, T., Bisset, W. P., Crowley, M., Cullen, J., Gryzmski, J., Haidvogel., *et al.* 2004. Biogeochemical impact of summertime coastal upwelling on the New Jersey shelf. Journal of Geophysical Research: Atmospheres, 109: C12S02.

Hansen, B. K., Bekkevold, D., Clausen, L. W., and Nielsen, E. E. 2018. The skeptical optimist: challenges and perspectives for the application of environmental DNA in marine fisheries. Fish and Fisheries, 19: 751–768.

Hansen, M. C., Tolker-Nielsen, T., Givskov, M., and Molin, SÃ¸r. 1998. Biased 16S rDNA PCR amplification caused by interference from DNA flanking the template region. FEMS Microbiology Ecology, 26: 141–149.

Hinks, G., and Barry, L. 2019. Ocean Trawling Survey, 2019. New Jersey Department of Environmental Protection, Division of Fish and Wildlife, Marine Fisheries Administration, Bureau of Marine Fisheries.

Hollingworth C. 2000. Ecosystem effects of fishing. ICES Journal of Marine Science, 57: 465–792.

Jerde, C. L. 2019. Can we manage fisheries with the inherent uncertainty from eDNA? Journal of Fish Biology, 10: 14218.

Jerde, C. L., Mahon, A. R., Chadderton, W. L., and Lodge, D. M. 2011. "Sight-unseen" detection of rare species using environmental DNA. Conservation Letters, 4: 150–157.

Jeunen, G.-J., Lamare, M. D., Knapp, M., Spencer, H. G., Taylor, H. R., Stat, M., Bunce, M., et al. 2020. Water stratification in the marine biome restricts vertical environmental DNA (eDNA) signal dispersal. Environmental DNA, 2: 99–111.

Kelly, R. P., Closek, C. J., O'Donnell, J. L., Kralj, J. E., Shelton, A. O., and Samhouri, J. F. 2017. Genetic and manual survey methods yield different and complementary views of an ecosystem. Frontiers in Marine Science, 3: 283.

Kelly, R. P., Shelton, A. O., and Gallego, R. 2019. Understanding PCR processes to draw meaningful conclusions from environmental DNA studies. Scientific Reports, 9: 12133.

Knudsen, S. W., Ebert, R. B., Hesselsøe, M., Kuntke, F., Hassingboe, J., Mortensen, P. B., Thomsen, P. F., et al. 2019. Species-specific detection and quantification of environmental DNA from marine fishes in the Baltic Sea. Journal of Experimental Marine Biology and Ecology, 510: 31–45.

Lafferty, K. D., Benesh, K. C., Mahon, A. R., Jerde, C. L., and Lowe, C. H. 2018. Detecting southern California's white sharks with environmental DNA. Frontiers in Marine Science, 5: 355.

Leonard, J. A., Shanks, O., Hofreiter, M., Kreuz, E., Hodges, L., Ream, W., Wayne, R. K., et al. 2007. Animal DNA in PCR reagents plagues ancient DNA research. Journal of Archaeological Science, 34: 1361–1366.

Levesque, J. C. 2019. Spatio-temporal patterns of the oceanic conditions and nearshore marine community in the Mid-Atlantic Bight (New Jersey, USA). PeerJ, 7: e7927.

Lodge, D. M., Turner, C. R., Jerde, C. L., Barnes, M. A., Chadderton, L., Egan, S. P., Feder, J. L., et al. 2012. Conservation in a cup of water: estimated biodiversity and population abundance from environmental DNA. Molecular Ecology, 21: 2555–2558.

Liu, Y., Wikfors, G. H., Rose, J. M., McBride, R. S., Milke, L. M., and Mercaldo-Allen, R. 2019. Application of environmental DNA metabarcoding to spatiotemporal finfish community assessment in a temperate embayment. Frontiers in Marine Science, 6: 674.

MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droege, S., Royle, J. A., and Langtimm, C. A. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology, 83: 2248–2255.

Murakami, H., Yoon, S., Kasai, A., Minamoto, T., Yamamoto, S., Sakata, M. K., Horiuchi, T., et al. 2019. Dispersion and degradation of environmental DNA from caged fish in a marine environment. Fisheries Science, 85: 327–337.

Nye, J. A., Link, J. S., Hare, J. A., and Overholtz, W. J. 2009. Changing spatial distribution of fish stocks in relation to climate and population size on the Northeast United States continental shelf. Marine Ecology Progress Series, 393: 111–129.

Politis, P. J., Galbraith, J. K., Kostovick, P., and Brown, R. W. 2014. Northeast Fisheries Science Center bottom trawl survey protocols for the NOAA Ship Henry B. Bigelow. Northeast Fish Sci Cent Ref Doc 14-06. 138 pp.

Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J., Yamahara, K. M., et al. 2016. Assessing vertebrate biodiversity in a kelp forest using environmental DNA. Molecular Ecology, 25: 527–541.

Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., and Coissac, E. 2011. ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. Nucleic Acids Research, 39: e145–e145.

Roussel, J.-M., Paillisson, J.-M., Tréguier, A., and Petit, E. 2015. The downside of eDNA as a survey tool in water bodies. Journal of Applied Biology, 52: 823–826.

Salter, I., Joensen, M., Kristiansen, R., Steingrund, P., and Vestergaard, P. 2019. Environmental DNA concentrations are correlated with regional biomass of Atlantic cod in oceanic waters. Communications Biology, 2: 461.

Sato, H., Sogo, Y., Doi, H., and Yamanaka, H. 2017. Usefulness and limitations of sample pooling for environmental DNA metabarcoding of freshwater fish communities. Scientific Reports, 7: 14860.

O'Shea, B., Mordue-Luntz, A. J., Fryer, R. J., Pert, C. C., and Bricknell, I. R. 2006. Determination of the surface area of a fish. Journal of Fish Diseases, 29: 437–440.

Schofield, O., Chant, R., Cahill, B., Castelao, R., Gong, D., Kahl, A., Kohut, J., et al. 2008. The decadal view of the mid-Atlantic bight from the COOLroom: is our coastal system changing? Oceanography, 21: 108–117.

Sigsgaard, E. E., Nielsen, I. B., Carl, H., Krag, M. A., Knudsen, S. W., Xing, Y., Holm-Hansen, T. H., et al. 2017. Seawater environmental DNA reflects seasonality of a coastal fish community. Marine Biology, 164: 128.

Spear, M. J., Embke, H. S., Krysan, P. J., and Zanden, M. J. V. 2020. Application of eDNA as a tool for assessing fish population abundance. Environmental DNA, 00: 1–9.

Stoeckle, M. Y., Das Mishu, M., and Charlop-Powers, Z. 2020. Improved environmental DNA reference library identifies overlooked marine fishes in coastal New Jersey, U.S.A. Frontiers in Marine Science, 7: 226.

Stoeckle, M. Y., Mishu, M. D., and Charlop-Powers, Z. 2018. GoFish: a versatile nested PCR strategy for environmental DNA assays for marine vertebrates. PLoS One, 13: e0198717.

Stoeckle, M. Y., Soboleva, L., and Charlop-Powers, Z. 2017. Aquatic environmental DNA detects seasonal fish abundance and habitat preference in an urban estuary. PLoS One, 12: e0175186.

Taberlet, P., Bonin, A., Zinger, L., and Coissac, E. 2018 Environmental DNA for Biodiversity Research and Monitoring. Oxford University Press, New York.

Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., and Kawabata, Z. 2012. Estimation of fish biomass using environmental DNA. PLoS One, 7: e35868.

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., and Willerslev, E. 2012. Detection of diverse marine fish fauna using environmental DNA from seawater samples. PLoS One, 7: e41732.

Thomsen, P. F., Møller, P. R., Sigsgaard, E. E., Knudsen, W., Jørgensen, O. A., and Willerslev, E. 2016. Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. PLoS One, 11: e0165252.

Walsh, H. J., Richardson, D. E., Marancik, K. E., and Hare, J. A. 2015. Long-term changes in the distributions of larval and adult fish in the Northeast U.S. shelf ecosystem. PLoS One, 10: e013782.

Yamamoto, S., Minami, K., Fukaya, K., Takahashi, K., Sawada, H., and Murakami, H. 2018. Environmental DNA as a "snapshot" of fish distribution: a case study of Japanese jack mackerel in Maizuru Bay, Sea of Japan. PLoS One, 11: e0149786.

Yates, M. C., Glaser, D., Post, J., Cristescu, M. E., Fraser, D. J., and Derry, A. M. 2020. The relationship between eDNA particle concentration and organism abundance in nature is strengthened by allometric scaling. Molecular Ecology, 1–15. 10.1111/mec.15543

Handling editor: Ian Bradbury