



Canadian Journal of Fisheries and Aquatic Sciences

Fish community surveys in eelgrass beds using both eDNA metabarcoding and seining: implications for biodiversity monitoring in the coastal zone

Journal:	<i>Canadian Journal of Fisheries and Aquatic Sciences</i>
Manuscript ID	cjfas-2021-0215.R1
Manuscript Type:	Article
Date Submitted by the Author:	23-Dec-2021
Complete List of Authors:	He, Xiaoping; Fisheries and Oceans Canada - Pacific Biological Station, Stanley, Ryan; Bedford Institute of Oceanography, Habitat Ecology Section Rubidge, Emily; Pacific Region Institute of Ocean Sciences Jeffery, Nicholas; Bedford Institute of Oceanography, Habitat Ecology Section Hamilton, Lorraine; Fisheries and Oceans Canada, Bedford Institute of Oceanography Westfall, Kristen; Fisheries and Oceans Canada - Pacific Biological Station Gilmore, Scott; Fisheries and Oceans Canada - Pacific Biological Station Roux, Louise-Marie; Fisheries and Oceans Canada - Pacific Biological Station Gale, Katie; Pacific Region Institute of Ocean Sciences Heaslip, Susan; Bedford Institute of Oceanography, Habitat Ecology Section Steeves, Royce; Gulf Fisheries Centre, Fisheries and Oceans Canada Abbott, Cathryn; Fisheries and Oceans Canada - Pacific Biological Station
Keyword:	12S rRNA, 16S rRNA, marine protected area, seagrass, seining
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

SCHOLARONE™
Manuscripts

**Fish community surveys in eelgrass beds using both eDNA metabarcoding and seining:
implications for biodiversity monitoring in the coastal zone**

Xiaoping He¹, Ryan R.E. Stanley², Emily M. Rubidge^{3,4}, Nicholas W. Jeffery^{2,5}, Lorraine C.
Hamilton², Kristen M. Westfall¹, Scott R. Gilmore¹, Louise-Marie D. Roux¹, Katie S.P. Gale³,
Susan G. Heaslip², Royce Steeves⁶, Cathryn L. Abbott^{1*}

- 1 Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC, Canada
- 2 Bedford Institute of Oceanography, Fisheries and Oceans Canada, Dartmouth, NS, Canada
- 3 Institute of Ocean Sciences, Fisheries and Oceans Canada, Sidney, BC, Canada
- 4 Department of Forest and Conservation Sciences, University of British Columbia, Vancouver,
BC, Canada
- 5 Department of Integrative Biology, University of Guelph, Guelph, ON, Canada
- 6 Gulf Fisheries Centre, Fisheries and Oceans Canada, Moncton, NB, Canada

* corresponding author

Abstract

Marine Protected Areas (MPAs) have been adopted globally as a tool to combat biodiversity loss and restore marine ecosystems. Successful application of MPAs will be predicated on the ability to monitor biodiversity in a synoptic and non-invasive manner. Environmental DNA (eDNA) methods have important advantages over traditional biodiversity survey methods for monitoring conservation areas. To evaluate the efficacy of eDNA metabarcoding for fish biodiversity monitoring, we sampled 19 coastal eelgrass beds in Canada, as eelgrass beds are known for high biodiversity and significant conservation value. At each site, beach seines were used to survey fish and water samples were collected contemporaneously for eDNA metabarcoding. In total, beach seining caught 32,672 individuals across 59 fish taxa and eDNA detected 129 fish taxa. eDNA captured site-level variation and detected higher species richness at both site and regional levels compared to seining. eDNA abundance had a positive association with capture abundance. Collectively these results highlight how eDNA metabarcoding offers an efficient approach for monitoring fish biodiversity in coastal eelgrass beds, thus providing a valuable and non-invasive tool for MPA planning and coastal monitoring.

Keywords: *12S rRNA*, *16S rRNA*, marine protected area, seagrass, seining

Introduction

Awareness of the implications of global biodiversity loss has increased over the past two decades, as has acknowledgement that biodiversity conservation should be part of broader economic and political discussions at both national and international levels (Worm et al. 2006; Cardinale et al. 2012). Indeed, combating biodiversity loss has become a point of international cooperation, with the Convention on Biological Diversity (CBD) setting various targets to combat and reduce loss (Thomas et al. 2014; Meehan et al. 2020). Marine Protected Areas (MPAs) have been adopted globally to conserve and restore marine ecosystems. Successful application and management of MPAs will be predicated on the ability to monitor and track changes in biodiversity in a synoptic and preferably non-invasive manner.

Coastal zones are dynamic ecosystems characterized by relatively high fish diversity (Suchanek 1994). They are at the interface of the terrestrial and marine realms and thus receive a wider range of cumulative impacts from human activities (Murphy et al. 2021). Seagrass beds in the coastal zone provide nursery, feeding, and foraging habitats for fish (Gillanders 2006), as well as provide other important ecosystem services including water purification, carbon sequestration, and shoreline erosion prevention (Cole and Moksnes 2016; Unsworth et al. 2019). Further, in Canada, eelgrass (*Zostera marina*) has been recognized as an ecologically significant species and is a key priority for conservation planning (DFO 2018a, 2019).

The Government of Canada has implemented an ambitious plan to protect marine biodiversity first by the renewed commitment to Aichi Target 11 (Schram et al. 2019) and later with a commitment to protect 25% of its ocean estate by 2025 and 30% by 2030. With these commitments comes the

need to develop and implement biodiversity monitoring programs to inform management. Monitoring biodiversity of coastal environments in Canada, which has the longest coastline in the world spanning over 240,000 km across three oceans, poses a significant challenge. The expanse and complexity of this coastline limits the ability to conduct systematic and comprehensive surveys to characterize biodiversity within nearshore marine habitats. The need to develop and implement biodiversity monitoring programs within coastal ecosystems at large spatial scales necessitates exploration of new approaches that can be deployed in a standardized fashion across Canada's expansive coastline.

Environmental DNA (eDNA) metabarcoding is a revolutionary approach for measuring biodiversity, which can rapidly and reliably characterize species composition from environmental samples without isolating target organisms (Taberlet et al. 2012; Rodríguez-Ezpeleta et al. 2021). With the development of improved filtration methods (Spens et al. 2017), more efficient fish-specific markers (e.g., Miya et al. 2015), and more accurate bioinformatics pipelines (e.g., Callahan et al. 2016), eDNA metabarcoding has been increasingly used to survey fish communities. Compared to traditional fish survey methods, eDNA metabarcoding offers some clear advantages. First, traditional capture-based and video-based methods generally lack the ability to sample the full spectrum of species and size classes (Cappo et al. 2004; Stoeckle et al. 2021), whereas the detection of extra-organismal DNA is less sensitive to the size-structure of sampled organisms (Taberlet et al. 2012; Rodríguez-Ezpeleta et al. 2021), enabling a much broader characterization of the fish community. Second, unlike capture-based methods, eDNA sampling is non-invasive, and itself imposes negligible negative effects on fish or fish habitats (Thomsen and Willerslev 2015). Third, species identification for eDNA data is based on DNA sequences, which

can be more accurate than morphology-based taxonomy methods for fish at early life stages (Pegg et al. 2006) and for morphologically similar but genetically different species when curated reference libraries are available (Danovaro et al. 2016; Goodwin et al. 2017). Lastly, eDNA samples are relatively easier and less costly to collect than traditional samples, especially for remote or otherwise difficult to access areas (Bohmann et al. 2014).

The evidence is building that eDNA metabarcoding may represent a decisive tool for measuring biodiversity within coastal environments. For sensitive, three-dimensional habitats, such as seagrass beds, the non-invasiveness of eDNA is of particular interest. Biodiversity associated with these ecosystems plays an integral role in their ecosystem function (Blake and Duffy 2012). In the nearshore where access is generally less restricted, eDNA has already proven an invaluable tool for evaluating and monitoring biodiversity (Lacoursière-Roussel et al. 2018; Aglieri et al. 2021). While studies have demonstrated the potential utility of eDNA as a biodiversity monitoring tool for eelgrass (Kelly et al. 2017; O'Donnell et al. 2017), its application in these ecosystems is still in its relative infancy.

The goal of this study was to evaluate the efficacy of eDNA metabarcoding as a fish biodiversity monitoring tool in eelgrass meadows through direct comparison to traditional fish biodiversity survey methods. To accomplish this evaluation we sampled 19 coastal sites in Atlantic and Pacific Canada. At each site, beach seines were used to assess fish diversity and compared to seawater sampled contemporaneously for eDNA metabarcoding. For eDNA metabarcoding, we used two fish-specific markers and built reference libraries for these two markers using Canadian fish species. Fish composition detected by eDNA and seining were compared, as were estimates of

regional (gamma) diversity, local (alpha) diversity, and species turnover (beta diversity) between the two sampling methods. Results were assessed and discussed in the context of the application of eDNA metabarcoding as a biodiversity monitoring tool in support of MPA planning and coastal monitoring in Canada.

Materials and methods

Site selection

We sampled 19 coastal sites selected based on the known presence of eelgrass beds and accessibility by boat or road (Fig. 1, Table S1). We sampled ten sites across approximately 2 degrees of longitude along the Atlantic coast of Nova Scotia, including four sites within the Eastern Shore Islands Area of Interest (AOI), an area under consideration for MPA establishment (Jeffery et al. 2020). We sampled nine Pacific sites on southern Vancouver Island, British Columbia (BC): seven on the eastern side of the island and two on the southwest end of the island. Five of the 9 sites in the Pacific are within the boundary of the proposed Southern Strait of Georgia National Marine Conservation Reserve (<https://www.pc.gc.ca/en/amnc-nmca/cnamnc-cnnmca/dgs-ssg/region-area>). Sampling on both coasts took place between July and September 2019.

Seining and fish identification

We sampled each site during daytime and within two hours of low tide. In the Atlantic, we used a 25 m long x 2 m high Danish bag seine net (19 mm stretch mesh in the wings and belly and 9 mm stretch mesh in the codend) for collections with 2-3 seine hauls at each site, separated by approximately 25 m. The seine was deployed from shore using a small boat (14' or 23' depending on site access and depth) and the net was hauled onshore by hand. The seine sampled from the

seabed to 2 m above bottom. In the Pacific, a 9.2 m long beach seine with a 4 mm stretch mesh throughout and a 3.1 m drop in the center was used. The net was deployed from shore and hauled onshore by hand. The net was set three times (each set adjacent to the previous) along an approximately 30 m length of eelgrass meadow. Nets used on each coast reflect the most commonly used seining gear for eelgrass in that region. Given that the objective of the study is to compare eDNA to traditional methods within each region, slight differences in regional catchability due to net specifications were not relevant to the analysis, particularly because species composition for each region is also different and therefore not directly comparable.

Captured fish hauled ashore were immediately transferred into dark totes or 20 L buckets filled with fresh seawater and aerators and then identified, and counted. Commonly encountered fish were identified and enumerated on-site before being returned to the capture site; taxa that were not readily identified to species in the field were also photographed and voucher specimens were collected for later morphological identification in the lab. After field identification, fish were transferred to aerated recovery buckets before release back into the ocean. Fish were sampled under Fisheries and Oceans Canada Scientific License (Pacific: #2018-502-00112; Atlantic: #332354) and following Animal Use Protocol 19-29 (Fisheries and Oceans Canada Maritimes Region).

Water collection and eDNA filtration

Three or four 1.0 L water samples were collected at each site prior to seining (to avoid contamination caused by the net or disturbed sediments). Water samples were collected by the same boat used for seining or by wading into the water. Adjustable metal hose clamps were used

to secure a cleaned and sterilized (using 6% v/v sodium hypochlorite solution) Nalgene bottle to a sampling pole made of a T-splitter attached to a 5' length of 1.5" PVC pipe. The lid was removed and the bottle was filled and rinsed with seawater at least three times (to ensure no trace bleach remained). The bottle was then held approximately 30 cm below the surface, filled with seawater, and closed. Samples were stored on ice in the dark for transport to the lab (Atlantic) or for immediate on-site filtration (Pacific). One field blank consisting of 1.0 L distilled water was collected at each site by pouring the water from one bottle into another (Atlantic) or by opening the bottle at the site for approximately the time required to collect an individual sample (Pacific).

Samples from both coasts were filtered using a peristaltic pump with L/S 15 or 25 Tygon tubing with 0.22 μm polyvinylidene fluoride (PVDF) Sterivex cartridge filters (Fisher Scientific). In the Pacific, samples were filtered on-site using an 18V Milwaukee brushless drill attached to a Masterflex Easy Load II SS pump head. In the Atlantic, samples were transported to the lab and filtered within 6 hours of collection using bench-top pumps. All tubing and surfaces were bleached using a 6% v/v sodium hypochlorite solution between samples and rinsed with tap and then distilled water to remove bleach residue. Additionally, at least 50 mL of seawater was run through the tubing prior to filtration to remove any remaining bleach solution. Filters were preserved by injecting approximately 200 μL Longmire buffer (Longmire et al. 1997) in the cartridge inlet using sterile syringes. Filters were then sealed and stored at $-20\text{ }^{\circ}\text{C}$ until DNA extraction.

DNA extraction and eDNA metabarcoding

DNA was extracted using DNeasy Blood & Tissue Kits (Qiagen) based on Spens et al. (2017) with minor modifications (Supplementary file 1). One DNA extraction control was included for each

batch of DNA extraction. In total, DNA was extracted from 87 samples, which consisted of 61 coastal water samples, 19 field controls for each of the 19 sites, one lab control consisting of 1.0 L distilled water filtered in the laboratory, and six DNA extraction controls.

Two markers targeting the mitochondrial DNA *12S* and *16S rRNA* genes were used for fish eDNA metabarcoding. For 12S, a new forward primer (12S_248F: CGTGCCAGCCACCGCGGTT) was designed instead of using the Miya et al. (2015) forward primer (MiFish-U-F) as it had higher sequence complementarity with Elasmobranchs, Osmeridae, and Gadiformes. For design of the 12S_248F primer, approximately 600 mtDNA genomes were downloaded from GenBank and the *12S rRNA* regions were extracted and aligned in Geneious 9.1.8 and the new primer location was selected by eye. The newly-designed forward primer and the reverse primer MiFish-U-R (CATAGTGGGGTATCTAATCCCAGTTTG) (Miya et al. 2015) amplified *ca.* 160 bp. The 16S primers (16S_Fish_F and 16S_Fish_R) amplified *ca.* 200 bp (McInnes et al. 2017). These markers were selected by doing an alignment for each of the *12S rRNA* and *16S rRNA* genes using sequences from a diverse selection of Canadian fish. Primer regions for three 12S markers (MiFish, Miya et al. 2015; Teleo, Valentini et al. 2016; and the 12S primer set mentioned above) and three 16S markers (16S fish-specific, Shaw et al. 2016; Ac16S, Evans et al. 2016; and 16S_Fish, McInnes et al. 2017) were identified and inspected by eye to select one primer set for each gene based on highest complementarity to target taxa. For eDNA metabarcoding, a dual-indexing approach with one-step PCR library preparation was adopted by using fusion primers containing: an Illumina adapter sequence, an index, a sequencing primer binding region, and a marker-specific primer sequence. The fusion primer sequences, except for marker-specific primer regions, followed Lacoursière-Roussel et al. (2018).

198
199 PCR amplification was performed in 25 μ L reactions containing 12.5 μ L 2x QIAGEN Multiplex
200 PCR Master Mix (Qiagen), 8.5 μ L water, 0.5 μ L of 10 μ M forward primer, 0.5 μ L of 10 μ M
201 reverse primer, and 3 μ L template. PCR thermal cycling for the 12S marker was 95°C for 15 min,
202 followed by 35 cycles of 94°C for 30 s, 58°C for 90 s and 72°C for 60 s, followed by a final
203 extension at 72°C for 10 min. The same cycling program was used for the 16S marker except that
204 40 amplification cycles were used. For each sample, four PCR reactions were performed at each
205 marker: three with DNA template and one as PCR negative control (i.e., with water as template).
206 PCR amplifications were checked on a QIAxcel (Qiagen) using the QIAxcel DNA Screening kit
207 with the 15bp/3kb QX Alignment marker to ensure no amplification in PCR negative controls. The
208 three PCR amplifications of each sample were pooled and then purified using an AxyPrep Mag™
209 PCR Clean-up Kit (Axygen). These purified PCR products were quantified using an AccuClear
210 Ultra High Sensitivity dsDNA Quantification Kit (Biotium) with a Synergy H1 microplate reader
211 (BioTek). Purified PCR products for each marker were combined into separate, single-marker
212 libraries, as follows: 30 ng or 50 ng of purified PCR product from each sample was added to the
213 multiplexed library for 12S and 16S, respectively. In cases where there was insufficient DNA to
214 meet the target amount, 10 μ L of purified PCR product was added. Each library was purified using
215 the AxyPrep Mag™ PCR Clean-up Kit again and then quantified using a Qubit™ dsDNA HS
216 Assay Kit (Thermo Fisher Scientific). Separate MiSeq runs were performed for the two markers
217 using MiSeq v2 (300 cycles) kits (Illumina).

218

219 **Bioinformatics and statistical analyses**

220 Raw sequence data were imported into QIIME2 using the import plugin (Bolyen et al. 2019).
221 Gene-specific primer sequences were trimmed using the cutadapt plugin with the parameters *--p-*
222 *error-rate* set to '0.11' and *--p-discard-untrimmed* set to 'True' for both markers (Martin 2011).
223 Trimmed sequences were then processed using the dada2 plugin (Callahan et al. 2016), producing
224 an amplicon sequence variant (ASV) table and a feature sequence file for each marker. For the
225 dada2 plugin, the parameters for *--p-trunc-len-f*, *--p-trunc-len-r*, and *--p-n-threads* were set to 116,
226 108 and 0 for 12S and 125, 125 and 0 for 16S. All other parameters were set to the default value.
227 We did not do OTU clustering as using ASVs offers some clear benefits relative to OTUs as
228 outlined in Callahan et al. (2017). In the raw ASV table for each marker, the following was done
229 to reduce potential false positives caused by unavoidable contamination and tag jumping (Schnell
230 et al. 2015; Zinger et al. 2019): rare ASVs in each sample were excluded (i.e., the numbers were
231 changed to 0) if the number of reads of an ASV in a sample was less than 0.1% of the total read
232 number in that sample or was less than 0.1% of the total read number of that ASV across all
233 samples. This threshold was used following previous fish eDNA metabarcoding studies (Hänfling
234 et al. 2016; Stoeckle et al. 2017).

235
236 A custom reference library was built for each marker for taxonomy assignment using the fuzzy-
237 set-theory-based method (Zhang et al. 2012). To build custom reference libraries, a Canadian bony
238 marine fish species list (Table S2) was generated by the following steps: downloading the
239 Canadian fish list from Brian Coad's website (<http://www.briancoad.com/>); retaining only bony
240 fish (i.e., Actinopterygii); removing freshwater fish; and adding missing marine fish species with
241 records in Canada based on OBIS (<https://obis.org/>). The approach of curating reference libraries

to include only those species known to occur within the focal area potentially limits the detection of invasive species or newly-occurring range-expanded species; however, it is known to greatly improve accuracy of taxonomy assignment (Zinger et al. 2019). *12S rRNA* and *16S rRNA* sequences from all Canadian bony marine fish species were then downloaded from NCBI. These data were quality screened to minimize the chance of retaining erroneous sequences by discarding those with very high genetic distances among conspecifics. Furthermore, identical haplotypes among species from the same family were grouped into species-groups (see details in Tables S3 and S4) and identical haplotypes among species from different families were discarded as likely errors.

Taxonomy assignments were performed for each marker using the fuzzy-set-theory-based method (Zhang et al. 2012) with the custom reference library and assignments were secondarily assessed by comparing them to the BLAST top hit method (using remote BLAST and the NCBI Nucleotide database). The fuzzy-set-theory-based taxonomy assignments were performed using the FuzzyID2 R package (Shi et al. 2018), and taxonomic status was assigned for ASVs that had a Fuzzy Membership Function (FMF) value ≥ 0.90 to a single species or species-group (Zhang et al. 2012). Given the generation of new custom libraries and that FuzzyID2 has not been widely used for eDNA data, we checked for discrepancies between taxonomy assignment by FuzzyID2 and BLAST top hit using the NCBI Nucleotide database as a means of flagging potential misassignments. In rare instances (i.e., 3.4% and 0.8% of assignments for 12S and 16S, respectively) for which assignments were different between the two methods, other information was used to determine taxonomy for these ASVs or the ASVs were removed (see Table S5 for details).

As one major objective of this study was to compare fish biodiversity between two methods, unassigned ASVs were discarded. For ASVs found in DNA extraction controls, the number of reads of that ASV in the control was subtracted from the number of reads of that ASV in samples. For ASVs with reads in field controls, the numbers of reads of that ASV in the field control was subtracted from the number of reads in the water samples from that site following Nguyen et al. (2015). For 12S data, three samples were removed due to suspected contamination from samples collected on the other coast that likely occurred when adding DNA template for PCR. Detailed numbers of reads in each sample at each step were provided in Tables S6 and S7.

For gamma and alpha diversity analyses, species richness (i.e., number of taxa detected) was analyzed at regional and site levels, respectively. Sample-size- and coverage-based species accumulation curves, with associated confidence intervals, were generated using the R package iNEXT (Hsieh et al. 2016). Here sample-size-based analyses compare species richness at equal sample sizes while coverage-based analyses compare species richness at equal sampling completeness (Chao and Jost 2012). For beta diversity analyses, samples from the two coasts were analyzed separately to compute Bray-Curtis dissimilarity distances, whereby seining data were used as-is while 12S and 16S ASV tables were collapsed into fish taxa tables and then normalized using the cumulative sum scaling (CSS) method to address different number of reads among samples (Paulson et al. 2013). Principal Coordinate Analyses (PCoA), Mantel tests, and adonis analyses were performed using QIIME2. We conducted Mantel tests to analyze correlations between Bray-Curtis community dissimilarity distances and geographic distances. We computed geographic distances as the minimum geographic spanning distance between sites (great circle distance (gcd)) and the seascape distance which considers the shortest or least cost path (lcp)

between two sites constrained within the marine setting. Distances were calculated in R using the `sf` package (Pebesma 2018) for `gcd` and the `gdistance` package (Etten 2017) for `lcd`, with the distance costs based on landforms developed from the `rnaturalearth` package (<https://github.com/ropensci/rnaturalearth>).

Linear mixed effects models were used to assess the relationship between relative abundances obtained by eDNA versus seining using taxa detected by both methods. The relative abundance of each taxon at each site was calculated in the seining data; the relative abundance of each taxon in each sample was calculated in the eDNA data and then average relative abundance of each taxon at each site was calculated; data were then log-transformed. Log-transformed eDNA relative abundance was set as the response variable, while log-transformed seining relative abundance was set as a fixed effect and species was set as a random effect for each model using the `lmer` function call of the R package `lme4` (Bates et al. 2015). The association between abundances were plotted using the `ggeffects` R package (Lüdtke 2021).

Results

Summary of seining data

Atlantic coast seining caught a total of 23,672 fish which consisted of 22 taxa (Table S8). Pacific coast seining caught 9,001 fish, but one was excluded as it was not identifiable, hence the final number analyzed was 9000, comprising 38 taxa (Table S9). Atlantic herring (*Clupea harengus*), sand lance (*Ammodytes* sp.) and three-spined stickleback (*Gasterosteus aculeatus*) dominated the species composition at Atlantic sites, while bay pipefish (*Syngnathus leptorhynchus*) dominated at Pacific sites. Three-spined stickleback was the only species caught on both coasts.

Summary of eDNA metabarcoding data

For the 12S marker, 6.44 million raw paired-end reads were generated for 87 samples. In the final 12S ASV table, there were 331 ASVs from a total of 3.36 million reads across 56 samples (Table S6). These ASVs were assigned to 80 fish species and 17 species-groups, the latter representing identical sequences shared by multiple fish species, of which 13 contained species from the same genus and four contained species from different genera within a family (Table S10).

For 16S, 9.39 million raw paired-end reads were generated. In the final 16S ASV table, there were 493 ASVs from a total of 7.48 million reads across 61 samples (Table S7). These ASVs were assigned to 82 fish species and nine species-groups. Of the species-groups, seven contained species from the same genus and two contained species from different genera within a family (Table S10).

Comparison between seining and eDNA data

Across all 19 sites, seining detected 59 fish taxa whereas eDNA detected 129 (combining data from both markers). These taxa were classified to the lowest taxonomic level possible. For seining, 88.1% (52/59) were morphologically identified to species level, 10.2% (6/59) to genus level only, and 1.7% (1/59) to family level only. For eDNA data, 79.8% (103/129) were assigned to species level and 20.2% (26/129) were assigned to a species-group. All fish families detected by seining were also detected by eDNA except Tetraodontidae (pufferfish), with eDNA detecting 44 fish families and seining detecting 28 (Fig. 2). Considering species level assignments only, the number of species detected by eDNA was twice that detected by seining (Fig. 2). However, seven of 52 species caught by seining were not similarly detected by eDNA, as follows: four were detected by

eDNA within a species-group, and three were not detected by eDNA at all. Importantly, only one or two individuals were netted for each of the three species caught by seines that were not detected by eDNA (namely, mackerel scad *Decapterus macarellus*, northern puffer *Sphoeroides maculatus*, and rough scad *Trachurus lathami*).

At the regional level, both eDNA and seining detected more fish taxa in the Pacific than in the Atlantic region. The 12S detected 42 fish taxa in the Atlantic region and 58 fish taxa in the Pacific region. The 16S detected 40 fish taxa in the Atlantic region and 54 fish taxa in the Pacific region. Combining the two genetic markers, eDNA metabarcoding detected 56 fish taxa in the Atlantic region and 78 fish taxa in the Pacific region. In comparison, seining captured 22 fish taxa in the Atlantic region and 38 fish taxa in the Pacific region.

Species accumulation curves showed species richness detected by eDNA was much higher than that detected by seining on both coasts using both rarefaction and extrapolation methods (Fig. 3). Differences in estimates of species richness between seining and all eDNA-based estimates (i.e., using either genetic marker or both) were significant in both the Atlantic and the Pacific, as their 95% CIs did not overlap. Sample coverage estimates for sampled sites for seining, 12S, 16S, and two markers combined data were 0.910, 0.934, 0.926 and 0.942 in the Atlantic and 0.928, 0.964, 0.949 and 0.965 in the Pacific. Coverages were higher for eDNA than seining on both coasts.

Seining caught 4-12 fish taxa per site in the Atlantic and 13-19 fish taxa in the Pacific, while using two markers eDNA detected 19-29 fish taxa per site in the Atlantic and 27-54 fish taxa in the Pacific (Fig. 4, Fig. S1). For sites within the Atlantic region, eDNA detected 25 ± 3 (Mean $\pm$ SD)

fish taxa per site and seining caught 8 ± 3 fish taxa per site. For sites within the Pacific region, eDNA detected 37 ± 9 fish taxa per site and seining caught 15 ± 2 fish taxa per site.

Principle Coordinate Analysis (PCoA) of Bray-Curtis dissimilarity distances showed that eDNA replicates from the same site clustered together for most sites, and eDNA metabarcoding clearly captured the site-level variation in species composition and diversity (Fig. 5). Adonis analyses showed that site explained 83%, 83%, 79% and 75% of variation in Bray-Curtis distance in the Atlantic 12S, Atlantic 16S, Pacific 12S and Pacific 16S datasets, respectively (Table S11). Mantel tests showed that species turnover based on beach seine data significantly correlated with geographic distances (gcd and lcp) in the Pacific (correlation coefficient of 0.66 and 0.48 respectively), whereas the correlations were not significant between geographic distances and Bray-Curtis dissimilarity distances in the Atlantic based on seining data (Table S11). For eDNA data, correlations between geographic distances (gcd and lcp) and Bray-Curtis distances were significant for all four eDNA datasets (i.e., Atlantic 12S, Atlantic 16S, Pacific 12S and Pacific 16S) with coefficients ranging between 0.19 and 0.33 (Table S11). Overall, there was a significant relationship between relative abundances of fish per taxon obtained by the two methods for each marker in each region (Fig. 6, Table S12), and the relationship was stronger in the Pacific than in the Atlantic for both markers, as the fixed factor explained more proportion of variance (i.e., the marginal R^2 were bigger) for the former (Table S12).

Assessing other available data to substantiate species detections by eDNA

As we detected many fish taxa by eDNA that were not caught by seining, species detected by eDNA in each region were checked using public databases to help understand whether these

detections are plausible or if they may be errors. The species were first checked using FishBase to determine if they occur in that region; when it was not clear, the species were further checked using OBIS records. Only four species detected by eDNA were not associated with an occurrence record in their respective region in at least one of these databases: giant wrymouth (*Cryptacanthodes giganteus*) (detected by both markers), longfin gunnel (*Pholis clemensi*) (detected by 12S), and the species-groups AM2_16S and AM3_16S. Both AM2_16S and AM3_16S include *Ammodytes hexapterus* and *Ammodytes personatus*, both of which are commonly referred to as Pacific sand lance. The detection of the four taxa in Atlantic samples was surprising as the two species are Pacific species, and the two species-groups have more records from the Pacific coast than the Atlantic coast. The ASVs assigned to giant wrymouth were identical to or had 1 bp difference from giant wrymouth sequences in the NCBI database. Although assignments for these ASVs had high confidence, the true identity of the ASVs could be from another species wrymouth (*Cryptacanthodes maculatus*) that has been observed in the area and for which no 12S or 16S sequences were available. Longfin gunnel is a Pacific species and has not been recorded in the Atlantic region; the 12S ASV we detected may be from a species whose sequence is not in the reference database. Although *A. hexapterus* is recorded in all three Canadian Oceans, it occurs much less frequently in the Atlantic according to OBIS. The assignments of 16S ASVs to AM2_16S and AM3_16S may be due to not having complete understanding of the 16S sequence diversity of the Atlantic *Ammodytes* genus (e.g., northern sand lance *A. dubius*, and American sand lance *A. americanus*).

The majority of fish taxa detected by eDNA were region-specific, with a few exceptions. Using the 12S marker, one species and two species-groups were detected at both coasts: three-spined

stickleback (also detected by seining on both coasts), CL1_12S, and GA1_12S. CL1_12S includes American shad (*Alosa sapidissima*) and twaite shad (*Alosa fallax*): American shad is native to the Atlantic region and was introduced to the Pacific and subsequently spread. Twaite shad is recorded in Canada, although records of this species are rare. The GA1_12S includes Pacific cod (*Gadus macrocephalus*), Atlantic cod (*G. morhua*), and Alaska pollock (*G. chalcogrammus*); the occurrence of GA1_12S on both coasts was not surprising as it includes both Pacific and Atlantic species. Similarly, one species and two species-groups were detected at both coasts using the 16S marker: three-spined stickleback, AM2_16S, and PL5_16S. Detection of AM2_16S in the Atlantic region was a bit surprising as we mentioned above. PL5_16S includes six species in the family Pleuronectidae (Table S10) and its occurrence in both regions was not surprising.

Discussion

Expanding marine conservation measures to combat biodiversity loss has brought a clear need for monitoring approaches that can develop baselines and track change. eDNA holds promise as a key tool for addressing this growing need by providing a non-invasive and comprehensive approach to monitoring biodiversity (Thomsen and Willerslev 2015). We evaluated the efficacy of eDNA metabarcoding for monitoring fish biodiversity in eelgrass meadows, a habitat sensitive to disturbance that supports high fish diversity and of high conservation value. We compared fish composition and relative abundance of fishes surveyed by beach seining and eDNA metabarcoding at 19 coastal eelgrass beds along the Pacific Northeastern and Atlantic Northwestern coasts in Canada. We found eDNA metabarcoding provided a more comprehensive assessment of fish species composition and characterization of both alpha and gamma fish diversity in coastal environments compared to traditional beach seining, and clearly captured site-level variation.

These results highlight the potential of eDNA metabarcoding as an effective, non-invasive approach for monitoring coastal fish biodiversity, including in sensitive ecosystems like eelgrass meadows, thus representing a promising tool for monitoring coastal conservation areas.

Many studies have demonstrated the utility of eDNA metabarcoding for characterizing fish diversity in marine ecosystems (e.g., Stat et al. 2019; Aglieri et al. 2021; Gold et al. 2021; Stoeckle et al. 2021), and it has been shown to accurately reflect known or observed patterns of community composition and species turnover at fine spatial and temporal scales (Lacoursière - Roussel et al. 2018; Jeunen et al. 2019; West et al. 2020; Stoeckle et al. 2021). While some studies have demonstrated that eDNA detected greater fish diversity than direct sampling methods (e.g., Gehri et al. 2021; Gold et al. 2021; Stoeckle et al. 2021), other studies have found eDNA detected comparable (e.g., Shaw et al. 2016) or even fewer fish taxa (e.g., Thomsen et al. 2016; DiBattista et al. 2017). In this study, the number of fish taxa detected by eDNA metabarcoding was approximately twice the number captured by beach seining across all samples, and nearly all fish taxa captured by seining were also detected by eDNA. Similarly, species richness detected by eDNA was much higher than by seining at both the site (alpha diversity) and regional (gamma diversity) levels.

Species captured only by seining were rare (one or two individuals captured) and relatively small in size, suggesting that eDNA concentrations for these species in the environment were too low to detect with our sampling method. In contrast, eDNA captured numerous species that were not observed in nets and are primarily considered rare in Canada. For example, eDNA from the Atlantic revealed the presence of two semi-tropical species spotfin butterflyfish (*Chaetodon*

ocellatus) and queen triggerfish (*Balistes vetula*); both of which were likely carried into northern waters via the northward flowing Gulf Stream, mirroring similar patterns of warm water species increasingly observed offshore on the Scotian Shelf (DFO 2018b; O'Neill 2021). On the Pacific coast, eDNA was able to detect several taxa of conservation interest that were not captured in the beach seines, including five species of Pacific salmon. For example, Chinook salmon (*Oncorhynchus tshawytscha*) was detected by eDNA at eight of nine sites but not captured in seines at any site. This is particularly important because Chinook salmon populations in the Salish Sea have declined 60% since 1984 (Pacific Salmon Commission 2019), so the use of non-invasive eDNA to better understand juvenile habitat use in coastal areas could greatly increase our ability to monitor the spatial distribution of this species without causing any capture mortality. Finally, Pacific results are corroborated with other eelgrass associated fish diversity studies in BC. For example, Iacarella et al. (2018) sampled 89 eelgrass meadows across the coast of BC by beach seines, covering a much larger geographic area than this study. Our eDNA metabarcoding results from just 9 sites detected 54 of 64 species (84%) captured by Iacarella et al. (2018). This comparison highlights the efficiency of eDNA metabarcoding for surveying eelgrass-associated fish communities.

When comparing methods it is important to consider both capture efficiency and sampling bias. Beach seining can be biased by the capture efficacy of the net and size of the mesh, which is often predicated on the size of the fish. Similarly, eDNA metabarcoding for fish diversity can also be biased due to template competition during PCR (Zinger et al. 2019), differing accuracies of taxonomy assignment methods (Hleap et al. 2021), and lack of resolution in the genetic markers for some species. Here we demonstrate that eDNA metabarcoding captured a broader spectrum of

species than seining, with the difference in selectivity even more pronounced when multiple markers were used: two markers detected 33% and 42% more species than 12S and 16S markers alone, respectively. Although the two markers lack of species identification for specific genera (e.g., rockfish, cod species), it was not specifically a concern because our study was trying to test whether eDNA can capture fish diversity in a comparison to direct sampling methods and not necessarily surprising given that there lack of markers available that can characterize fish diversity across many taxa, especially in temperate ecosystems. This highlights that eDNA metabarcoding can provide a more comprehensive overview of fish biodiversity than traditional seining.

Although eDNA metabarcoding has been widely used to survey fish biodiversity, there is currently no consensus for a standardized eDNA metabarcoding protocol and bioinformatic pipelines (Spens et al. 2017; Prodan et al. 2020; Zaiko et al. 2021). Results can be affected by changes at one or more of the many steps in the protocol: sample collection, water filtration, DNA extraction, genetic marker selection, library preparation, sequence quality control, feature (e.g., ASV vs. OTU) table construction, and taxonomy assignment. Among those steps, taxonomy assignment is especially critical for eDNA metabarcoding that is strongly affected by the reference library and assignment method used. Accurate taxonomy assignment to species level is more challenging than to higher taxonomic levels (Hleap et al. 2021), but it is critical and often the target of biodiversity monitoring. Here we minimized erroneous assignments by building custom reference libraries and using two taxonomy assignment methods, leading to what we argue to be confident taxonomic assignment of fish metabarcoding reads to species level or species-group. The protocol used in this study provides an important basis for fish biodiversity monitoring in coastal ecosystems.

Quantifying spatial patterns in species assemblage and turnover is an important aspect for biodiversity monitoring. These patterns help to reveal the commonness or rarity of species or assemblages and changes in these patterns can help to understand how ecosystem function responds to environmental (i.e., climate change) and management (e.g., conservation regulations) contexts. We found that eDNA captured spatial variation in species assemblages, with fidelity among site-level replicates clustered in PCoA analyses. PCoA and adonis analyses showed strong site effects on eDNA-based species composition and abundance, supporting the validity of the eDNA approach to characterize patterns in beta (turnover) diversity. For both the Pacific and Atlantic coasts, correlations of compositional dissimilarity and geographic distance were overall weak, suggesting that factors other than geography contributed to site-level variation. Given the relatively limited geographic extent of sampling (< 110 km and 175 km of coastline for the Pacific and Atlantic coasts, respectively) this result is not necessarily surprising, but importantly eDNA did capture variation associated with common physical attributes including clustering of sites that were more exposed and not sheltered by headlands or islands on the Atlantic Coast (i.e., PLS, CAB, RSE). Collectively, these results indicate that eDNA is a sensitive tool to capture patterns in beta diversity for coastal monitoring and conservation planning.

The ability of eDNA metabarcoding to estimate relative or absolute species abundance remains a key question with high value in fisheries and conservation science. The relative abundances of fish captured by the two methods in this study exhibit a positive, yet variable relationship. Potential factors leading to this weak correlation include that eDNA read abundances per species within samples are affected by technical variation during library preparation and sequencing as well as the taxonomic complexity of the sample. The amount of eDNA in a sample is also impacted by

environmental factors (e.g., Buxton et al. 2017), fish biomass, and life-stage (e.g., Maruyama et al. 2014) and thus may not show a simple relationship with fish capture abundance. Despite several factors influencing the relationship between eDNA and capture abundance, studies including ours have shown metabarcoding can generate a significant, and in some cases strong, association between read counts and biomass (Stoeckle et al. 2021; Russo et al. 2021). It remains possible that eDNA read counts could be used to quantify fish numbers and our results suggest that at minimum, eDNA could be used to evaluate relative differences in abundance among sites or sampling events, but further research is needed to better understand this relationship and factors affecting it.

The relative ease with which eDNA water samples can be collected without damaging or disturbing sensitive ecosystems makes eDNA metabarcoding a promising tool to characterize species composition and distribution at large temporal and spatial scales. This is especially applicable within complex and/or sensitive areas, like eelgrass beds that are of key conservation concern for their roles in providing fish habitat and blue carbon storage. Because eDNA collection can be standardized in different ecosystems and geographic locations, unlike conventional capture-based methods (as gear types vary across habitat types), eDNA based biomonitoring holds significant promise to be deployed at scale (e.g., within the Canadian Coastal Conservation Network). Overall, our results suggest that eDNA can be a useful tool for rapidly assessing fish diversity in the coastal zone and within sensitive three-dimensional habitats, where data availability is relatively poor; in Canada there are few comprehensive, annual coastal biodiversity surveys performed on a broad scale compared to the offshore environment, where fisheries-dependent and -independent surveys are regularly carried out. Our study provides an important empirical basis to demonstrate the application of eDNA for biodiversity characterization and on

which to apply eDNA metabarcoding as a tool to characterize alpha, beta, and gamma fish biodiversity in temperate coastal ecosystems and marine conservation areas in Canada. With the continued development of fish reference libraries, and the standardization of eDNA metabarcoding protocols and bioinformatics pipelines, eDNA metabarcoding can provide a key tool to support monitoring of Canadian marine conserved and coastal areas.

Acknowledgements

The authors thank Shawn Roach, John O'Brien, Catalina Gomez, Kara Layton, Ed Horne, Geoff Lowe, and Josephine Iacarella for assistance in the field, Melisa Wong, Benedikte Vercaemer, Jennifer Yakimishyn, and Lily Burke for providing information on eelgrass bed locations and field guides of commonly observed fish, Angela Schulze for help with MiSeq sequencing, and Daphne Themelis for identifying Atlantic fish voucher specimens. We thank Jennifer Sunday for advice on filtration. Bob Gregory and Dave Cote provided valuable seining training and discussions on eelgrass bed fish communities.

Competing interests statement

The authors declare no conflict of interest.

Contributors' statement

C.L.A., E.M.R., R.R.E.S. and N.W.J. conceived this study. E.M.R., R.R.E.S., N.W.J., K.S.P.G., L.M.D.R., and S.G.H. did field work. R.S. designed a new primer. S.R.G., X.H. and C.L.A. selected primers for this study. L.C.H., X.H. and L.M.D.R. did molecular lab work. X.H., R.R.E.S.,

K.M.W. and S.R.G. did data analyses. X.H., R.R.E.S., E.M.R., N.W.J. and C.L.A. wrote the first draft. All authors contributed to the final version of the paper.

Funding statement

Funding for this study was provided through a Genomics Research and Development Initiative grant.

Data availability statement

MiSeq sequences were submitted to NCBI SRA database (Accession number: PRJNA740347). All code associated with R for this manuscript is available online (github.com/rystanley/biodiversity_edna).

References

- Aglieri, G., Baillie, C., Mariani, S., Cattano, C., Calò, A., Turco, G., Spatafora, D., Di Franco, A., Di Lorenzo, M., Guidetti, P., and Milazzo, M. 2021. Environmental DNA effectively captures functional diversity of coastal fish communities. *Mol Ecol* **30**(13): 3127–3139. doi:10.1111/mec.15661.
- Bates, D., Mächler, M., Bolker, B., and Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* **67**(1): 1–48. doi:10.18637/jss.v067.i01.
- Bessey, C., Jarman, S.N., Berry, O., Olsen, Y.S., Bunce, M., Simpson, T., Power, M., McLaughlin, J., Edgar, G.J., and Keesing, J. 2020. Maximizing fish detection with eDNA metabarcoding. *Environmental DNA* **2**(4): 493–504. doi:https://doi.org/10.1002/edn3.74.

- 585 Blake, R.E., and Duffy, J.E. 2012. Changes in biodiversity and environmental stressors influence
586 community structure of an experimental eelgrass *Zostera marina* system. *Marine Ecology*
587 *Progress Series* **470**: 41–54. doi:10.3354/meps10006.
- 588 Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W., and de
589 Bruyn, M. 2014. Environmental DNA for wildlife biology and biodiversity monitoring.
590 *Trends in Ecology & Evolution* **29**(6): 358–367. doi:10.1016/j.tree.2014.04.003.
- 591 Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander,
592 H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod,
593 A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J.,
594 Cope, E.K., Silva, R.D., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M.,
595 Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons,
596 S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste,
597 H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D.,
598 Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciulek, T.,
599 Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher,
600 M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L.,
601 Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian,
602 S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Priesse, E., Rasmussen, L.B.,
603 Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R.,
604 Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi,
605 A., Turnbaugh, P.J., Ul-Hasan, S., Hooft, J.J.J. van der, Vargas, F., Vázquez-Baeza, Y.,
606 Vogtmann, E., Hippel, M. von, Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C.,
607 Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight,

- 608 R., and Caporaso, J.G. 2019. Reproducible, interactive, scalable and extensible
609 microbiome data science using QIIME 2. *Nat Biotechnol* **37**(8): 852–857.
610 doi:10.1038/s41587-019-0209-9.
- 611 Buxton, A.S., Groombridge, J.J., Zakaria, N.B., and Griffiths, R.A. 2017. Seasonal variation in
612 environmental DNA in relation to population size and environmental factors. *Sci Rep* **7**(1):
613 46294. doi:10.1038/srep46294.
- 614 Bylemans, J., Gleeson, D.M., Hardy, C.M., and Furlan, E. 2018. Toward an ecoregion scale
615 evaluation of eDNA metabarcoding primers: A case study for the freshwater fish
616 biodiversity of the Murray–Darling Basin (Australia). *Ecology and Evolution* **8**(17): 8697–
617 8712. doi:https://doi.org/10.1002/ece3.4387.
- 618 Callahan, B.J., McMurdie, P.J., and Holmes, S.P. 2017. Exact sequence variants should replace
619 operational taxonomic units in marker-gene data analysis. *ISME J* **11**(12): 2639–2643.
620 doi:10.1038/ismej.2017.119.
- 621 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. 2016.
622 DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods*
623 **13**(7): 581–583. doi:10.1038/nmeth.3869.
- 624 Cappel, M., Speare, P., and De'ath, G. 2004. Comparison of baited remote underwater video
625 stations (BRUVS) and prawn (shrimp) trawls for assessments of fish biodiversity in inter-
626 reefal areas of the Great Barrier Reef Marine Park. *Journal of Experimental Marine*
627 *Biology and Ecology* **302**(2): 123–152. doi:10.1016/j.jembe.2003.10.006.
- 628 Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P., Narwani, A.,
629 Mace, G.M., Tilman, D., Wardle, D.A., Kinzig, A.P., Daily, G.C., Loreau, M., Grace, J.B.,

- 630 Larigauderie, A., Srivastava, D.S., and Naeem, S. 2012. Biodiversity loss and its impact
631 on humanity. *Nature* **486**(7401): 59–67. doi:10.1038/nature11148.
- 632 Chao, A., and Jost, L. 2012. Coverage-based rarefaction and extrapolation: standardizing samples
633 by completeness rather than size. *Ecology* **93**(12): 2533–2547. doi:10.1890/11-1952.1.
- 634 Cole, S.G., and Moksnes, P.-O. 2016. Valuing multiple eelgrass ecosystem services in Sweden:
635 fish production and uptake of carbon and nitrogen. *Front. Mar. Sci.* **2**: 121.
636 doi:10.3389/fmars.2015.00121.
- 637 Danovaro, R., Carugati, L., Berzano, M., Cahill, A.E., Carvalho, S., Chenuil, A., Corinaldesi, C.,
638 Cristina, S., David, R., Dell’Anno, A., Dzhenbekova, N., Garcés, E., Gasol, J.M., Goela,
639 P., Féral, J.-P., Ferrera, I., Forster, R.M., Kurekin, A.A., Rastelli, E., Marinova, V., Miller,
640 P.I., Moncheva, S., Newton, A., Pearman, J.K., Pitois, S.G., Reñé, A., Rodríguez-Ezpeleta,
641 N., Saggiomo, V., Simis, S.G.H., Stefanova, K., Wilson, C., Lo Martire, M., Greco, S.,
642 Cochrane, S.K.J., Mangoni, O., and Borja, A. 2016. Implementing and innovating marine
643 monitoring approaches for assessing marine environmental status. *Front. Mar. Sci.* **3**: 213.
644 doi:10.3389/fmars.2016.00213.
- 645 DFO. 2018a. Design strategies for a network of marine protected areas in the Scotian Shelf
646 Bioregion. Available from <https://waves-vagues.dfo-mpo.gc.ca/Library/40688070.pdf>.
- 647 DFO. 2018b. Maritimes research vessel survey trends on the Scotian Shelf and Bay of Fundy.
- 648 DFO. 2019. Design strategies for the Northern Shelf Bioregional marine protected area network.
649 Available from <https://waves-vagues.dfo-mpo.gc.ca/Library/40817787.pdf>.
- 650 DiBattista, J.D., Coker, D.J., Sinclair-Taylor, T.H., Stat, M., Berumen, M.L., and Bunce, M. 2017.
651 Assessing the utility of eDNA as a tool to survey reef-fish communities in the Red Sea.
652 *Coral Reefs* **36**(4): 1245–1252. doi:10.1007/s00338-017-1618-1.

- Etten, J. van. 2017. R Package gdistance: Distances and routes on geographical grids. *Journal of Statistical Software* **76**(1): 1–21. doi:10.18637/jss.v076.i13.
- Evans, N.T., Olds, B.P., Renshaw, M.A., Turner, C.R., Li, Y., Jerde, C.L., Mahon, A.R., Pfrender, M.E., Lamberti, G.A., and Lodge, D.M. 2016. Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. *Mol Ecol Resour* **16**(1): 29–41. doi:10.1111/1755-0998.12433.
- Gehri, R.R., Larson, W.A., Gruenthal, K., Sard, N.M., and Shi, Y. 2021. eDNA metabarcoding outperforms traditional fisheries sampling and reveals fine-scale heterogeneity in a temperate freshwater lake. *Environmental DNA* **3**: 912–929. doi:https://doi.org/10.1002/edn3.197.
- Gillanders, B.M. 2006. Seagrasses, Fish, and Fisheries. *In* Seagrasses: Biology, Ecology and Conservation. *Edited by* A.W.D. Larkum, R.J. Orth, and C. Duarte. Springer Netherlands, Dordrecht. pp. 503–505. doi:10.1007/978-1-4020-2983-7_21.
- Gold, Z., Sprague, J., Kushner, D.J., Zerecero Marin, E., and Barber, P.H. 2021. eDNA metabarcoding as a biomonitoring tool for marine protected areas. *PLoS One* **16**(2): e0238557. doi:10.1371/journal.pone.0238557.
- Goodwin, K.D., Thompson, L.R., Duarte, B., Kahlke, T., Thompson, A.R., Marques, J.C., and Caçador, I. 2017. DNA sequencing as a tool to monitor marine ecological status. *Front. Mar. Sci.* **4**: 107. doi:10.3389/fmars.2017.00107.
- Hänfling, B., Lawson Handley, L., Read, D.S., Hahn, C., Li, J., Nichols, P., Blackman, R.C., Oliver, A., and Winfield, I.J. 2016. Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Mol Ecol* **25**(13): 3101–3119. doi:10.1111/mec.13660.

- 676 Hleap, J.S., Littlefair, J.E., Steinke, D., Hebert, P.D.N., and Cristescu, M.E. 2021. Assessment of
677 current taxonomic assignment strategies for metabarcoding eukaryotes. *Mol Ecol Resour.*
678 doi:10.1111/1755-0998.13407.
- 679 Hsieh, T.C., Ma, K.H., and Chao, A. 2016. iNEXT: an R package for rarefaction and extrapolation
680 of species diversity (Hill numbers). *Methods in Ecology and Evolution* 7(12): 1451–1456.
681 doi:<https://doi.org/10.1111/2041-210X.12613>.
- 682 Iacarella, J.C., Adamczyk, E., Bowen, D., Chalifour, L., Eger, A., Heath, W., Helms, S., Hessing-
683 Lewis, M., Hunt, B.P.V., MacInnis, A., O'Connor, M.I., Robinson, C.L.K., Yakimishyn,
684 J., and Baum, J.K. 2018. Anthropogenic disturbance homogenizes seagrass fish
685 communities. *Glob Chang Biol* 24(5): 1904–1918. doi:10.1111/gcb.14090.
- 686 Jeffery, N.W., Heaslip, S.G., Stevens, L.A., and Stanley, R.R.E. 2020. Biophysical and ecological
687 overview of the Eastern Shore Islands Area of Interest (AOI). Available from
688 <https://waves-vagues.dfo-mpo.gc.ca/Library/40885045.pdf>.
- 689 Jeunen, G.-J., Knapp, M., Spencer, H.G., Lamare, M.D., Taylor, H.R., Stat, M., Bunce, M., and
690 Gemmell, N.J. 2019. Environmental DNA (eDNA) metabarcoding reveals strong
691 discrimination among diverse marine habitats connected by water movement. *Mol Ecol*
692 *Resour* 19(2): 426–438. doi:10.1111/1755-0998.12982.
- 693 Kelly, R.P., Closek, C.J., O'Donnell, J.L., Kralj, J.E., Shelton, A.O., and Samhouri, J.F. 2017.
694 Genetic and manual survey methods yield different and complementary views of an
695 ecosystem. *Front. Mar. Sci.* 3: 283. doi:10.3389/fmars.2016.00283.
- 696 Lacoursière-Roussel, A., Howland, K., Normandeau, E., Grey, E.K., Archambault, P., Deiner, K.,
697 Lodge, D.M., Hernandez, C., Leduc, N., and Bernatchez, L. 2018. eDNA metabarcoding

- 698 as a new surveillance approach for coastal Arctic biodiversity. *Ecology and Evolution*
699 **8**(16): 7763–7777. doi:10.1002/ece3.4213.
- 700 Longmire, J.L., Maltbie, M., and Baker, R.J. 1997. Use of “Lysis Buffer” in DNA isolation and its
701 implication for museum collections. Museum of Texas Tech University.
702 doi:10.5962/bhl.title.143318.
- 703 Lüdecke, D. 2021, April 30. ggeffects: Create Tidy Data Frames of Marginal Effects for “ggplot”
704 from Model Outputs [R package ggeffects version 1.1.0]. Comprehensive R Archive
705 Network (CRAN). Available from <https://CRAN.R-project.org/package=ggeffects>
706 [accessed 1 June 2021].
- 707 Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads.
708 *EMBnet.journal* **17**: 10–12. doi:10.14806/ej.17.1.200.
- 709 Maruyama, A., Nakamura, K., Yamanaka, H., Kondoh, M., and Minamoto, T. 2014. The release
710 rate of environmental DNA from juvenile and adult fish. *PLoS ONE* **9**(12): e114639.
711 doi:10.1371/journal.pone.0114639.
- 712 McInnes, J.C., Jarman, S.N., Lea, M.-A., Raymond, B., Deagle, B.E., Phillips, R.A., Catry, P.,
713 Stanworth, A., Weimerskirch, H., Kusch, A., Gras, M., Cherel, Y., Maschette, D., and
714 Alderman, R. 2017. DNA metabarcoding as a marine conservation and management tool:
715 a circumpolar examination of fishery discards in the diet of threatened albatrosses. *Front.*
716 *Mar. Sci.* **4**: 277. doi:10.3389/fmars.2017.00277.
- 717 Meehan, M.C., Ban, N.C., Devillers, R., Singh, G.G., and Claudet, J. 2020. How far have we come?
718 A review of MPA network performance indicators in reaching qualitative elements of
719 Aichi Target 11. *Conservation Letters* **13**(6): e12746.
720 doi:<https://doi.org/10.1111/conl.12746>.

- 721 Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J.Y., Sato, K., Minamoto, T., Yamamoto, S.,
722 Yamanaka, H., Araki, H., Kondoh, M., and Iwasaki, W. 2015. MiFish, a set of universal
723 PCR primers for metabarcoding environmental DNA from fishes: detection of more than
724 230 subtropical marine species. *R Soc Open Sci* **2**(7): 150088. doi:10.1098/rsos.150088.
- 725 Murphy, G.E.P., Dunic, J.C., Adamczyk, E.M., Bittick, S.J., Côté, I.M., Cristiani, J., Geissinger,
726 E.A., Gregory, R.S., Lotze, H.K., O'Connor, M.I., Araújo, C.A.S., Rubidge, E.M.,
727 Templeman, N.D., and Wong, M.C. 2021. From coast to coast to coast: ecology and
728 management of seagrass ecosystems across Canada. FACETS. Canadian Science
729 Publishing 1840 Woodward Drive, Suite 1, Ottawa, ON K2C 0P7. doi:10.1139/facets-
730 2020-0020.
- 731 Neckles, H.A. 2015. Loss of eelgrass in Casco Bay, Maine, linked to Green Crab disturbance.
732 doi:10.1656/045.022.0305.
- 733 Nguyen, N.H., Smith, D., Peay, K., and Kennedy, P. 2015. Parsing ecological signal from noise in
734 next generation amplicon sequencing. *New Phytol* **205**(4): 1389–1393.
735 doi:10.1111/nph.12923.
- 736 O'Donnell, J.L., Kelly, R.P., Shelton, A.O., Samhouri, J.F., Lowell, N.C., and Williams, G.D.
737 2017. Spatial distribution of environmental DNA in a nearshore marine habitat. *PeerJ* **5**:
738 e3044. doi:10.7717/peerj.3044.
- 739 O'Neill, M.E. 2021. Characterizing the relationship between species richness and the seasonal
740 phenomenon of tropical fish dispersal in New England waters. MSc Thesis, University of
741 Massachusetts Boston.

- 742 Pacific Salmon Commission. 2019. Annual report of catch and escapement for 2018. Available
743 from [https://www.psc.org/download/35/chinook-technical-committee/11776/tcchinook-](https://www.psc.org/download/35/chinook-technical-committee/11776/tcchinook-19-1.pdf)
744 [19-1.pdf](https://www.psc.org/download/35/chinook-technical-committee/11776/tcchinook-19-1.pdf).
- 745 Paulson, J.N., Stine, O.C., Bravo, H.C., and Pop, M. 2013. Robust methods for differential
746 abundance analysis in marker gene surveys. *Nat Methods* **10**(12): 1200–1202.
747 doi:10.1038/nmeth.2658.
- 748 Pebesma, E. 2018. Simple features for R: Standardized support for spatial vector data. *The R*
749 *Journal* **10**(1): 439–446. doi:10.32614/RJ-2018-009.
- 750 Pegg, G.G., Sinclair, B., Briskey, L., and Aspden, W.J. 2006. MtDNA barcode identification of
751 fish larvae in the southern Great Barrier Reef – Australia. *Scientia Marina* **70**(S2): 7–12.
752 doi:10.3989/scimar.2006.70s27.
- 753 Prodan, A., Tremaroli, V., Brolin, H., Zwinderman, A.H., Nieuwdorp, M., and Levin, E. 2020.
754 Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. *PLoS*
755 *One* **15**(1): e0227434. doi:10.1371/journal.pone.0227434.
- 756 Rodriguez-Ezpeleta, N., Morissette, O., Bean, C.W., Manu, S., Banerjee, P., Lacoursière-Roussel,
757 A., Beng, K.C., Alter, S.E., Roger, F., Holman, L.E., Stewart, K.A., Monaghan, M.T.,
758 Mauvisseau, Q., Mirimin, L., Wangenstein, O.S., Antognazza, C.M., Helyar, S.J., de Boer,
759 H., Monchamp, M.-E., Nijland, R., Abbott, C.L., Doi, H., Barnes, M.A., Leray, M.,
760 Hablützel, P.I., and Deiner, K. 2021. Trade-offs between reducing complex terminology
761 and producing accurate interpretations from environmental DNA: Comment on
762 “Environmental DNA: What’s behind the term?” by Pawlowski et al., (2020). *Molecular*
763 *Ecology* **30**(19): 4601–4605. doi:10.1111/mec.15942.

- Rodríguez-Ezpeleta, N., Zinger, L., Kinziger, A., Bik, H.M., Bonin, A., Coissac, E., Emerson, B.C., Lopes, C.M., Pelletier, T.A., Taberlet, P., and Narum, S. 2021. Biodiversity monitoring using environmental DNA. *Mol Ecol Resour* **21**(5): 1405–1409. doi:10.1111/1755-0998.13399.
- Russo, T., Maiello, G., Talarico, L., Baillie, C., Colosimo, G., D’Andrea, L., Di Maio, F., Fiorentino, F., Franceschini, S., Garofalo, G., Scannella, D., Cataudella, S., and Mariani, S. 2021. All is fish that comes to the net: metabarcoding for rapid fisheries catch assessment. *Ecol Appl* **31**(2): e02273. doi:10.1002/eap.2273.
- Schnell, I.B., Bohmann, K., and Gilbert, M.T.P. 2015. Tag jumps illuminated--reducing sequence-to-sample misidentifications in metabarcoding studies. *Mol Ecol Resour* **15**(6): 1289–1303. doi:10.1111/1755-0998.12402.
- Schram, C., Ladell, K., Mitchell, J., and Chute, C. 2019. From one to ten: Canada’s approach to achieving marine conservation targets. *Aquatic Conservation: Marine and Freshwater Ecosystems* **29**(S2): 170–180. doi:https://doi.org/10.1002/aqc.3133.
- Shaw, J.L.A., Clarke, L.J., Wedderburn, S.D., Barnes, T.C., Weyrich, L.S., and Cooper, A. 2016. Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. *Biological Conservation* **197**: 131–138. doi:10.1016/j.biocon.2016.03.010.
- Shi, Z.-Y., Yang, C.-Q., Hao, M., Wang, X.-Y., Ward, R.D., and Zhang, A.-B. 2018. FuzzyID2: A software package for large data set species identification via barcoding and metabarcoding using hidden Markov models and fuzzy set methods. *Mol Ecol Resour* **18**(3): 666–675. doi:10.1111/1755-0998.12738.

- 786 Spens, J., Evans, A.R., Halfmaerten, D., Knudsen, S.W., Sengupta, M.E., Mak, S.S.T., Sigsgaard,
787 E.E., and Hellström, M. 2017. Comparison of capture and storage methods for aqueous
788 microbial eDNA using an optimized extraction protocol: advantage of enclosed filter.
789 *Methods in Ecology and Evolution* **8**(5): 635–645. doi:[https://doi.org/10.1111/2041-](https://doi.org/10.1111/2041-210X.12683)
790 [210X.12683](https://doi.org/10.1111/2041-210X.12683).
- 791 Stat, M., John, J., DiBattista, J.D., Newman, S.J., Bunce, M., and Harvey, E.S. 2019. Combined
792 use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity.
793 *Conservation Biology* **33**(1): 196–205. doi:[10.1111/cobi.13183](https://doi.org/10.1111/cobi.13183).
- 794 Stoeckle, M.Y., Adolf, J., Charlop-Powers, Z., Dunton, K.J., Hinks, G., and VanMorter, S.M. 2021.
795 Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance
796 in coastal New Jersey, USA. *ICES Journal of Marine Science* **78**(1): 293–304.
797 doi:[10.1093/icesjms/fsaa225](https://doi.org/10.1093/icesjms/fsaa225).
- 798 Stoeckle, M.Y., Soboleva, L., and Charlop-Powers, Z. 2017. Aquatic environmental DNA detects
799 seasonal fish abundance and habitat preference in an urban estuary. *PLoS One* **12**(4):
800 e0175186. doi:[10.1371/journal.pone.0175186](https://doi.org/10.1371/journal.pone.0175186).
- 801 Suchanek, T.H. 1994. Temperate coastal marine communities: biodiversity and threats. *American*
802 *Zoologist* **34**(1): 100–114. doi:[10.1093/icb/34.1.100](https://doi.org/10.1093/icb/34.1.100).
- 803 Taberlet, P., Coissac, E., Hajibabaei, M., and Rieseberg, L.H. 2012. Environmental DNA. *Mol.*
804 *Ecol.* **21**(8): 1789–1793. doi:[10.1111/j.1365-294X.2012.05542.x](https://doi.org/10.1111/j.1365-294X.2012.05542.x).
- 805 Thomas, H.L., Macsharry, B., Morgan, L., Kingston, N., Moffitt, R., Stanwell-Smith, D., and
806 Wood, L. 2014. Evaluating official marine protected area coverage for Aichi Target 11:
807 appraising the data and methods that define our progress. *Aquatic Conservation: Marine*
808 *and Freshwater Ecosystems* **24**(S2): 8–23. doi:<https://doi.org/10.1002/aqc.2511>.

- 809 Thomsen, P.F., Møller, P.R., Sigsgaard, E.E., Knudsen, S.W., Jørgensen, O.A., and Willerslev, E.
810 2016. Environmental DNA from seawater samples correlate with trawl catches of subarctic,
811 deepwater fishes. *PLoS One* **11**(11): e0165252. doi:10.1371/journal.pone.0165252.
- 812 Thomsen, P.F., and Willerslev, E. 2015. Environmental DNA – An emerging tool in conservation
813 for monitoring past and present biodiversity. *Biological Conservation* **183**: 4–18.
814 doi:10.1016/j.biocon.2014.11.019.
- 815 Unsworth, R.K.F., Bertelli, C.M., Cullen-Unsworth, L.C., Esteban, N., Jones, B.L., Lilley, R.,
816 Lowe, C., Nuuttila, H.K., and Rees, S.C. 2019. Sowing the seeds of seagrass recovery using
817 hessian bags. *Front. Ecol. Evol.* **7**: 311. doi:10.3389/fevo.2019.00311.
- 818 Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., Bellemain, E.,
819 Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp,
820 G.H., Geniez, P., Pont, D., Argillier, C., Baudoin, J.-M., Peroux, T., Crivelli, A.J., Olivier,
821 A., Acqueberge, M., Le Brun, M., Møller, P.R., Willerslev, E., and Dejean, T. 2016. Next-
822 generation monitoring of aquatic biodiversity using environmental DNA metabarcoding.
823 *Mol Ecol* **25**(4): 929–942. doi:10.1111/mec.13428.
- 824 Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine,
825 A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short,
826 F.T., and Williams, S.L. 2009. Accelerating loss of seagrasses across the globe threatens
827 coastal ecosystems. *PNAS* **106**(30): 12377–12381. National Academy of Sciences.
828 doi:10.1073/pnas.0905620106.
- 829 West, K.M., Stat, M., Harvey, E.S., Skepper, C.L., DiBattista, J.D., Richards, Z.T., Travers, M.J.,
830 Newman, S.J., and Bunce, M. 2020. eDNA metabarcoding survey reveals fine-scale coral

reef community variation across a remote, tropical island ecosystem. *Mol Ecol* **29**(6): 1069–1086. doi:10.1111/mec.15382.

Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B.C., Lotze, H.K., Micheli, F., Palumbi, S.R., Sala, E., Selkoe, K.A., Stachowicz, J.J., and Watson, R. 2006. Impacts of biodiversity loss on ocean ecosystem services. *Science* **314**(5800): 787–790. doi:10.1126/science.1132294.

Zaiko, A., Greenfield, P., Abbott, C., von Ammon, U., Bilewitch, J., Bunce, M., Cristescu, M.E., Chariton, A., Dowle, E., Geller, J., Ardura Gutierrez, A., Hajibabaei, M., Haggard, E., Inglis, G.J., Lavery, S.D., Samuiloviene, A., Simpson, T., Stat, M., Stephenson, S., Sutherland, J., Thakur, V., Westfall, K., Wood, S.A., Wright, M., Zhang, G., and Pochon, X. 2021. Towards reproducible metabarcoding data: Lessons from an international cross-laboratory experiment. *Mol Ecol Resour*. doi:10.1111/1755-0998.13485.

Zhang, A.-B., Muster, C., Liang, H.-B., Zhu, C.-D., Crozier, R., Wan, P., Feng, J., and Ward, R.D. 2012. A fuzzy-set-theory-based approach to analyse species membership in DNA barcoding. *Mol Ecol* **21**(8): 1848–1863. doi:10.1111/j.1365-294X.2011.05235.x.

Zinger, L., Bonin, A., Alsos, I.G., Bálint, M., Bik, H., Boyer, F., Chariton, A.A., Creer, S., Coissac, E., Deagle, B.E., Barba, M.D., Dickie, I.A., Dumbrell, A.J., Ficetola, G.F., Fierer, N., Fumagalli, L., Gilbert, M.T.P., Jarman, S., Jumpponen, A., Kauserud, H., Orlando, L., Pansu, J., Pawlowski, J., Tedersoo, L., Thomsen, P.F., Willerslev, E., and Taberlet, P. 2019. DNA metabarcoding—Need for robust experimental designs to draw sound ecological conclusions. *Molecular Ecology* **28**(8): 1857–1862. doi:10.1111/mec.15060.

Fig. 1 Map of the 19 coastal eelgrass beds sampled in the Pacific (a) and Atlantic (b) regions of Canada. Site abbreviations are defined in Table S1. The Eastern Shore Islands Area of Interest, an area of high eelgrass bed density and a potential future Marine Protected Area, is shown in blue in the Atlantic region (source: Fisheries and Oceans Canada). Map projections are NAD 1983 CSRS Statistics Canada Lambert (national), NAD 1983 CSRS BC Environment Albers (Pacific), and NAD 1983 UTM 20N (Atlantic region). Coastlines are the Statistics Canada 2011 Census Boundary File (national, Atlantic), and Canadian Hydrographic Service 2015 high water line (Pacific).

Fig. 2 Venn diagrams comparing the fish taxa detected by eDNA and seining at family and species levels. For the lower panel, only taxa identified to the species level were considered.

Fig. 3 Sample-size- (left plots) and coverage-based (right plots) species accumulation curves for seining, 12S, 16S, and the two markers combined eDNA data using interpolation (solid line) and extrapolation (dashed line) methodologies. Data were formatted to the incidence-frequency datatype and the curves were generated using the iNEXT R package.

Fig. 4 Combined species richness for seining, 12S, 16S, and the two markers combined eDNA data for Atlantic coast (a) and Pacific coast (b).

Fig. 5 Principal Coordinate Analyses (PCoA) of Bray-Curtis dissimilarity distances to analyze site differences based on seining and eDNA data for each marker and each coast. Plots a, c and e share the same color legend, as do plots b, d and f.

877

878 **Fig. 6** Relationship between relative abundances obtained by seining and eDNA metabarcoding.

879 For seining data, relative abundances of each taxon at each site were calculated. For eDNA data,

880 relative abundances of each taxon in each sample were calculated and then averaged for each site.

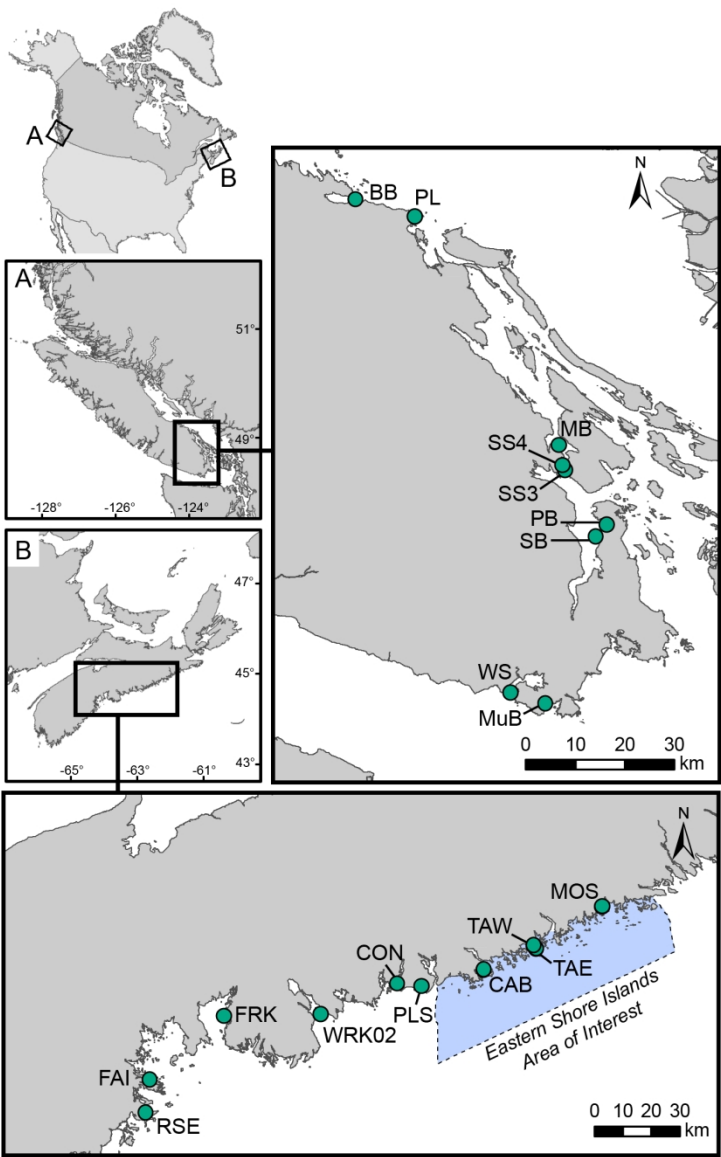
881 Linear regression analyses between log-transformed relative abundances were performed using

882 the lme4 R package and predictions of eDNA data were generated using the ggeffects R package.

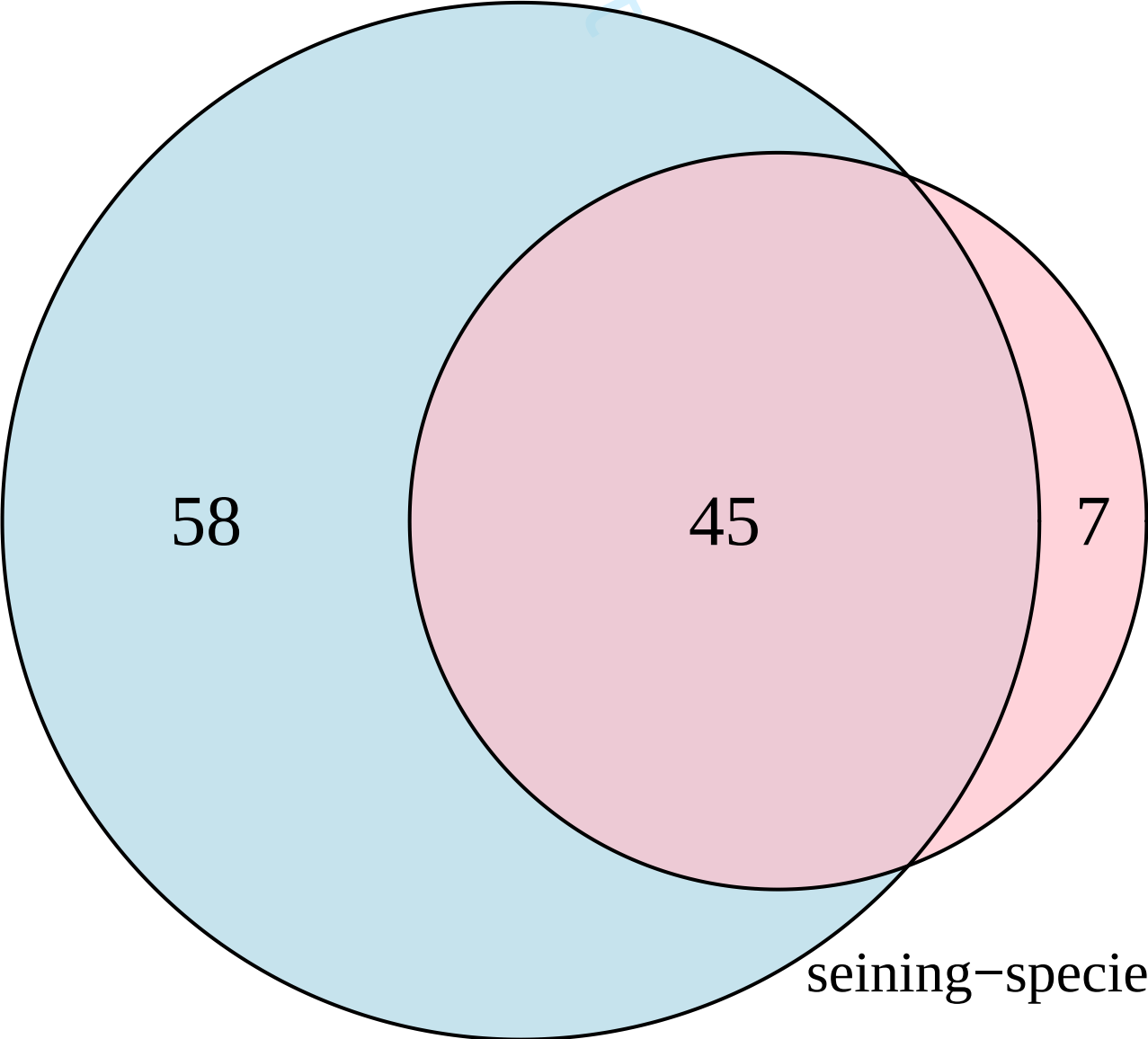
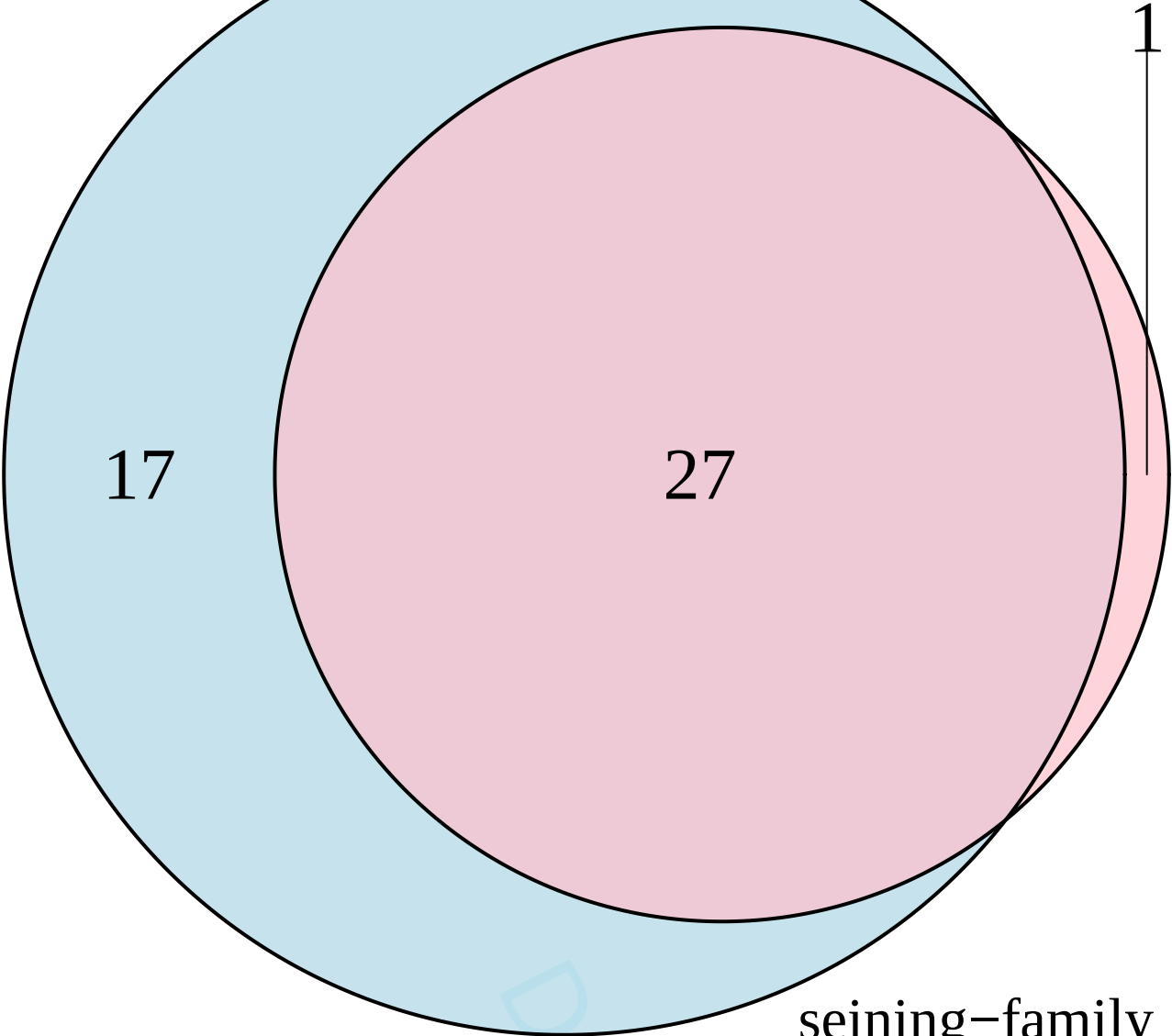
883 Blue points are actual log-transformed relative abundances. The solid lines show estimates of

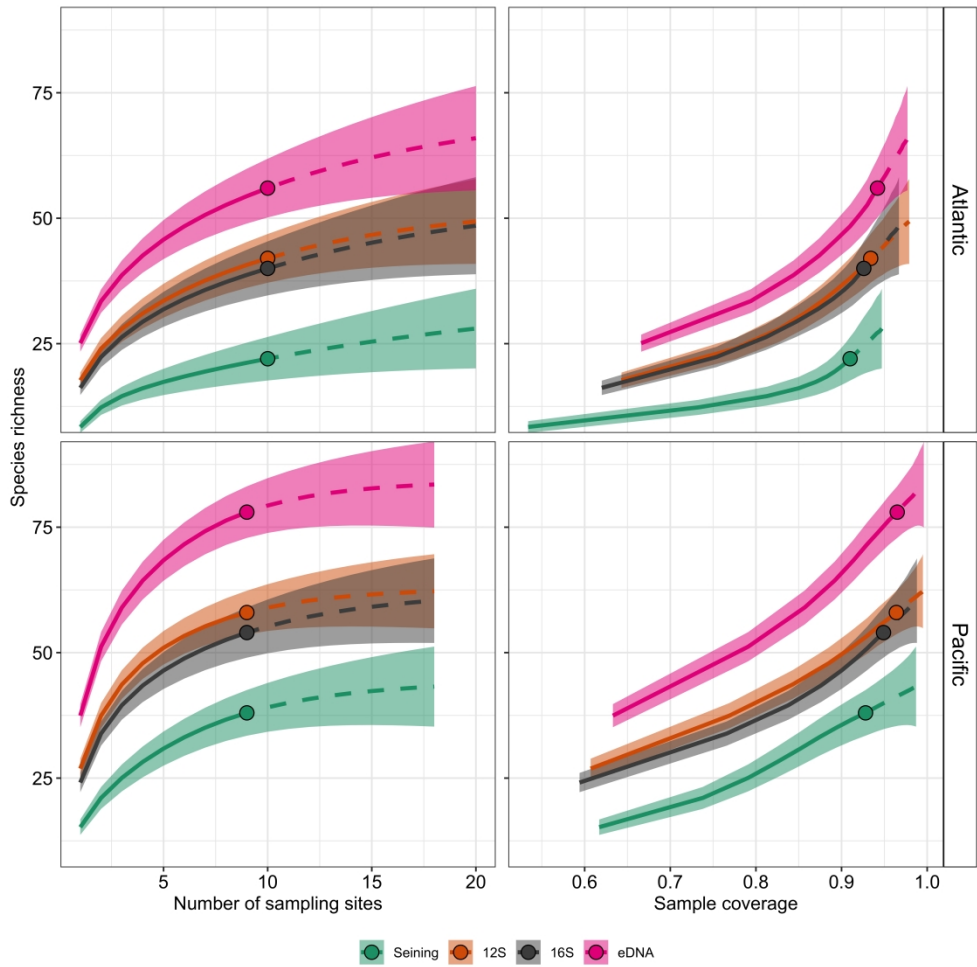
884 eDNA relative abundance based on seining relative abundance.

Draft



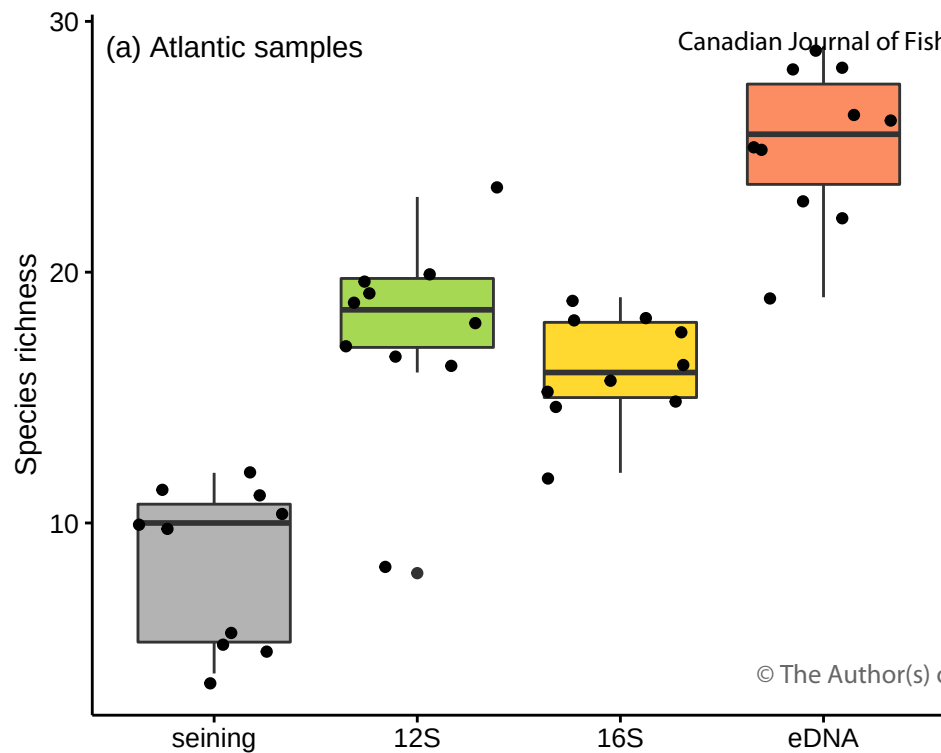
329x501mm (118 x 118 DPI)





1905x1905mm (72 x 72 DPI)

(a) Atlantic samples



(b) Pacific samples

