Supplemental material for *Sunday et al.* Networked eDNA sampling is a data-rich approach for scaling biodiversity monitoring

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Table S1. Read counts throughout quality control steps. Each row shows results from a unique NextSeq sequencing run for all but the final column. Final column shows total reads associated with each year after a first run was conducted that included “redoes” for some samples that had too few sequences in the first run.

| | Samples | Raw | Cutadapt | Filter and Trim | Denoised | Merged | No singletons | Nochimeras | Reads after non-fish taxa were removed | Fish reads after “redoes” included |
|---|---|---|---|---|---|---|---|---|---|---|
| 2021 | 377 | 166,342,010 | 158,113,702 | 156,087,534 | 155,801,267 | 76,794,703 | 75,861,834 | 69,524,860 | 58,782,197 | 58,782,197 |
| 2022 | 381 | 167,492,564 | 146,448,608 | 144,527,948 | 144,343,820 | 70,802,731 | 70,464,678 | 65,877,781 | 61,884,043 | 122,681,735 |
| 2023 | 365 | 156,914,334 | 141,586,560 | 140,356,072 | 140,175,531 | 68,940,061 | 68,317,855 | 61,104,294 | 58,036,338 | 74,843,378 |
| 2024 | 384 | 162,088,284 | 146,943,192 | 145,013,872 | 144,688,383 | 71,022,683 | 70,642,165 | 63,794,246 | 61,093,540 | 62,035,750 |

Table S2. Fish handling permit information for each partner group.

| Contributor name | location | Permit reference |
|---|---|---|
| Scott Gabara | Juneau region, AK | ADFG CF-21-051 03/15/2021-08/31/2021<br>ADFG CF-22-041 03/29/2022-10/31/2022 |
| Lia Domke and Ginny Eckert | Southeast AK region | ADFG CF-21-062 (For |

| | | |
|---|---|---|
| | | seining in 2021)<br>ADFG CF-22-070 (in 2022).<br>UAF IACUC (#892147) |
| Zachary Monteith | Central Coast, BC | Department of Fisheries and Oceans Canada XMCFR 14 2021 |
| Angeleen Olson | Inner Coast, BC | Department of Fisheries and Oceans Canada XR 80 2021 |
| Jennifer Yakimishyn | Pacific Rim National Park Reserve, BC | DFO scientific licence (FIN: 124588), XR 137 2021, XR 167 2022, WR 284 2023, XR 266 2024, XR 317 2025 |
| Kathryn Sobocinski | Padilla Bay, WA | Washington Department of Fish and Wildlife Scientific Collection Permit 20-199<br>Western Washington University Institutional Animal Care and Use Committee Protocol 20-006 |
| Jennifer Ruesink | Willapa Bay, WA | Washington Department of Fish and Wildlife Scientific Collection Permit 20-311<br>University of Washington Institutional Animal Care and Use Committee protocol 3363-02<br>NOAA Scientific Research Permit 20047-2R |
| Fiona Tomas Nash | Yaquina and Coos Bays, OR | Oregon's Department of Fish and Wildlife permit number 25758. |
| Jay Stachowicz | Bodega Bay, CA | California Dept of Fish and Wildlife Specific Use Permit *S-211340004-24177-001* |
| Brent Hughes | Drakes Estero, CA | California Department of Fish and Wildlife: S-201820002-20182-001<br>National Park Service: PORE-20 18-SCI-0038 |
| Margot Buchbinder | San Francisco Bay, CA | California Department of Fish and Wildlife Specific Use Permit: S-211010004-21125-001 |
| Karl Koehler and Kevin Hovel | San Diego region, CA | California Department of Fish and Wildlife Specific Use Permit: S-190320001-20188-001 |
| Olivia Graham and Lillian Aoki | San Juan Islands, WA | Collections made under the auspices of state statute (House Bill 68, R.C.W.28.77.230, 1969 Revision R.C.W.28B.20.320), with FHL as the managing agency. |

| | | |
|---|---|---|
| | | UW IACUC (#4238-15) |

Figure S1. Photograph of filtration set-up for field processing of water samples onto a Sterivex filter.

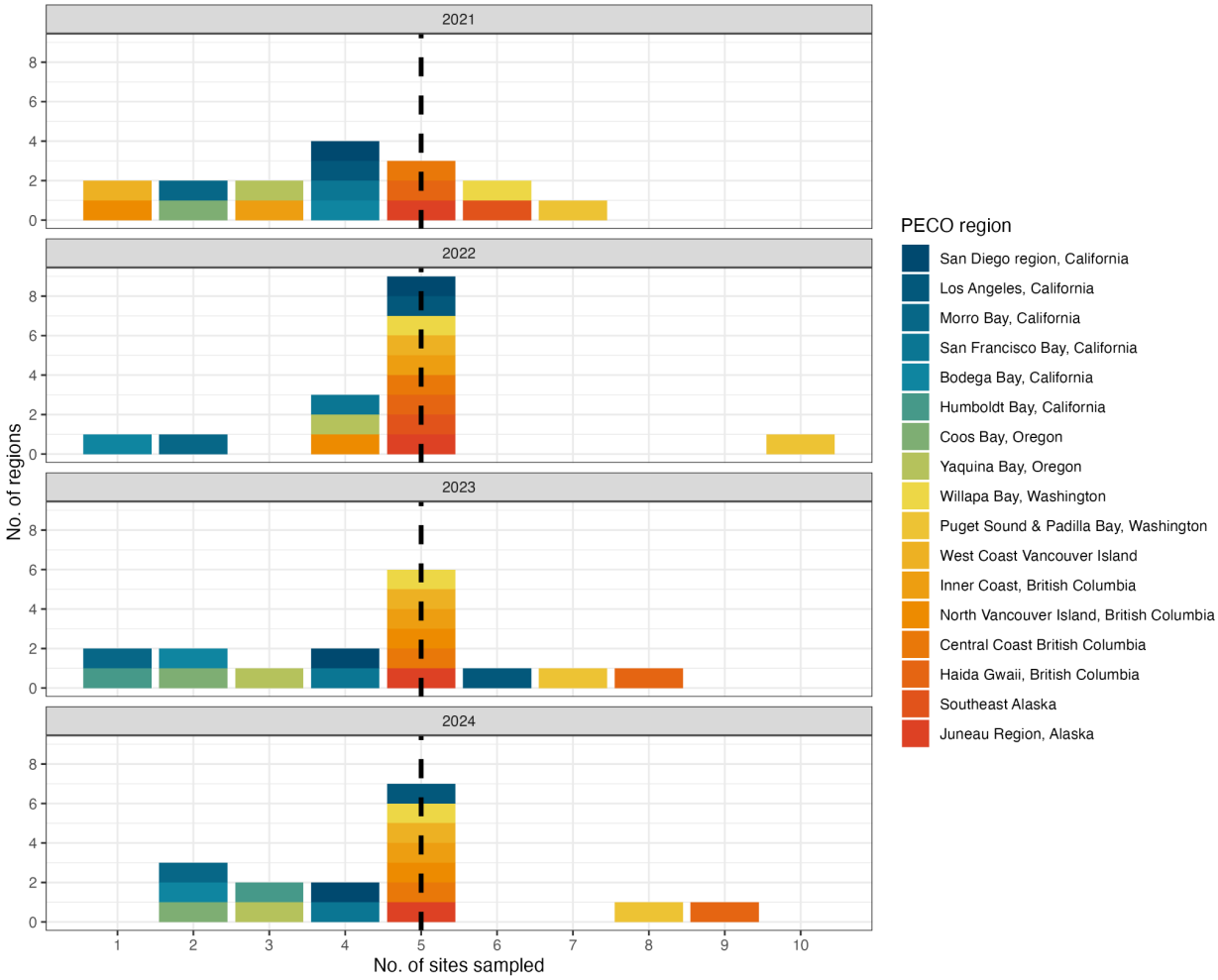


Figure S2. Number of sites sampled per year and region in PECO. The dashed line indicates our target of five sites per region.

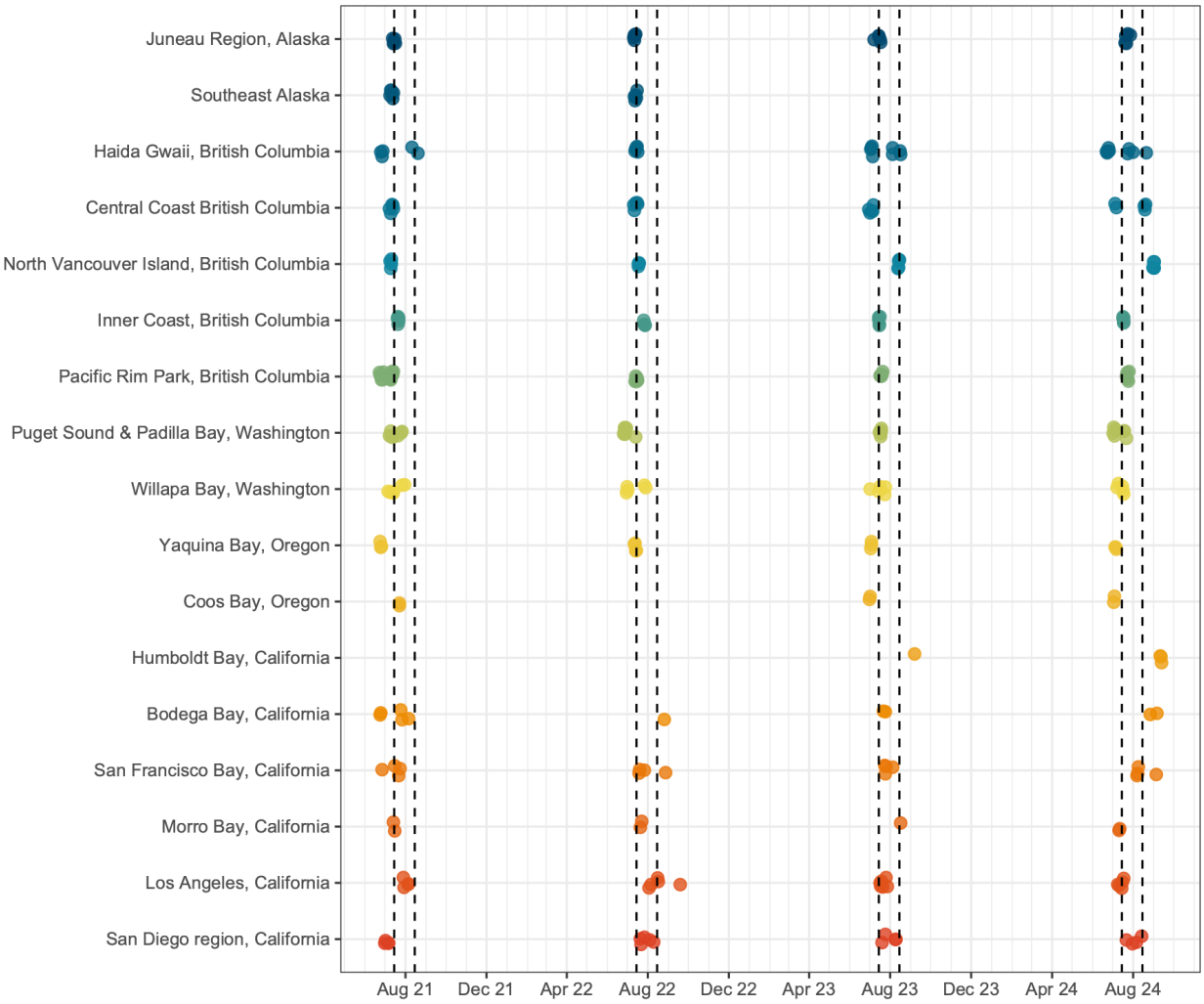


Figure S3. Sample collection dates for sites in PECO. Dashed lines indicate the targeted sampling window between July 15 and August 15 of each year.

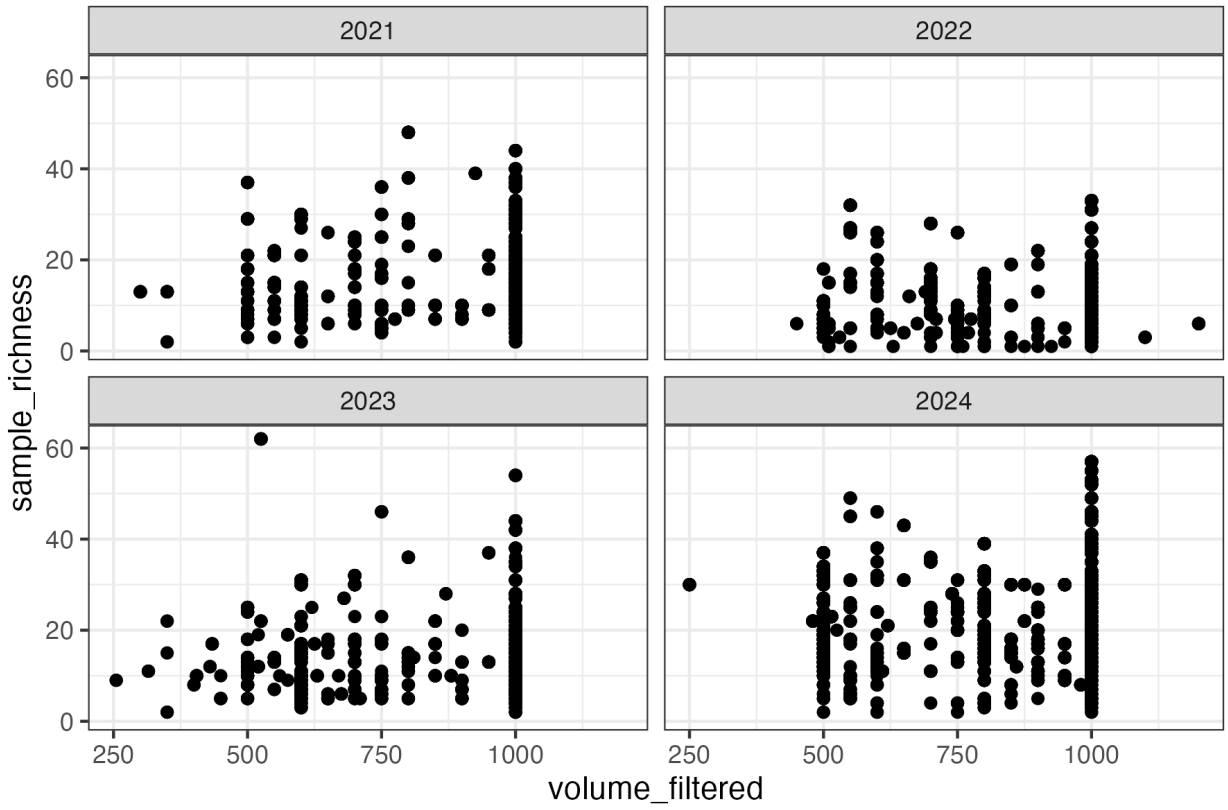


Figure S4. Per sample ASV richness as a function of volume sampled. Field researchers filtered seawater with a goal of processing 1000ml but stopped between 500ml and 1000ml when filters became clogged. This protocol resulted in variation in volume filtered among our samples, mostly between 500ml and 1000ml, but no strong pattern in ASV richness as a function of volume filtered.

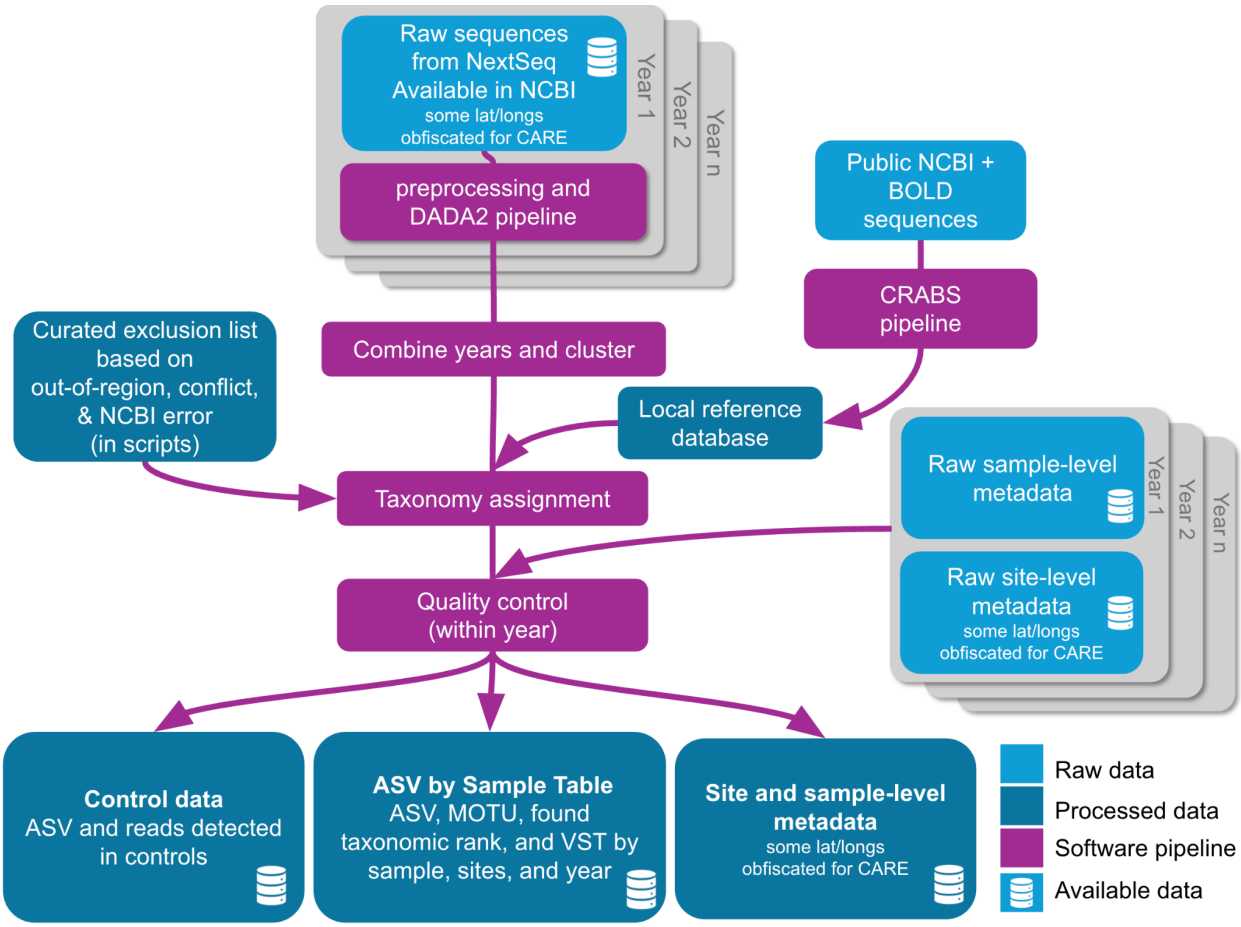


Figure S5. Schematic flow diagram of PECO analytical pipeline. Raw data are shown in light blue, processed data in dark blue, and purple elements represent reproducible data processing pipeline. Raw and processed datasets available are noted with the "datastack" icon, although some data require additional permissions to respect CARE principles (Collective Benefit, Authority to Control, Responsibility, and Ethics). Namely, datasets collected on Haida territory (Haida Gwaii region) require permission before further use, and latitude and longitudes of sites are obfuscated to 0.1 degrees of latitude. These are noted in the public records. New generated sequences are available in the National Center for Biotechnology Information (NCBI), and raw and processed data files and full analytical pipeline are available at Zenodo.

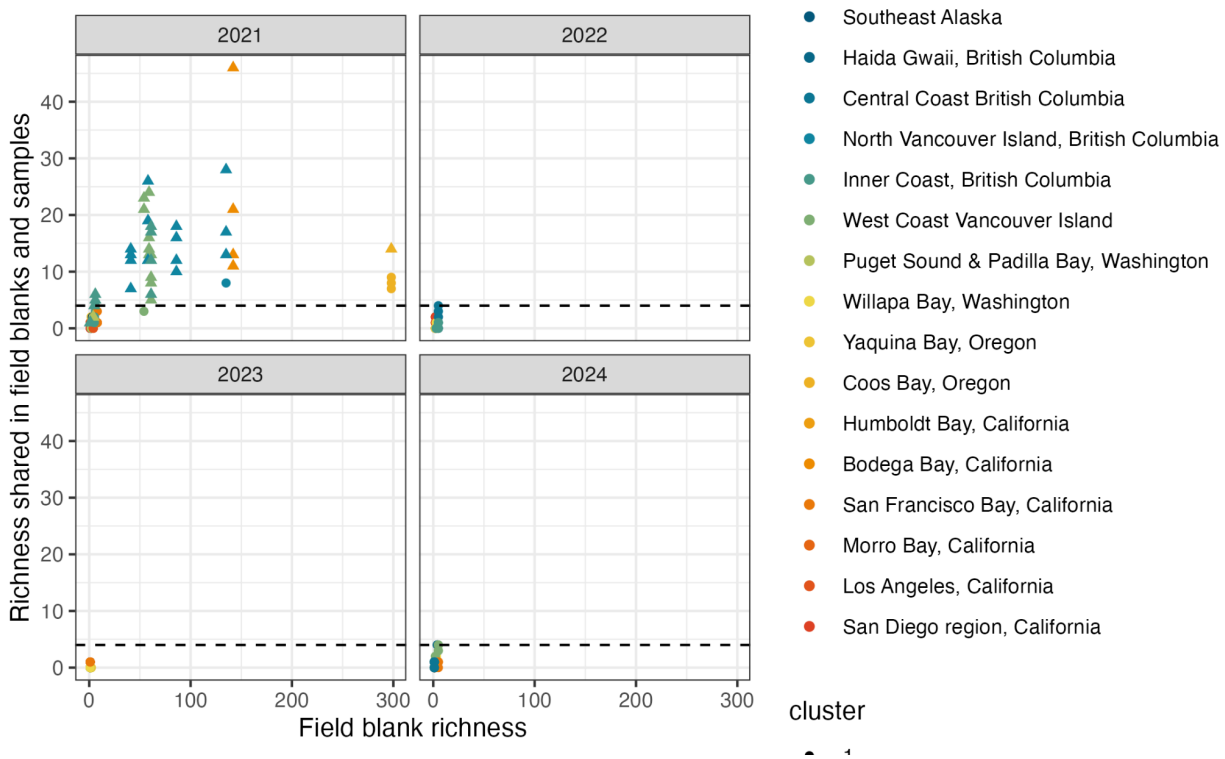


Figure S6. Relationship between ASVs from field blanks in samples and total ASVs in field blanks. Samples from several sites in 2021 had high ASV richness in field blanks (x-axis) and associated high field-blank content in samples associated with those field blanks (y-axis, ~4 samples associated with each field blank). Dotted line indicates the threshold by which we initially considered flagging these samples for removal, and symbol indicates the inclusion of samples into the same community assemblage using a clustering algorithm (see Fig. S7).

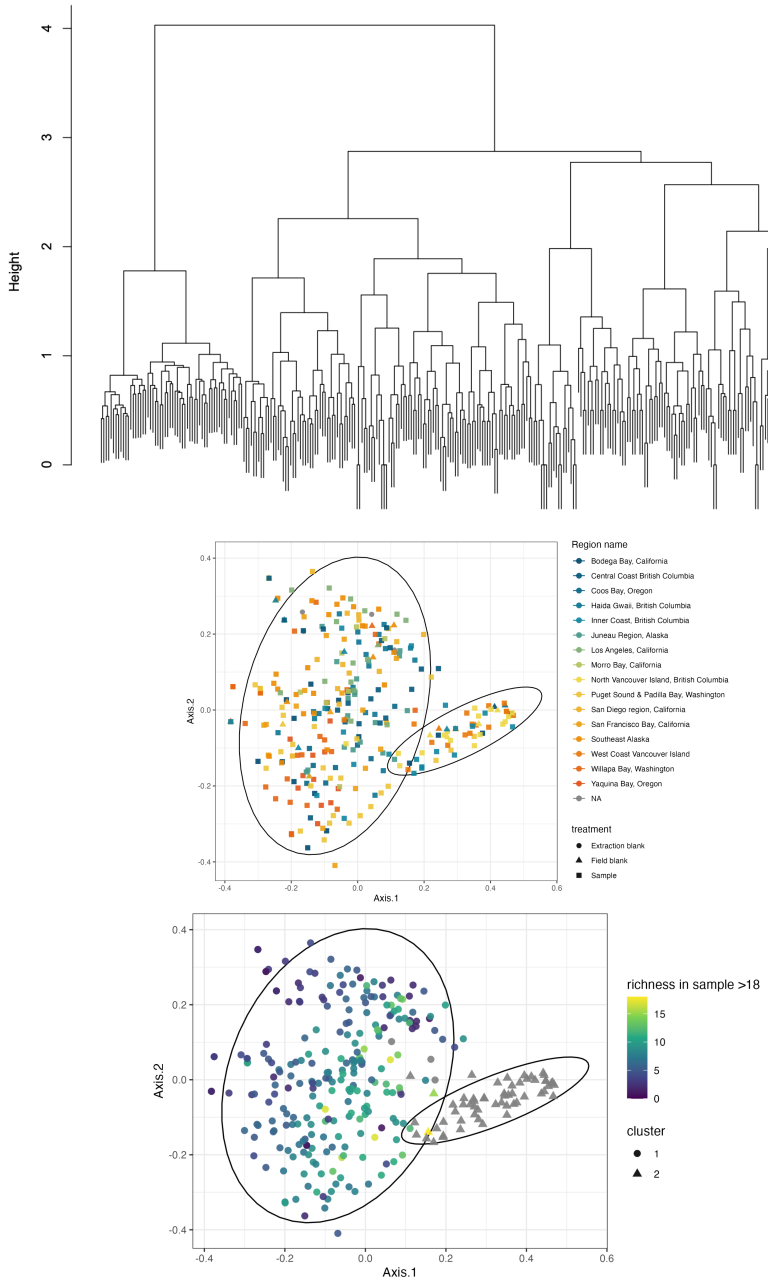


Fig. S7. Hierarchical clustering of 2021 data shows clear clustering of putative contaminants in 2021. Dendrogram in (a) shows two major clusters using Ward's minimum variance clustering, input data were all samples, field blanks, and extraction blanks in 2021. (b-c) PCoA of samples shows some field blanks, extraction blanks, and samples clustering together and associated with high richness, grey values in (c) are samples with richness > 20 ASV.

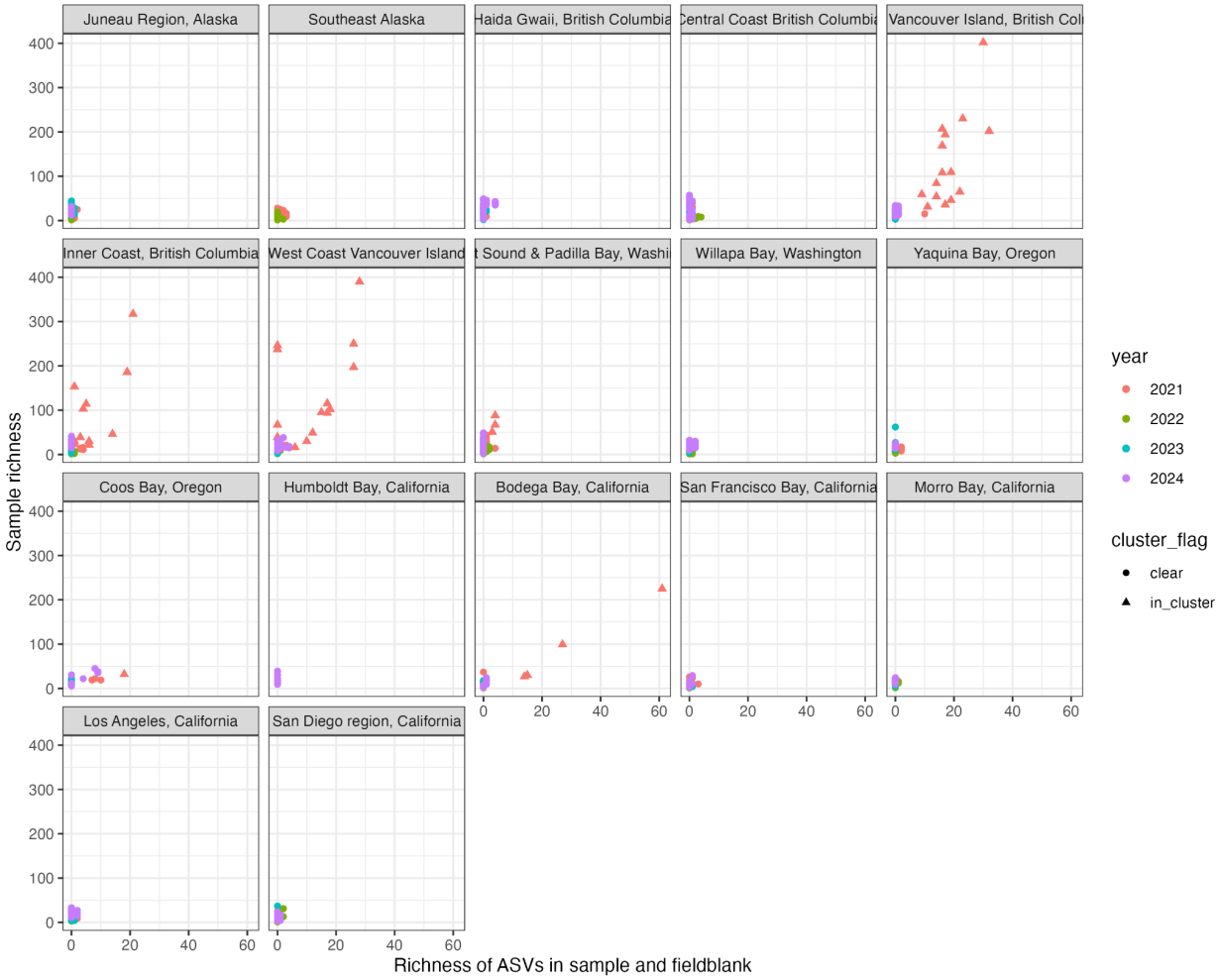


Fig. S8. Richness of ASVs in field blanks and samples showing flagged samples for removal. Some samples still had 5-10 ASVs in common with field blanks even after removal but these samples did not have particularly high richness and did not cluster with contaminated field blanks.

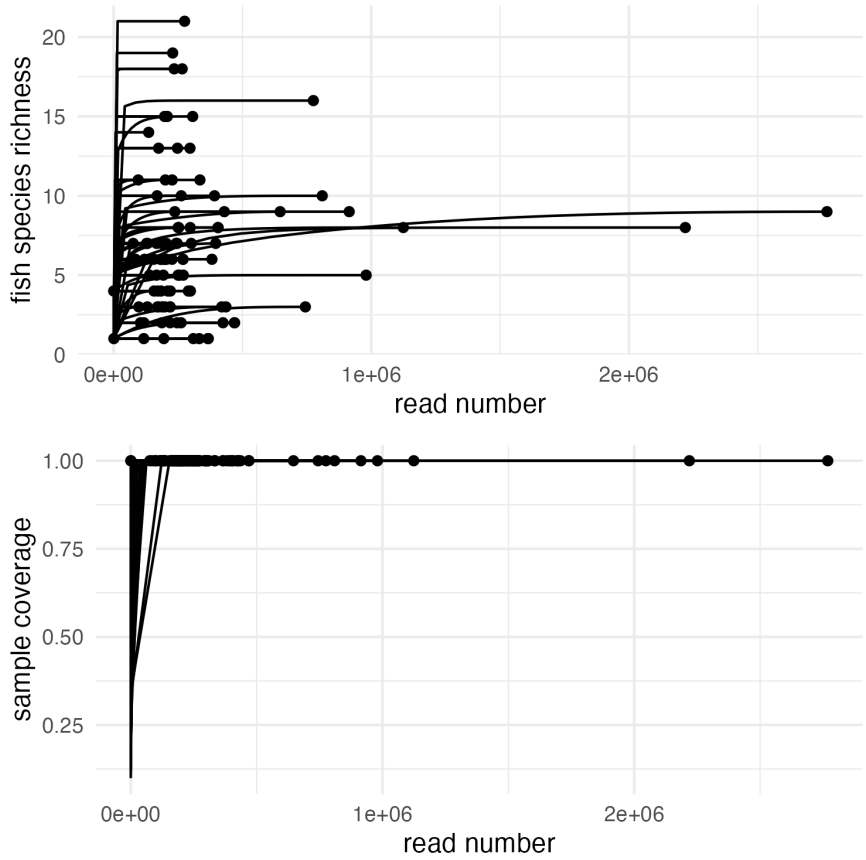


Fig. S9. Sequence rarefaction curves showing taxonomic richness as a function of read number after quality control for a random 10% subset of 100 samples. Panel (a) shows increase in taxonomic richness with number of reads (all fish OTUs included, most identified to species level), point indicates achieved read number per sample, after quality control and removal of non-fish ASVs. Panel (b) shows estimated sample coverage which was 1 (maximum) for all samples  All values are from abundance-based rarefaction procedures conducted using iNext, no extrapolation shown.

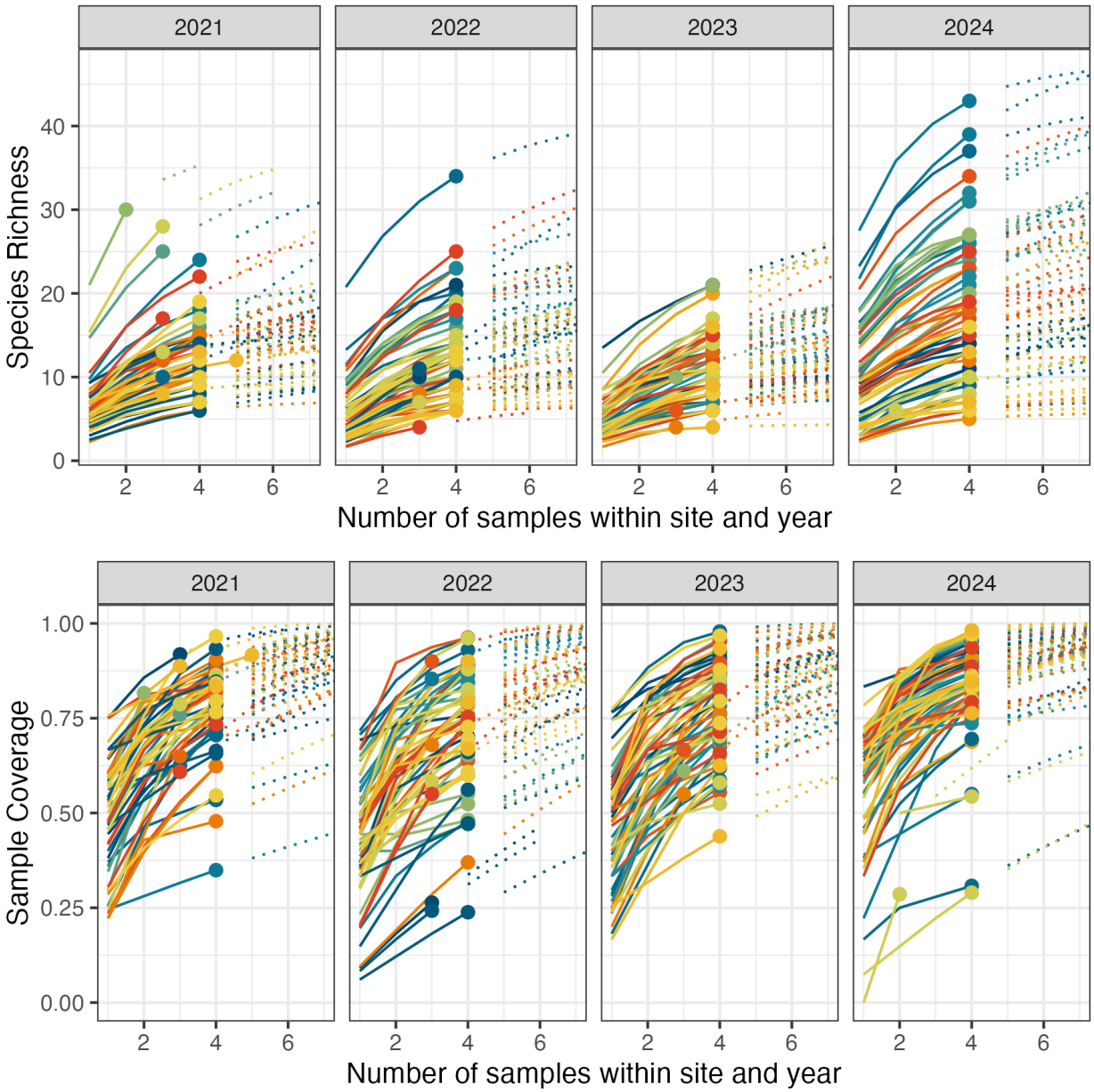


Fig. S10. Sample rarefaction curves showing species richness and sample coverage as a function of samples within sites. Upper panels show increase in taxonomic richness with number of samples at a site, point indicates achieved species richness per site. Lower panels show the relationship between sample number and estimated sample coverage. All values are from incidence-based rarefaction procedures based on taxon presence within in sample using iNext, solid line shows rarefaction and dotted lines show extrapolations.

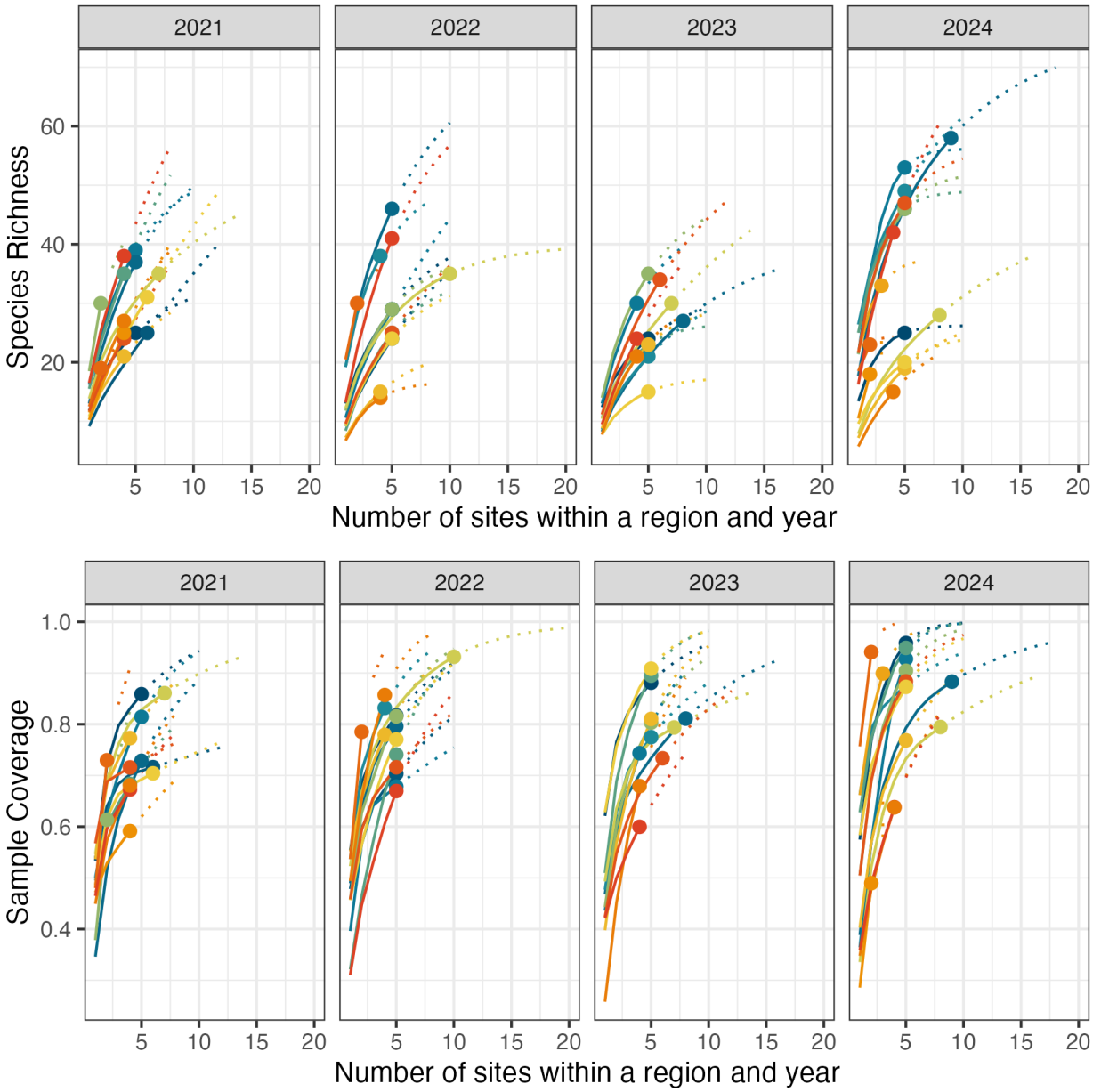


Figure S11. Site rarefaction curves showing species richness and sample coverage as a function of sites within regions. Upper panels show increase in taxonomic richness with number of sites in a region, points indicate total observed species richness per region. Lower panels show the relationship between site number and estimated sample coverage in the region. All values are from incidence-based rarefaction procedures based on taxon presence within each site using iNext; solid line shows rarefaction and dotted lines show extrapolation.