Developing fish abundance and ecosystem indices from environmental DNA (eDNA)

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Introduction

Fisheries-independent surveys form the foundation of fisheries stock assessments worldwide. On the West Coast, data collected from multi-species surveys such as the West Coast Groundfish Bottom Trawl Survey (WCGBTS) and the Integrated West Coast Pelagics Survey (IWCPS) inform abundance indices and thereby support stock assessments for dozens of species. However, there are tens of additional fish species that are missed or inconsistently measured by these surveys and developing new surveys for additional species is both time- and cost-prohibitive. Therefore, it is important to develop innovative and cost-effective methods that can extract additional information about a broader swath of marine species and ecosystem components without expanding survey needs.

Over the past two decades, rapid advances in molecular technologies have unlocked new methods for observing ocean ecosystems. In particular, environmental DNA (eDNA), in which residual genetic material is collected from environmental media such as water, air, or soil, has developed as a data source useful for non-invasively detecting and quantifying a wide range of taxa in diverse habitats (e.g., Dougherty et al. 2016, Keller et al. 2020, Shelton et al. 2019, 2022). This document is intended to begin the process of considering eDNA-derived information as a valid data source for informing fisheries management advice under the Pacific Fisheries Management Council (PFMC).

Document Structure

We divide this report into three substantive sections. In the first, we provide a high level introduction to the theory and practice of eDNA methods. This section includes a summary of some of the basics of eDNA practice and supporting literature. We begin by describing the types of observations that can be derived from eDNA samples and continue to outline the processes of the ‘ecology of DNA’ (Barnes & Turner 2016) including the production, transport, and loss of eDNA and the implication of these processes for fisheries surveys. Finally, and most importantly, we describe the theoretical and experimental support for a strong connection between eDNA concentrations and fish biomass. To our knowledge eDNA has not been previously presented to or reviewed by any PFMC body, and we view this section as necessary background information for advisory bodies considering eDNA in the Council process.

In the second section we describe a detailed application of eDNA approaches to building an abundance index for Pacific hake along the US west coast. We first describe details of the survey design and then provide detailed comparisons between eDNA and acoustic trawl survey from one survey year (2019) before discussing the construction of

an eDNA index time-series across three surveys (2019, 2021, 2023). Finally, we introduce and discuss the major technical considerations that were required to include the eDNA index as a sensitivity in the 2025 Pacific hake assessment (Johnson et al. 2025), highlighting the steps necessary for making this approach generally applicable for including an eDNA index for nearly any other fish stock. The Pacific hake stock is managed jointly with Canada under the terms of the Pacific Hake Treaty (Johnson et al. 2025), not under the PFMC, but this species provides a large amount of traditional survey and fisheries information in which to draw out comparisons between more traditional information surveys and eDNA.

The third section discusses how the eDNA samples collected and analyzed for Pacific hake can be potentially repurposed to provide information on other groundfish and coastal pelagic species. We discuss the range of additional molecular data that are being generated from the existing samples and results from pilot projects that inform how these observations can provide information parallel to the DNA data available for hake. We discuss both the potential advantages and important caveats for using eDNA observations and outline considerations for including eDNA information in fisheries management advice.

Goals

The science of eDNA analysis is relatively young and the use of eDNA for management advice is younger still. Here, the proponents seek advice from the SSC on the applicability of eDNA methods to fisheries management topics within the PFMC process. A threshold of particular importance for eDNA in fisheries management is (1) recognition that eDNA concentrations and biomass are mechanistically related. Beyond this threshold, it may be desirable to (2) determine the conditions under which eDNA concentrations may be reliably used in an assessment, or (3) the conditions under which additional side-by-side methods comparisons might be required. Finally, (4) the SSC may wish to suggest future research avenues that would best serve the needs of assessors in beginning to use eDNA data in a quantitative context.

1. Background and Fundamentals of Environmental DNA

What is environmental DNA (eDNA)?

Nearly all cells in all organisms contain the unique DNA sequences that encode the genetic information that allow an organism to survive, grow, and reproduce. Inevitably and necessarily throughout a multi-cellular organism's life, cells for most tissues will die and be replaced repeatedly with new cells. Some of the old cells and the associated DNA may be reabsorbed by the organism, but many are released into the environment in a range of forms (e.g., sloughed skin or scales, blood, feces, slime). Such shed cells as well as free DNA (i.e., DNA not associated with individual cells or cellular organelles) can be collected from the environment via sampling of air, soil, or water (Taberlet et al. 2012a, Thomsen & Willerslev 2015). We collectively refer to DNA collected from the environment and examined for genetic sequences as environmental DNA (eDNA). The following section discusses the surprisingly rich and sophisticated range of information that can be gleaned from such eDNA samples. As our focus is on fisheries and fisheries-relevant taxa, we focus on eukaryotic DNA (i.e., DNA arising from cells with a nucleus as well as cellular organelles).

Sources and types of eukaryotic DNA

Because almost every cell in a fish's body contains a full complement of its DNA, useful information can arise from a cell shed from anywhere (gut lining, skin, muscle, etc.). Moreover, because the billions of cells in a fish each contain billions of bits of genetic information (nucleotides, the A, G, C, T making up DNA), it only takes a tiny fraction of the total available information in DNA to distinguish one species from another. Generally 75 to 200 base pairs (bp) is enough. Therefore, DNA isolated from an environmental sample can generally be traced back to a single species or potentially even finer resolutions (e.g., sub-species, population, or even individual; see Parsons et al. 2018, Andres et al. 2023).

The vast majority of DNA sequence in a cell is in the nucleus (generally billions of bp), but in addition each cell contains many mitochondria, each of which has a relatively small amount of its own DNA sequence (ca. 12-13,000bp for vertebrate taxa). In vertebrates, mitochondrial DNA (mtDNA) is haploid (a single copy of the genome per mitochondria) while nuclear DNA is diploid for most animal species (two copies of the genome per cell). But while there is a single nucleus in a cell, there may be hundreds to thousands of mitochondria within a single cell (Robin & Wong 1988). This means that there are often many more copies of a given mtDNA sequence than a nuclear sequence

per cell. For purposes of surveying fish using eDNA, it is possible to construct a molecular assay to target DNA sequences in the nucleus or mtDNA or both (Zhang et al. 2020, Kumar et al. 2022). At present, most investigations of eDNA target mtDNA in metazoans (e.g., Liu et al. 2026; Claver et al. 2026) because of its abundance, its ubiquity, its diversity, and because of the wide availability of reference databases for matching detected sequences to known species.

Molecular methods review

We will not summarize the process of filtering water, preserving samples, and subsequently extracting the DNA present in a given sample. These are standard field and methodological techniques that are well described in accompanying literature (see e.g., Ramon-Laca et al. 2021, Allan et al. 2025, Shaffer et al. 2025).

Having collected an eDNA sample and isolated DNA, the researcher can choose from a set of analytical tools with which to query the sample. In general, queries take one of two forms: single-species or multi-species assays.

In a single-species assay the purpose of the query is narrow: what is the concentration of DNA of a species of interest in a sample? Akin to some COVID tests and other biomedical tools, this approach uses either quantitative polymerase chain reaction (qPCR), droplet digital PCR (ddPCR), or equivalent techniques to yield concentration estimates. These tools differ in their specifics, but all share the common approach of measuring the concentration of target molecules with fluorescent dye, which lights up only in contact with target molecules and where fluorescence is proportional to the molecule's concentration in the sample. To use such tools one uses or designs de novo PCR primers and associated fluorescent probes that only match the DNA sequence of the target species (e.g., Sassoubre et al. 2016; Roux et al. 2020).

By contrast a multi-species assay asks a broader question: which species are present in my sample and what is the relative abundance of their eDNA? This approach uses primers designed to amplify via PCR a target group of species, which might be a single genus, all vertebrates, or could be as broad as all eukaryotes (Leray et al. 2013). The result after PCR is a mix of amplicons (short sections of DNA produced by the PCR reaction) derived from the various species present within the sample. Only sequences matching the primer will be replicated by PCR and be present after the reaction and therefore the primer sequence is crucial in targeting specific groups (e.g., fish or marine mammals or the genus *Sebastes*; e.g., Miya et al. 2015, Matthews et al. 2025, Ushio et al. 2025). Turning this mix into useful information requires sequencing the mixed pool of amplicons, resulting in millions of reads of short DNA sequences, each of which can be matched to a known species identity using a reference database. This multi-species technique is known as metabarcoding (Figure 1.1).

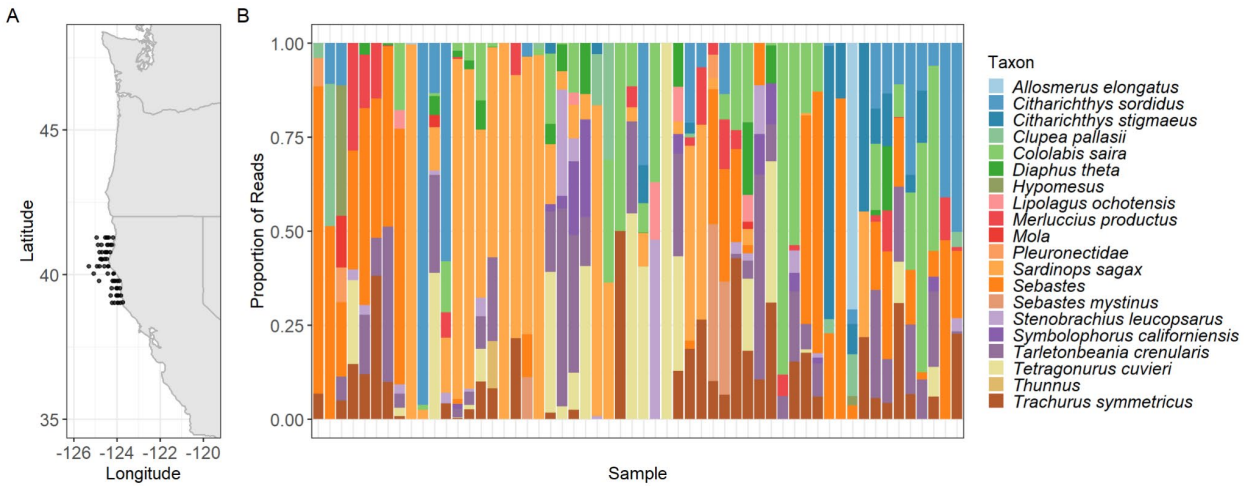

Figure 1.1: An example of metabarcoding data derived from NOAA-collected surface samples (panel A), sequenced at the 12S mtDNA gene region and taxonomically annotated (panel B). Individual bottle samples are reflected in columns in panel B, with sequence-read proportions for each taxon summing to one in each sample. Only the most common 20 species are shown, for illustrative purposes. Unpublished data.

Metabarcoding has proved a powerful and efficient way to survey the living world (Taberlet et al. 2012b, Valentini et al. 2015), but properly analyzing the resulting data requires more care than does the analysis of single-species observations. Three complications arise relevant to the present application in stock assessment.

1. Metabarcoding sequence reads are compositions, proportions that sum to one (McLaren et al. 2019). As such, species are not independent of each other within a sample, and, without additional auxiliary information, read-counts are not interpretable on their own as indicators of absolute DNA abundance

2. A given primer set does not amplify every species equally efficiently, often resulting in substantial observation bias and requiring correction (Shelton et al. 2023). Fortunately this bias is consistent and correctable in practice; the main source of bias appears to be primer-template mismatches (Shaffer et al. 2025), such that species with template molecules perfectly matching primer sequences amplify optimally and amplification efficiency declines dramatically with increasing numbers of mismatches.

3. The third is not an inherent property of metabarcoding data but instead a downstream question of analysis. One needs to match read sequences with known species in a reference database in order to make those sequences useful. This process, known as taxonomic annotation, depends in part on the completeness of the underlying database

but also on the diversity of the targeted stretch of DNA itself (Blackman et al. 2023). For fish species in the temperate Pacific the completeness of databases is not a significant issue because the total number of species relevant to the question at hand is relatively small and these are relatively well-documented taxa. Despite the relative completeness of the reference database for these species, it remains true that closely related species may be identical at a given genetic amplicon – and therefore not individually distinguishable on the basis of the amplicon used. In such cases, eDNA sequences would be annotated at the genus level, but more practically one could instead target a faster-evolving (and thus more genetically diverse) region of the genome to better distinguish closely related species where necessary (e.g., Ledger et al. 2025, Matthews et al. 2025).

The Ecology of eDNA

We view eDNA data – and more broadly, ecological observations of all kinds – as arising from an underlying biological process of interest (see Shelton et al. 2016). For example, we may see a DNA concentration (in copies/L of water) for a target species in a water sample (the observation). This concentration only imperfectly reflects something about the world that we wish to know (e.g., the spatial distribution of that species' DNA, or perhaps the biomass contributing DNA to the sample, the process). As a result, linking observed eDNA concentration to biomass — a core task of stock assessment — requires 1) distinguishing observation from process and 2) understanding the process by which eDNA behaves in the world (i.e., the ecology of eDNA). The first of these elements is not unique to eDNA studies, and because it is already a routine part of existing stock assessment, we need not treat it further here.

As for the process model, the eDNA concentration in a water sample reflects an integration of production, transport, and loss mechanisms (Barnes & Turner 2016; here, we separate transport and loss as conceptually distinct, recognizing that transport away from the sampling domain is a form of loss of signal more generally). Although the production of eDNA by individual animals varies over time and circumstance (Brasseale et al. 2025), for groups of individuals it is sensible to average over space to derive an approximate steady-state for per-individual production (Jacobson et al. submitted). General sources of loss include decay (generally via microbial degradation), with reported first-order decay constants across aquatic systems ranging from 0.05 to 0.10 per hour (Sassoubre et al. 2016), equivalent to a half-life of roughly 7 to 14 hours. Other sources of loss include sinking beyond the scope of sampling (i.e., transport in the z-dimension).

Although a full treatment of eDNA fate-and-transport is out of the present scope, it is useful to consider eDNA shed into the environment as analogous to a signal that

attenuates with distance away from the source (akin to radio, sound, light, etc.). At steady state – which again assumes a consistent average signal over some relevant space and time – this creates a smoothed signal relative to the source organisms themselves, though in general the distribution of DNA often closely follows the distribution of organisms (Andruszkiewicz et al. 2019) . In dynamic marine environments, eDNA transport is well-characterized by the dominant velocity vectors (Xiong et al 2025), which in a given case may be the sum of the vectors of dominant water-movement currents and of the movements of the source organisms themselves.

The approximate characteristic length scale of smoothing in a transport-dominated environment is L = v/k, where v is the dominant velocity vector (m/s) and k is the first-order loss constant (1/s). If we choose an example velocity of 0.1m/s and loss as $2*10^{-5}$ s^{-1}, we get a characteristic distance of 5km. This gives an order-of-magnitude estimate of the distance over which a point source of eDNA attenuates and which approximates the distance over which two sampling points can be considered independent estimates of a common underlying eDNA concentration. Note that this is actually an overestimate of the characteristic distance, both because diffusion will dilute the signal from a point source further and because attenuation is happening along three spatial dimensions simultaneously (rather than the simplified single dimension presented above).

Accordingly, the scale of smoothing (relative to source organism biomass) is likely to be shorter than realistic spatial intervals of sampling in the ocean or the relevant scales of biological inference for purposes of stock assessment. Simulation independently suggests the same conclusion (Jacobson et al. in review), where a mechanistic model of shedding, organismal movement, decay, and observation variability indicates strong correlation between biomass and eDNA concentration at resolutions of 50-100km but not at smaller spatial resolutions (5-10km, within which smoothing due to transport of both water and individual movement obscures the correlation).

Empirical Examples

For purposes of developing an index to inform a stock assessment, the key quantity of interest is the average eDNA concentration in space and time relative to the abundance of the target source organism. That is, over what spatial and temporal scale does the concentration of eDNA reflect the abundance of the source species? A recent meta-analysis by Rourke et al. (2022) suggests widespread support for a positive connection between biomass and DNA concentration across many systems. In the following we summarize a range of specific laboratory and field observations that support the connection between biomass and DNA concentration.

Within an enclosed system, such as a tank, we expect a steady state eDNA concentration to reflect biomass fairly precisely. And indeed it does. Jo and Yamanaka

(2022) provide the most highly replicated example of the relationship between eDNA concentration and biomass in an isolated system (Figure 1.2). Regardless of the methodological details of each particular eDNA analysis (across different assays, filter configurations, etc.) the authors found a strong and consistent relationship between eDNA concentration and numeric abundance (here, a proxy for biomass).

Ledger and colleagues (2024) report the results of a multispecies tank study using Pacific gadids and metabarcoding (Figure 1.3), and consistent with Jo and Yamanaka (2022), show strong and consistent relationships between eDNA metabarcoding read proportions and proportional biomass. However, using the absolute abundance of one species (via qPCR) to estimate the abundances of the other two, the authors report a considerably weaker relationship between biomass and eDNA concentration than might be expected (see Figure 1.3, lower panels). This may in part be an artifact of scale – eDNA concentrations are often reported on a log scale, given the exponential nature of the observation model (e.g., Jo & Yamanaka 2022) – but may also reflect stochasticity in the relationship between individual fish biomass and detected eDNA concentration.

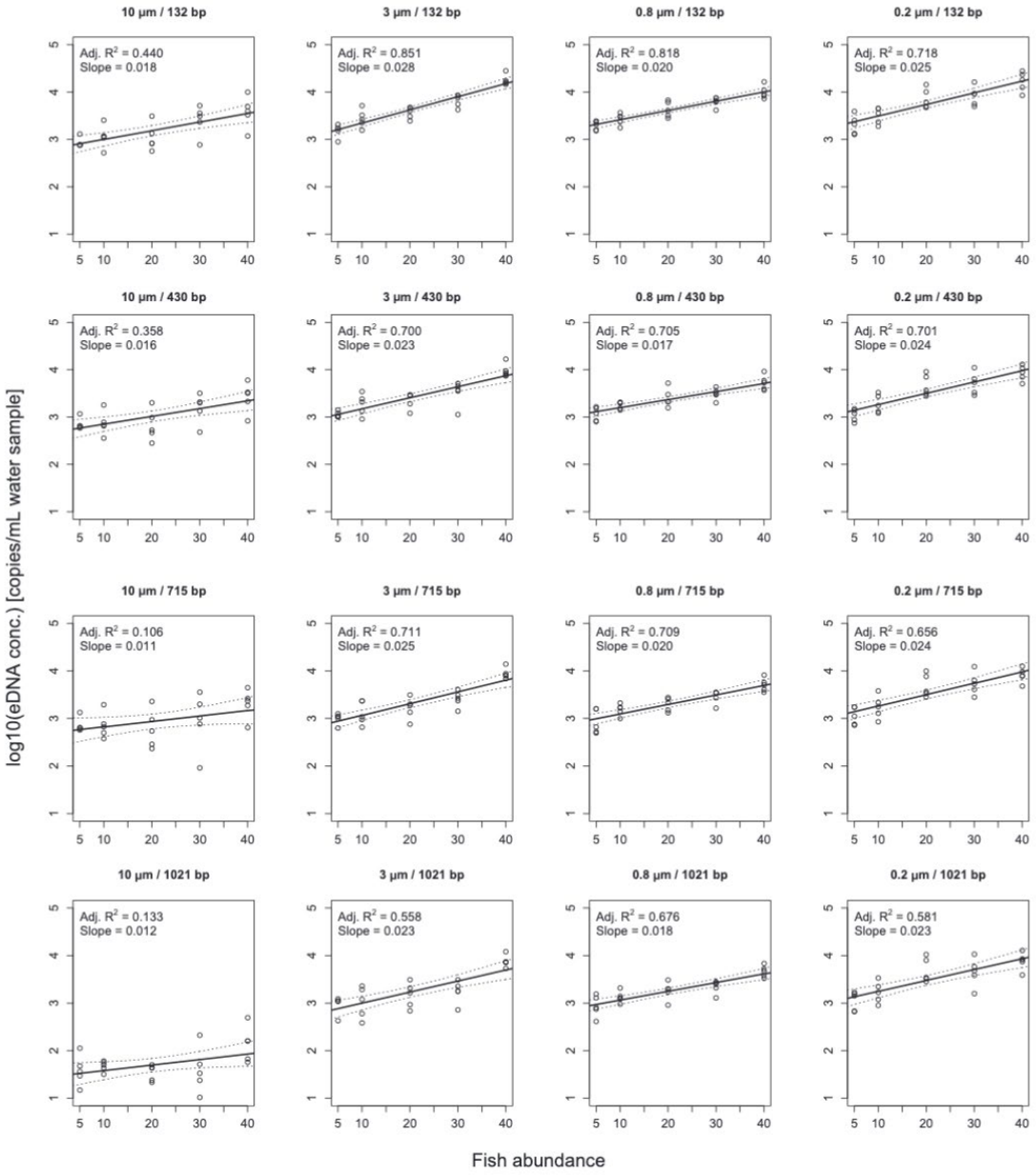

FIGURE 1.2: qPCR eDNA concentration vs abundance (number of zebrafish per tank) for a variety of eDNA fragment lengths (facets in rows) and filter pore sizes (facets in columns). (from Jo and Yamanaka 2022, Figure 2).

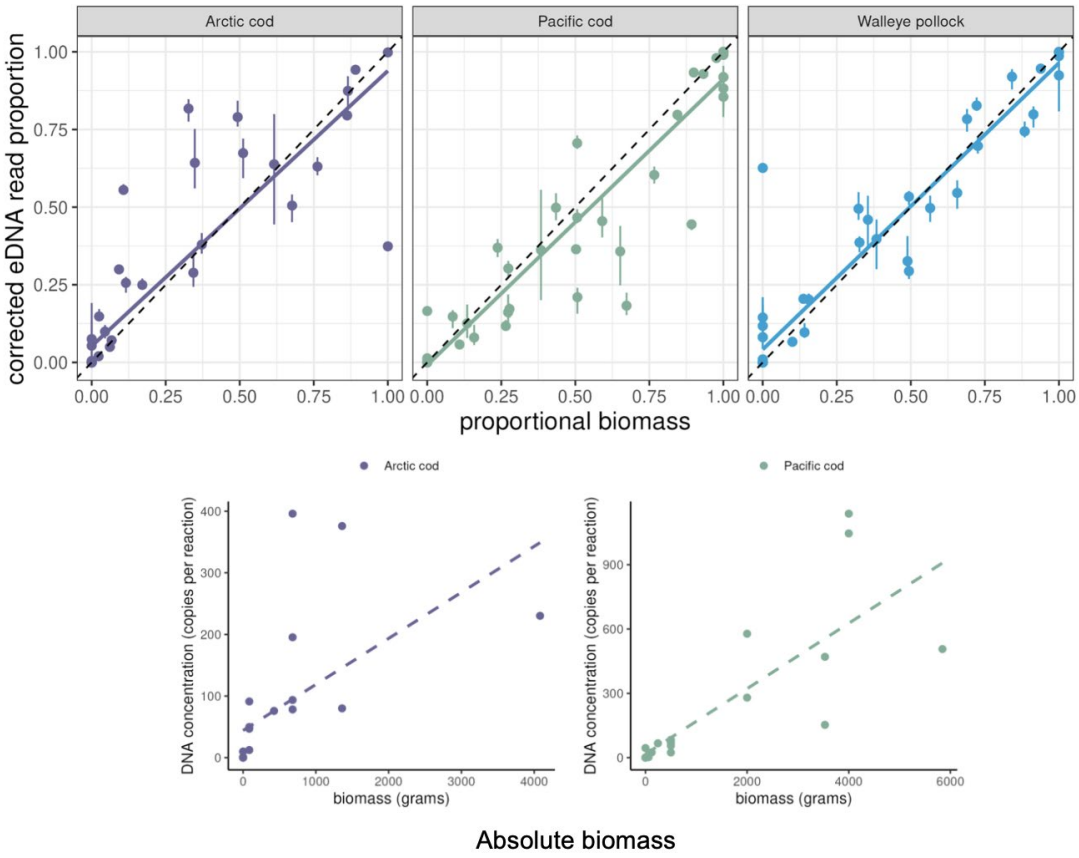

Figure 1.3: Metabarcoding-derived relationships between fish biomass and eDNA, in tanks. Top panels: proportional biomass (x-axis) and eDNA sequence reads (y-axis). Bottom panels: absolute biomass (x-axis) and eDNA concentration (y-axis). (from Ledger et al. 2024)

Sampling in a real-world field setting – as opposed to sampling in a tank of known volume – raises the possibility of a mismatch between the spatial scale of sampling and the spatial scale of the species (and the eDNA signal) of interest. Salter et al. (2019) provide an instructive example of the importance of aligning these spatial scales in order to yield meaningful results (Figure 1.4). There, in a qPCR study targeting Atlantic cod (*Gadus morhua*), the authors found little point-by-point correlation between

traditional CPUE or biomass and eDNA concentration, but at somewhat larger local/regional scales (ca. 50km bins) a quite strong relationship.

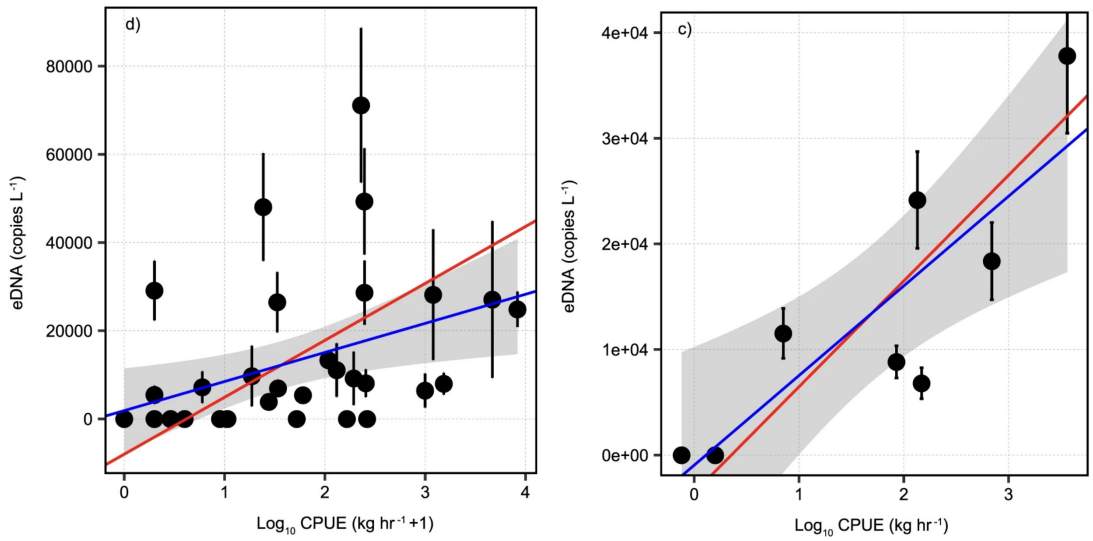

Figure 1.4: Field-based qPCR eDNA concentrations and CPUE for Atlantic Cod at two different spatial scales. Point samples (left) and regional means (right). (from Salter et al. 2019).

Shelton et al. (2019) report qPCR results for Chinook salmon in Skagit Bay, Washington, showing similar patterns (Fig. 1.5): point-to-point comparisons (in space and time) with catches from shore-based seine hauls had a strong but noisy correlation with eDNA concentration, while averaging across sampling sites (within sampling months; sites maximum distance 20km apart) yielded a stronger relationship.

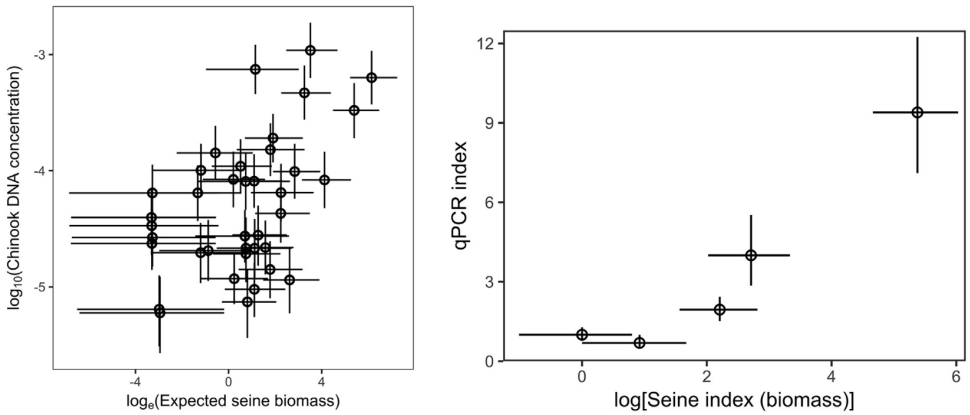

Figure 1.5: Field-based qPCR eDNA concentrations and expected biomass for Chinook at two different spatial/temporal scales. Individual site/month point-by-point comparisons (left panel) and monthly means (across sites; right panel). From Shelton et al. (2019).

Various other authors have assessed correlations between metabarcoding results and biomass – notably Mark Stoeckle and colleagues (Stoeckle et al. 2021, 2024, but see also West et al. 2024) – outside of the context of a formal quantitative framework, making it difficult to generalize those results for utility in stock assessments (but see Yates et al 2023, on allometric scaling in eDNA studies). As a general rule, broadly distributed, high-biomass fish species tend to show strong correlations between catch proportions and metabarcoding proportions (Stoeckle et al. 2021, 2024), while rarer and patchier species do not (West et al. 2024), again likely due to the alignment of spatial scale between observation and process. And across large spatial scales, species-specific spatial patterns of eDNA concentration tend to mirror closely the estimated biomass distributions of the species themselves (Guri et al. In Press).

Finally, integrated models, in which multiple datastreams inform the estimate of a common latent parameter such as biomass, offer an attractive way of using eDNA in combination with traditional datastreams – in the process highlighting areas where the datasets do or do not agree (Guri et al. 2024, Claver et al. 2026). Most relevant to stock assessments, Guri et al. (2024) developed a hierarchical model for two different forms of eDNA data – ddPCR for a few fish species and metabarcoding targeting a wider variety of commercial fishes – and commercial trawl catches in a set of Norwegian fjords over a three-year period. By recursively splitting the dataset into training vs. test years, the authors were able to make out-of-sample predictions of trawl catch based upon the molecular observations alone (Figure 1.6).

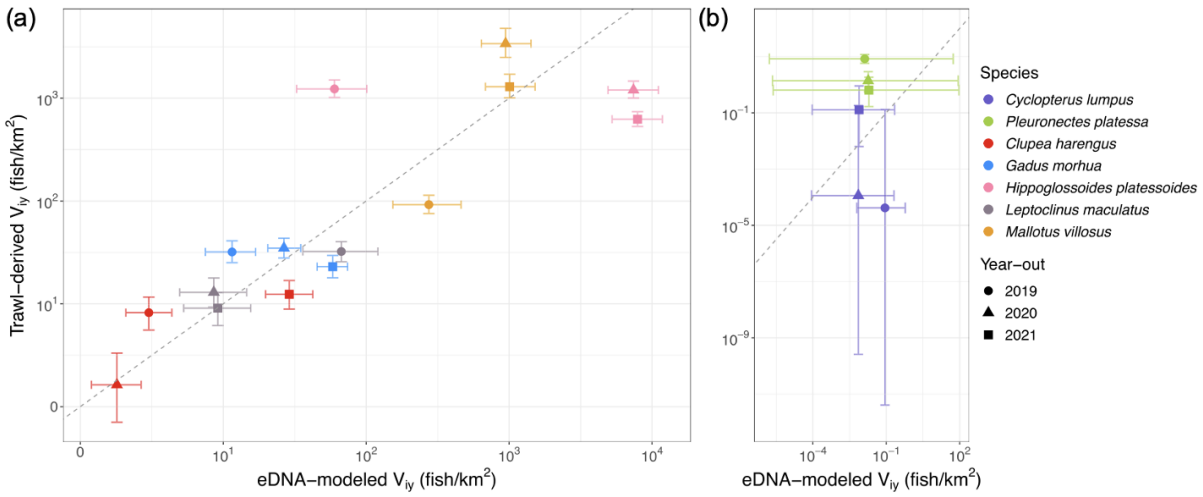

FIGURE 1.6: Predictions of trawl catches vs empirical trawl catches for a set of commercial fish species in Norwegian fjords. Colors are species, and symbols represent predictions for different years (following a recursive, out-of-sample prediction for catch in a third year, given the model fit of the other two years). Panel (a) reflects species for which sufficient observations were available; panel (b) illustrates the pathology of

predictions based upon very few observations for species not well sampled by either the trawl or the molecular methods. (From Guri et al. 2024).

Conclusions for part 1

This section outlined the basics of eDNA observations and provided a brief summary of the current state of the literature on the ‘ecology of eDNA’. The most salient points for fisheries and management are: 1) the DNA signal of taxa in the ocean is distributed somewhat more smoothly in space and time relative to source organisms, 2) due to fairly rapid decay/loss of DNA in the environment (on the order of hours) and limited transport distance (meters to tens of km), this smoothing happens at scales of (at most) tens of km. This means that DNA observed in the environment arose from individuals that are relatively nearby in space and/or time. Furthermore, the literature suggests that, when considered over the appropriate spatial and temporal scale, there is a relatively close coupling between biomass and DNA concentration.

2. Application: eDNA and Pacific Hake

In this section we discuss the application of eDNA methods to an abundant and important fisheries species on the US West Coast, Pacific hake (*Merluccius productus*, hereafter hake). We use data from a qPCR assay for hake applied to a large number of eDNA samples collected along the coast of California, Oregon, and Washington between 2019 and 2025. We present relevant aspects of the analyses below, but more details on all aspects can be found in published literature (Shelton et al. 2022, 2025, Ramon-Laca et al. 2021). A broader description of the hake acoustic-trawl survey can be found in the annual cruise reports (e.g., Grandin et al. 2020), in NOAA Fisheries' West Coast Offshore Scientific Surveys webpage, as well as in publications (Ressler et al. 2007, Malik et al. 2020).

Most importantly for the purpose of this review, the eDNA samples and data collected during the hake survey represent a rich dataset to compare existing survey information to new eDNA-based survey methods. Furthermore, the samples collected during the hake survey can and have been repurposed to examine the distribution of eDNA for other (non-hake) taxa (see Part 3), including for coastal pelagic species such as sardine and anchovy. The sampling considerations discussed for hake below are general and would need to be met for application of eDNA data to inform any other species.

Pacific hake biology

A general description of the biology of hake can be found elsewhere (Methot and Dorn 1995, Ressler et al. 2007, Malick et al. 2020, Grandin et al. 2020). Hake is one of the most abundant fish species in the California current ecosystem (CCE). They support fishery harvests of hundreds of thousands of metric tons annually and play an important role as both predator and prey in the CCE (Edwards et al. 2026). They are found at highest abundance near the shelf break (between bottom depths of approximately 150 and 300m) and in water depth between 50 and 500m though they are observed in deep offshore waters occasionally and occasionally in surface waters. Hake are well known for their fluctuations in abundance driven by sporadic large recruitment events (Vestfals et al. 2021) as well as their significant shifts in distribution among years (Malick et al. 2020).

Design considerations and eDNA samples

In 2019, eDNA sampling was first added to the existing biennial Pacific hake acoustic trawl survey. We collected water at oceanographic sampling stations along latitudinal transect lines crossing the continental shelf with bottom depths starting at 50m and reaching more than 3000m. However, CTD casts only reached a maximum depth of

500m and so the deepest collection at each station was within 10m of the sea floor or 500m. The sampling domain in 2019 spanned from the latitude of San Francisco Bay (38.7 N) north to Cape Flattery (49.4 N) and encompassed an area of approximately 86,000 km^2 (Figure 2.1; Shelton et al. 2022). From 2021 through 2023, the southernmost sampling began at Point Conception, California (34.5 N), with the northernmost edge of sampling ending at Cape Flattery in 2021 and 2025 and in southern B.C. in 2023 (Fig. 2.1). In 2025, the hake survey was combined with the Southwest Fisheries Science Center Coastal Pelagic Species survey to form the Integrated West Coast Pelagic Survey (IWCPS); the southernmost sampling in 2025 started in the Southern California Bight with surface samples only, and CTD transects that collected water samples for eDNA at depth beginning near Point Conception. Note that the latitude of transects and the longitudinal extent of the CTD stations sampled for eDNA vary somewhat among years (Fig. 2.1). This fact will affect the creation of an eDNA time-series below.

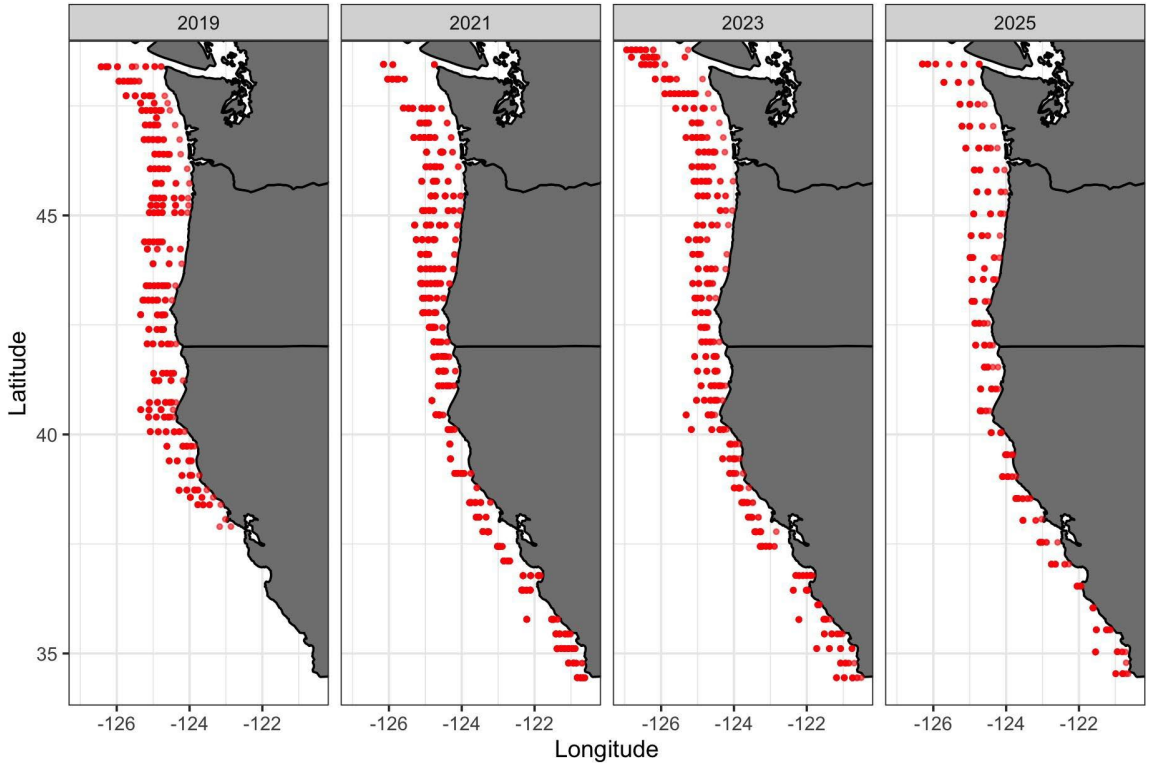

Figure 2.1: CTD stations sampled for eDNA for 2019-2025. Each location shown was sampled at the surface and at subsurface stations. Additional, surface-only sampling stations from 2025 are not shown.

In 2019, water samples from the surface were collected via the through hull pump (collecting water at approximately 3m depth) as well as via Niskin bottles deployed at 50, 100, 150, 300, and 500m depths. Each water sample has two, 2.5L replicates per station-depth combination. In subsequent years (2021 and 2025) we replaced the

100m samples with water collected at 200m. Water samples were collected over the course of the survey (spanning late June to early September in each year). In total, we have collected between 1597 and 2228 water samples annually representing approximately 200 stations with subsurface samples per year. In addition, we processed a range of eDNA control samples to document and account for different kinds of possible contamination that can occur during the sampling and laboratory processing. An important caveat for the 2019-2023 eDNA surveys is that water sampling does not co-occur with acoustic sampling for hake; it is not physically possible to simultaneously deploy CTDs and run acoustic transects. For the 2019, 2021, and 2023 survey years, CTDs were collected during nighttime operations while acoustic transects were recorded during the day. In 2025, samples were collected during the day, during breaks interspersed along acoustic transects. While the time gap between acoustic and eDNA collections on any transect was generally small (generally less than 12 hours and nearly always within 24 hours), the lack of precisely paired sampling restricts the ability to make comparisons of the two observation types at the level of individual observations (see also below).

To our knowledge this is the most extensive temporal and spatial eDNA sampling data set for fisheries anywhere in the world. However, for this to be a useful survey, the collected samples need to satisfy several basic design criteria. First, the sampled area must meet closure assumptions - the target population does not meaningfully change or experience new entries/exits during the specific observation period. Second, there must be an adequate number of samples collected to describe the population. Both of these requirements are true for all types of surveys. Finally, and most specifically for eDNA, we need to be able to assert that there are negligible differences in processes that affect eDNA quantification (i.e. decay, production, etc.) across the sampling domain.

For hake, the water samples closely mirror the areas and times surveyed using acoustic-trawl (AT) methods. The samples span the core latitudinal, longitudinal, and water depth range of Pacific hake. Following the assumptions of the AT survey, the hake biomass from areas offshore of the end of the acoustic transect or inshore (water depths shallower than 50m) of the survey are considered negligible. Similarly, depths shallower than 50m or deeper than 500m are assumed to contain negligible hake biomass. Surface water samples may include eDNA from larvae or young-of-the-year hake. Therefore, surface samples were excluded from the index generation process. Some hake were certainly present beyond the northern or southern boundaries of the eDNA sampling (e.g., the survey extends into Canadian waters, using a Canadian research vessel), but the sampled latitudinal range contains the core range of hake abundance. As such, the available sampling represents a large enough component of the range to provide information about abundance and trend. Experimental evidence

suggests that there is some variation in the production and decay rates of eDNA as a function of temperature and other environmental covariates in both laboratory and field conditions (see e.g., Collins et al. 2018, McCartin et al. 2022, Jo et al. 2019, Andruszkiewicz et al. 2021). For building an index across a time-series, the relevant concern is whether the environmental conditions shift significantly among years and thereby induce changes to production and decay rates that obscure or bias estimates of DNA concentration. According to available literature, the magnitude of change in production and decay in response to water temperature (a change of at most a few degrees C in any given year) should be very minor. However, we acknowledge that further research is certainly needed to investigate possible complications from changing rates.

Building an eDNA index

We use spatio-temporal index standardization (see e.g., Shelton et al. 2014, Thorson et al. 2015) to generate an eDNA index for hake. This style of index standardization is currently used for most west coast groundfish stocks (see e.g., Anderson et al. 2025; Wetzel et al. 2026), and the same general considerations inform the use of such approaches for trawl, acoustic-trawl, or an eDNA survey.

There are three main differences between the index standardization approaches currently used for West Coast groundfish and the approach necessary in the case of eDNA data. First, models are constructed for DNA concentration, not trawl catches, and require distinct observation models to reflect the laboratory processes inherent in generating eDNA observations (Shelton et al. 2022, Liu et al. 2026, Vignette A). For hake qPCR observations, this means we need to account for zero observations as well as positive observations and translate outputs from a qPCR machine into DNA concentrations (see Vignette A for a worked example for qPCR eDNA observations). Second, unlike a trawl survey or other methods that involve physically catching, and therefore depleting, individuals, water samples can be collected in replicate at a single location. This potentially allows for an additional hierarchical level representing small scale variation that is not generally possible using catch data. Finally, and likely most importantly, eDNA samples are collected at discrete locations in three dimensions (latitude, longitude, depth) rather than the two dimensional (latitude, longitude) spatial domain associated with the benthic trawl survey. This necessitates sharing and integrating information across depths. Acoustic observations from the hake survey also need to deal with the three dimensions but these tend to be addressed in a fundamentally different way than eDNA or benthic trawl information.

For building eDNA indexes, we have developed several related index standardization approaches. We outline each approach below, but all follow the same basic structure: 1) construct and estimate a spatio-temporal statistical model; 2) from the estimated model, make projections to a standardized set of locations and depths; 3) repeat step 2 many times to incorporate uncertainty in parameter estimates in the projections; 4) combine information across space and depths to arrive at a synthetic eDNA index.

Shelton et al. (2022) constructed a full Bayesian spatial model for hake DNA concentration. Modeling qPCR observations from 2019, they constructed a model using generalized additive models, including fixed environmental covariates (e.g., bottom depth), offsets for laboratory processing (e.g., dilution rates for inhibition), a depth-specific spatial field for each sampled depth (i.e., 3, 50, 100, 150, 300, and 500m), and a random effect for replicate bottles sampled at each location. They then projected DNA concentration to the centroids of a standardized spatial grid (a 5 x 5km resolution grid) at 50m intervals between 50 and 500m. To flatten this 3D set of predictions into a 2D surface, they summed the DNA concentrations across prediction depths to provide a summary of the DNA copies for each grid cell. This projection and summing process was repeated across many draws from the posterior distribution to provide uncertainty bounds on the individual depth predictions and summed index values for each grid cell. This index has units of DNA copies per liter per 5km grid cell. Note that there are other projection and summarization methods that could be used to generate an eDNA index with different units that can be considered equivalently proportional to biomass.

To compare eDNA results with acoustic trawls, we constructed a parallel spatial statistical model for the acoustic trawl observations (see Shelton et al. 2022). They used the depth integrated output provided by the AT survey in discrete 0.10nm spatial segments along each transect and estimated a comparable spatial model to the eDNA observations providing a depth integrated estimates of total hake age 2+ biomass for each spatial location between 50 and 500m. After estimating the spatial model, they projected the biomass estimates to the same 5km grid used for the eDNA observations. This enabled pairwise comparisons between the predicted biomass from the AT survey and the eDNA index (see Figs 2-3 in Shelton et al. 2022; reproductions provided as Figs 2.2 and 2.3 below). The predictions were further aggregated over larger spatial scales (1 degree latitudinal bins) to understand how eDNA and acoustic biomass estimates covaried over large spatial scales (Fig 2.3).

Broadly, areas of high biomass as assessed in the acoustic survey visually correspond with areas of high DNA concentration (Fig 2.2). At the 5 km grid scale, the two predictions are positively correlated (ρ = 0.55[0.53, 0.57]; posterior mean and 90% CI; Fig 2.2A) but at the one degree of latitude scale the correlation between the methods

increased substantially, (ρ = 0.88[0.65, 0.96]; Fig. 2.3). Evidence of concordance between eDNA and acoustic-derived biomass extended to spatial distribution (measured via center of gravity; see Fig. 4 in Shelton et al. 2022).

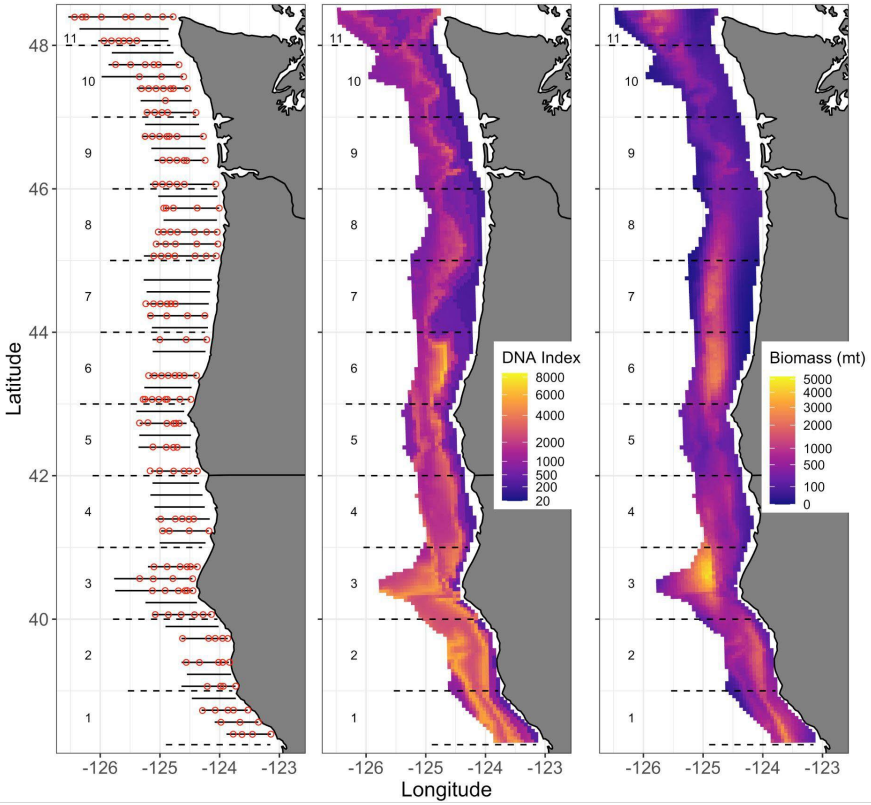

Figure 2.2: *Left panel:* survey locations for 2019 (circles show eDNA sampling locations, lines show acoustic transects). *Center:* depth-integrated index of hake DNA. *Right:* hake biomass from acoustic surveys. Both DNA and acoustic estimates are mean predicted values projected to a 5 km grid and include information between 50 and 500 m deep. All panels show one degree latitudinal bins (numbered) used to aggregate abundance estimates over larger spatial scales (from Shelton et al. 2022).

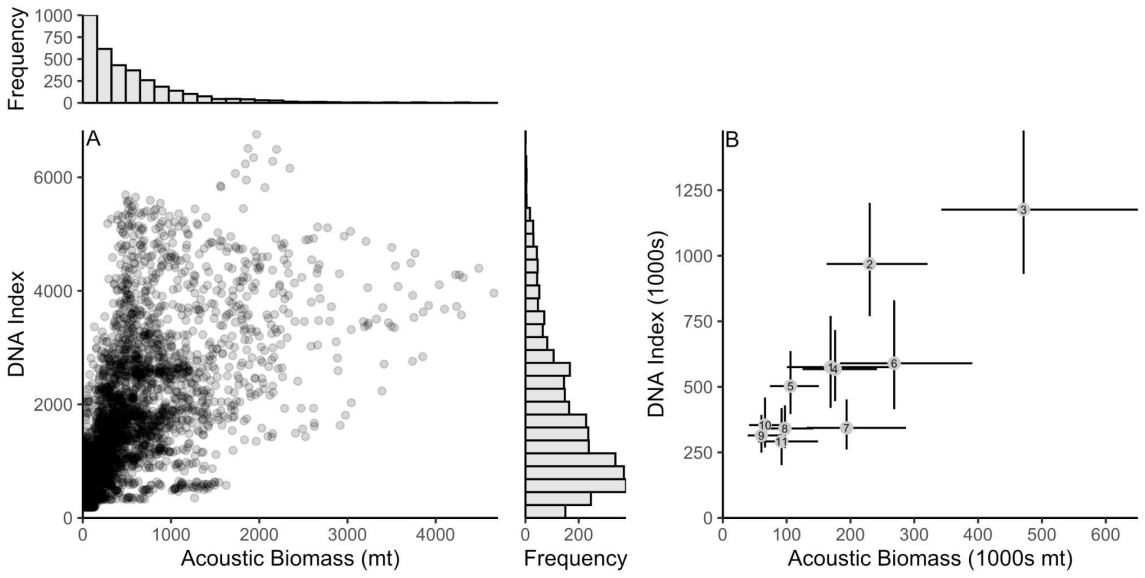

Figure 2.3: Pairwise comparison between DNA and acoustics-derived biomass. *A:* Posterior mean prediction from each method among the 5x5km grid cells. Marginal histograms show posterior mean values for individual cells in each method (correlation: ρ = 0.55[0.53,0.57]). *B:* Correlation between methods among the 11, one degree latitude bins (ρ = 0.88[0.65, 0.96]). Numbers indicate regions identified in Figure 2.1. From Shelton et al. (2022).

Generating an eDNA index time-series

The work based on the 2019 hake survey suggests correspondence between the acoustic and eDNA surveys, particularly at larger spatial scales. However, the provided index is intended as an index of relative abundance, not absolute abundance. Extending the comparison to subsequent years is necessary to evaluate whether the relationship between the eDNA index and abundance estimates from other indices is preserved over time.

To develop an index for 2019, 2021, and 2023, Shelton et al. (2025) used a spatio-temporal model broadly similar to the approach used by Shelton et al. (2022). Like Shelton et al. (2022), the 2025 approach uses a spatio-temporal index standardization approach but some of the statistical and estimation details differ. For example, the 2025 approach differed in using a likelihood based optimization (implemented in TMB) as opposed to a full Bayesian approach (implemented in Stan). This difference has modest implications for the interpretation of uncertainty bounds on parameters and index values. Structurally, the largest difference between the approaches was that instead of using an independent spatial field for each water depth, it developed a factor analysis method similar to Thorson et al. (2015) to construct smooth spatial fields for observed

depth that are a weighted average of a smaller number of shared spatial fields. This is a significant technical change, but the approaches do not meaningfully change the mechanics of building an index nor its interpretation. As in previous approaches the predicted value for each 5km grid cell was predicted at the centroid of each cell in 50m increments between 50 and 500m and then summed to generate a depth-integrated value for each grid cell. We show the mean spatial predictions for the entire spatial domain in Fig. 2.4.

For constructing an index time-series, Shelton et al. (2025) made several additional assumptions about the domain occupied by hake in each year. Most meaningfully, the spatial extent of the hake eDNA samples varied over time. 2019 samples do not extend south of approximately San Francisco Bay, while samples in 2023 extended into southern Canadian waters (Fig. 2.1). To enable fair comparisons among years, we project our estimated model to a latitudinal range shared among all years (a “core” area) as well as a “south” area shared between 2021 and 2023 (Fig. 2.5). In addition to the latitudinal variation among years, the longitudinal extent also changes modestly among years. This is most evident in the area around Cape Mendocino in Fig. 2.5. The longitudinal extent is defined by the acoustic transects; areas onshore or offshore from the ends of acoustic transects contain, by assumption, zero hake. These areas are defined externally from the eDNA samples by the extent of the acoustic transects and so for comparability, Shelton et al. (2025) followed the year to year variation defined by hake trawl survey. If these observations were used to build eDNA indexes for other taxa, alternate projection domains may be appropriate.

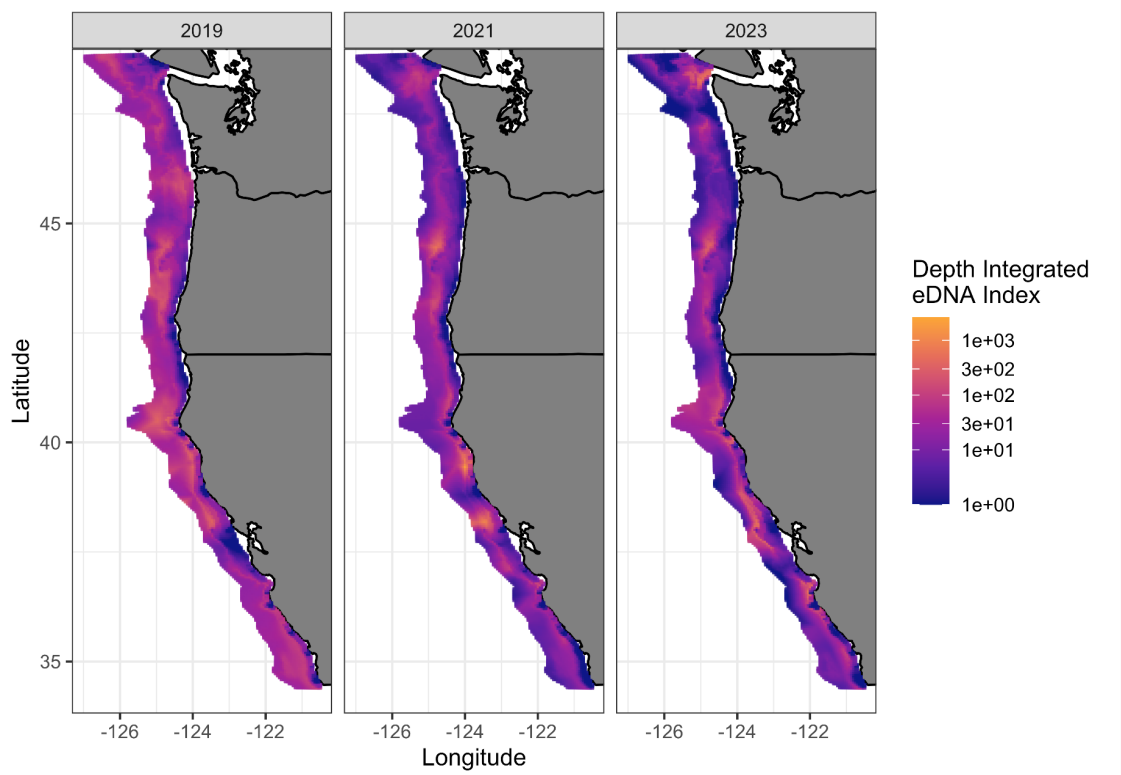

Figure 2.4: Predicted depth-integrated eDNA index values for each year at the 5km grid resolution. Predictions for the entire spatial domain are shown.

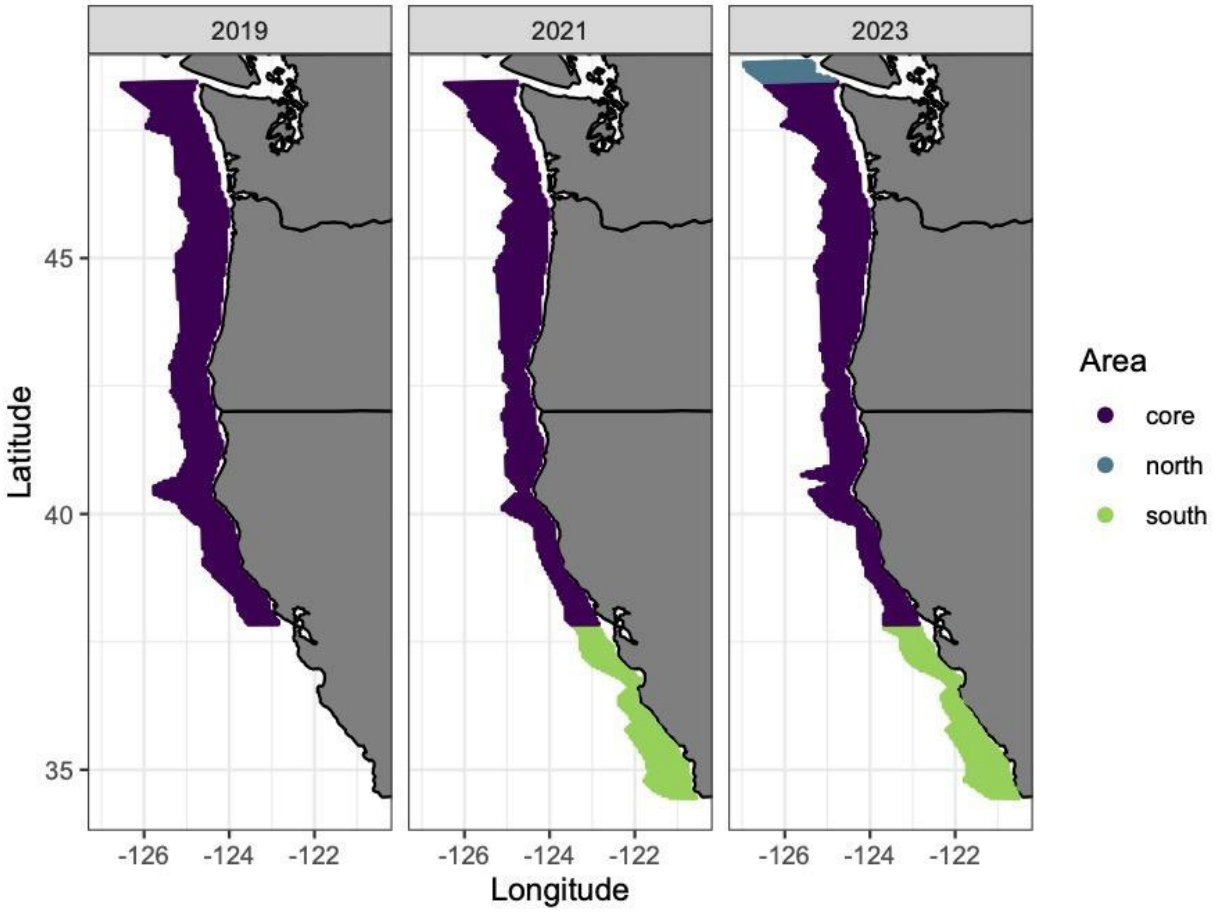

Figure 2.5: Prediction areas used for generating an eDNA index in each year. Boundaries for each area are based on the acoustic transect extent (longitudinal boundaries) and the latitudinal extent of eDNA sampling (reproduced from Shelton et al. 2025)

To generate an annual relative abundance index, Shelton et al. (2025) summed the values for each grid cell within the boundaries of “core” or “south” areas to arrive at an eDNA index time-series for each area (Fig. 2.6). The abundance values derived from the acoustic-trawl survey are presented (Fig. 2.7; see Johnson et al. 2025, their Fig. b). Overall, both indices suggest a marked decline in relative abundance between 2019 and 2023 with both indices falling by slightly more than one-third over that time (eDNA declining from an index value of 307 to 182 (1,000s), about a 41% decline; trawl survey declining from about 1.6 Mt to 0.9 Mt: about a 43% decline; FIg. 2.7). However, the eDNA suggests that the majority of the decline occurred between 2019 and 2021 while the trawl survey indicates the majority of the decline occurred between 2021 and 2023 (Figs 2.6 - 2.7).

However, there are additional significant similarities between the surveys. Most notably, the distribution of hake shifted substantially south from 2019 to 2021 and again from 2021 to 2023. Both surveys show very low hake abundances in Washington state (Fig 2.7, see Johnson et al. 2025 their Figure 2).

An important component of building an abundance index for inclusion in a stock assessment is providing information about the uncertainty surrounding index estimates. The approach derives the eDNA index using a single model linking the lowest level of observations to predicted concentration (see Shelton et al. 2025). As such, it should propagate uncertainty appropriately. In addition, they explore a range of alternative model structures, most notably varying the construction of the spatio-temporal fields, to explore the sensitivity of results to modeling choices (see Fig. 2.6). Overall, the uncertainty for the eDNA index was relatively low with coefficient of variation around 0.06 or 0.07 (2019: mean 307, SD =18.6, CV = 0.06; 2021: mean = 203, SD = 14.6, CV = 0.07; 2023: mean =182, SD = 10.8, CV = 0.06; 1000s). In general, spatio-temporal index standardization tends to provide narrower uncertainty bounds than other standardization methods (e.g., Shelton et al. 2014, Thorson et al. 2015), and ensuring these uncertainty bounds are appropriately capturing the uncertainty around the estimates deserves more attention.

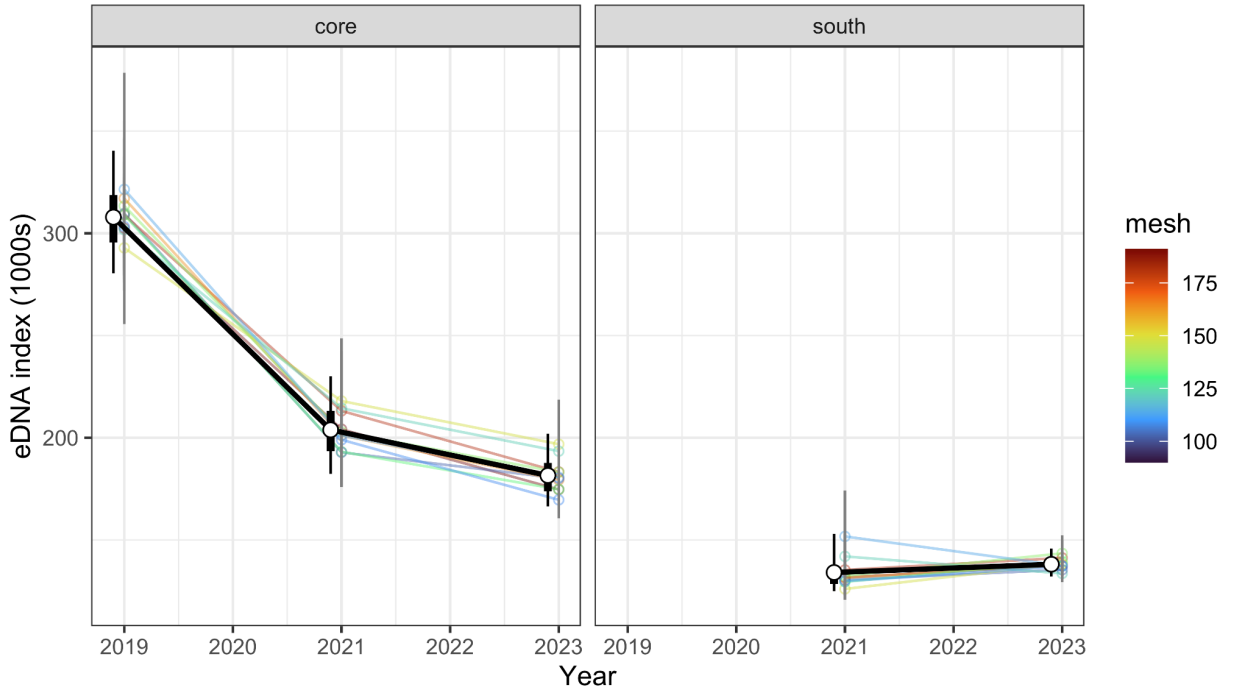

Figure 2.6: Predicted eDNA index for two regions between 2019 and 2023. The black line shows the model chosen for inclusion in the stock assessment with mean, interquartile range and 90% CI shown. The finer lines show alternate spatio-temporal

model constructions considered across a range of spatial mesh densities used in model estimation (from Shelton et al. 2025).

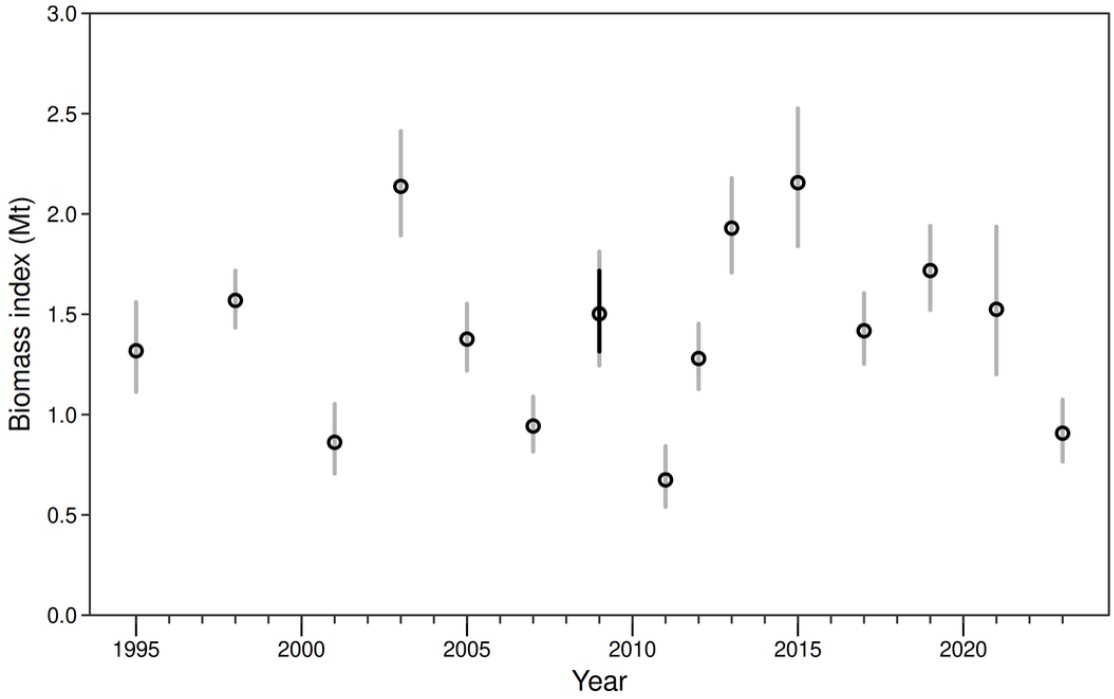

Figure 2.7: Acoustic survey biomass index of age-2+ fish (Mt). Approximate 95% confidence
intervals are based on sampling variability (from Johnson et al. 2005; their Fig. b)

Incorporating an eDNA index in a stock assessment

After creating an eDNA index, it is important to consider how to combine the information from an abundance index with the other components of an assessment. In this section we will outline the decisions faced by Johnson et al. (2025) for including the eDNA index as a sensitivity for the base model run of the hake stock assessment. Baetscher et al. (2025) provides an extended discussion of many aspects of incorporating eDNA indexes into stock assessments.

First, based on the information presented in part 1, the eDNA index was considered a relative abundance index, not an absolute abundance index. This is consistent with the evidence from part 1 and for the analysis of hake data from 2019 supporting a proportionality between biomass and eDNA concentration. Second, the eDNA index

was included as an independent abundance index. This decision was made despite the fact that the observations were collected from the same ship and generally the same time as the acoustic trawl survey. In the absence of creating a single joint abundance index that reflected both the trawl and eDNA observation, treating the two abundance indexes as independent appears to be a practicable approach that does not require additional data source weighting considerations.

There are two remaining aspects of including an eDNA index that are significantly more complicated and deserve more consideration: catchability and selectivity. As these topics are general points of contention for many data sources and have not been discussed in the context of eDNA (but see Baetscher et al. 2025), we devote a section to each component.

Catchability considerations

We define catchability as the relative proportion of a fish population that is sampled by a unit of survey or fishing effort. The options for the treatment of catchability in stock assessment models within the PFMC process include fixing it to an externally-derived value (e.g., acoustic trawl survey in the Pacific sardine assessment; Kuriyama et al. 2024), analytically computing it contingent on the other assessment parameters (e.g., bottom trawl survey in the canary rockfish assessment; Langseth et al. 2023), and estimating it as a free parameter (e.g., Haltuch et al. 2019). Regardless of the chosen treatment, catchability can be interpreted as a scaling coefficient between a relative index of abundance and absolute population abundance (Baetscher et al. 2025). In the case of a biomass survey (e.g., trawl survey), catchability can be used to scale the biomass index to the biomass estimate generated in the assessment model. For an eDNA index, catchability has a less straightforward interpretation because it relates molecule concentrations to population abundance or biomass. To our knowledge, scaling coefficients between eDNA concentration and organism abundance have not been empirically estimated. However, this does not necessarily limit the use of an eDNA index because catchability often links relative population indices, such as those from longline (Lennox et al. 2017), acoustic (Kotwicki et al. 2015), or submersible surveys (Dick and He 2019), to total population abundance.

Catchability can incorporate considerations beyond gear availability, such as the overlap between survey footprint and the spatial distribution of the population (Swain and Sinclair 1994). For example, acoustic trawl survey catchability in the Gulf of Alaska walleye pollock example is modeled to reflect the proportion of the population that spawns in the Shelikof Strait (Rogers et al. 2025).

Catchability can be estimated imprecisely when time-series are short or there is high uncertainty in the index data, which may lead to biased estimates of absolute biomass, especially when there are no other informative data sources in an integrated stock assessment. Prior information on catchability may be derived, for example, from gear-efficiency experiments, independent estimates of availability (e.g., the fraction of the stock within the survey's spatial, depth, or habitat footprint), or absolute abundance estimates from visual, tagging, or acoustic methods. An informative prior may help scale population biomass to external evidence while still estimating catchability where the data are informative, as opposed to fixing it, which is useful in propagating uncertainty around catchability.

The 2025 Pacific hake assessment estimates catchability for the acoustic trawl biomass index (Johnson et al. 2025). The model incorporating the eDNA index representing age 1 and older fish is presented as a sensitivity analysis. In that model, catchability for the eDNA index was derived as an additional parameter (posterior median q = 0.148). The authors note that catchability for the eDNA index is more difficult to interpret than the acoustic trawl biomass index because it scales DNA molecules to population biomass.

Selectivity considerations

Selectivity is often defined as the probability that a fish is captured when encountered by a fishing or survey gear and it can be a function of length or age. Like catchability, it generally incorporates some notion of the availability of different sized fish to the gear. Unlike catchability, it is not used to relate the proportion of the population to total population abundance, but rather to express the proportion of a length or age class that is captured. In stock assessments selectivity can be modeled using various forms, for example as a logistic function of length. Selectivity parameters can be estimated within the assessment model or fixed to some externally estimated or empirical values.

The functional form and the parameters of a selectivity curve usually reflect how the survey or fishing gear interact with the fish. In the case of an eDNA survey, selectivity should represent the probability of observing the DNA of a fish of age a given that the fish was present at the sampling location. However, selectivity of an eDNA survey may be confounded by processes that are currently unknown or difficult to measure such as age- or size-dependent DNA shedding rates, or by the presence of larvae at the sampling location.

Unlike most fishery-independent surveys used to inform stock assessments, eDNA surveys have the potential to detect individuals across all life stages, including larvae. Conventional age-based and size-based selectivity formulations are generally designed to characterize the sampling of post-recruitment fish and, in part, avoid sensitivity to the

high and often variable natural mortality rates experienced by early life stages. Consequently, these selectivity formulations may not adequately account for the contribution of larval DNA to an eDNA index. One approach to address this issue is to exclude samples collected in depths or locations known to represent larval habitat from the data used to construct the eDNA index.

In the 2025 Pacific hake assessment, eDNA survey selectivity was fixed at 1 for all age classes age-1 and older. This specification assumes that age-specific differences in eDNA shedding rates are negligible and that all fish age-1 and older are equally available to the eDNA survey (Johnson et al. 2025). Selectivity for Pacific hake younger than 1 year old (e.g., larvae and young-of-the-year) was fixed at 0, effectively excluding their DNA from the index. This assumption is reasonable because larvae and young-of-the-year Pacific hake are typically distributed near the surface, and surface collected DNA samples were excluded from the analysis used to construct the eDNA index.

3. Application: eDNA methods for other taxa

Thus far we have outlined the methods underlying the production of eDNA observations and detailed their application to produce an eDNA index for a single taxon (Pacific hake) from a single-species qPCR assay. In this section, we present several examples and developing applications to other taxa derived from both qPCR and metabarcoding approaches.

Approaches & developing information

The key to developing an eDNA index is in the ability to derive estimates of DNA concentration. There are three basic approaches to developing estimates of DNA concentration from eDNA observations: 1) Directly measure DNA concentration for a focal taxon using qPCR (as in the hake example) or similar technologies (e.g., ddPCR); 2) Pair direct measures of DNA for a single taxon (i.e., qPCR) with multi-species metabarcoding that provides a measure of relative abundance estimates. Use the DNA concentration of the qPCR species to anchor the quantitative estimates of concentrations of the non-qPCR species; 3) Conduct replicated metabarcoding for samples to account for variability and bias within and among samples to derive relative quantification of DNA copies for individual species within metabarcoding data. Aside from these approaches for deriving DNA concentrations from molecular observations alone, recent years have witnessed the development of integrated models that combine DNA observations with other survey methods for ecological inferences, we briefly note two examples of this work.

qPCR

The methods for qPCR and estimation of eDNA biomass using qPCR data is described in detail for hake in the above sections. Additionally, by leveraging samples from the hake and now IWCPS, we have been able to retrospectively quantify sardine and anchovy in samples collected on the surveys in 2021, 2023, and 2025 (see surface observations in Fig. 3.1). As is appropriate for coastal pelagic species, nearly all eDNA is observed at the shallowest samples (Fig. 3.2 for an anchovy example). Working with the SWFSC stock assessment team, eDNA biomass indices are in development for these two species as well, and there are ongoing efforts to evaluate the information relative to other indices from traditional survey data (acoustic-trawl).

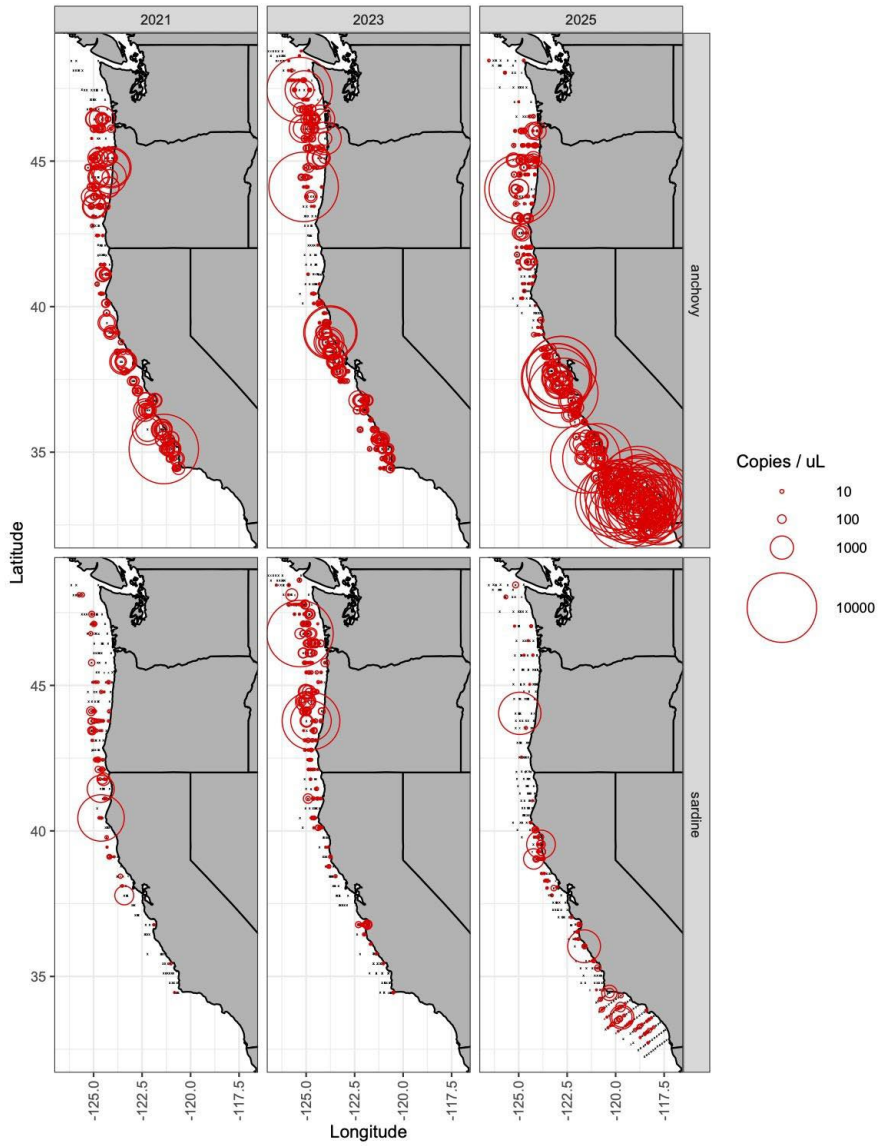

Figure 3.1. Anchovy (top) and sardine (bottom) DNA concentration (DNA copies / uL) observed in surface waters from the 2021, 2023, and 2025 survey years. Each circle represents the average concentration collected from a single 2.5 L water sample. Samples collected via CTD (i.e., non-surface samples) not shown. For visualization purposes, values >10,000 copies/uL are plotted as 10,000 copies / uL. Dots indicate sample locations. Note no samples from the southern California bight were collected in 2021 or 2023.

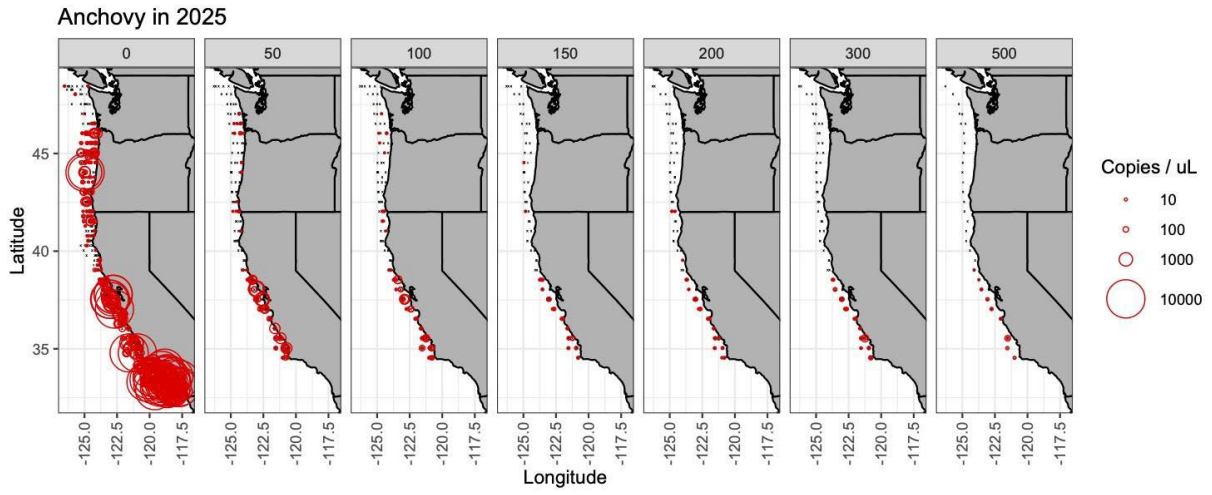

Figure 3.2. Average observed anchovy DNA concentration (DNA copies / uL) between the surface (0) and 500m (facets) in 2025. Each circle represents the average concentration collected from a single 2.5 L water sample. For visualization purposes, values >10,000 copies/uL are plotted as 10,000 copies / uL. Dots indicate sample locations. Note that no subsurface samples were collected from the southern California bight.

qPCR + Metabarcoding

Metabarcoding alone, depending on target amplicon, can provide information on tens to hundreds of species. For example, we have observations from two different metabarcoding amplicons in the mtDNA targeting vertebrates (MarVer (Valsecchi et al. 2020) and MiFishU (Miya et al. 2015)) for a subset of samples from our 2019-2025 hake/IWCPS surveys. In the 548 samples analyzed from 2019, 211 unique fish and 21 unique marine mammal species were detected in at least one sample. Of those, 45 fish and 11 marine mammal species were observed in 10 or more unique samples. Processing of 2021 and 2023 samples are incomplete at present, but initial results show similar detection patterns. Importantly, these markers are unable to differentiate among most rockfish species (genus *Sebastes*) but we have developed and tested a rockfish-specific metabarcoding marker to fill this gap (Matthews et al. 2025). We are applying this marker to identify rockfish species in the 2019-2025 samples currently and expect to be able to detect and quantify dozens of rockfish species soon.

Despite the utility of metabarcoding to detect species presence, as noted in section 1 and above, metabarcoding observations only provide relative abundance estimates. To provide information about abundance, we need to estimate DNA concentration, not relative abundance. Direct measurements of DNA concentration for hake, northern anchovy, and Pacific sardine (Figs 3.1 - 3.2) can be used to anchor relative abundance estimates provided by metabarcoding to generate DNA concentration estimates for the

fish community. Guri et al. (*In Press*) used hake qPCR observations from 2019 and metabarcoding observations for > 300 samples to estimate DNA concentration for 13 fish species. This integrated model incorporated qPCR and metabarcoding observations as well as information about primer amplification biases (see Shelton et al. 2023) to generate an estimate of DNA concentration for a taxonomically diverse group of species (six taxa are shown in Fig. 3.3). Spatial and depth distributions of DNA largely mirror available literature about the distribution of all 13 taxa.

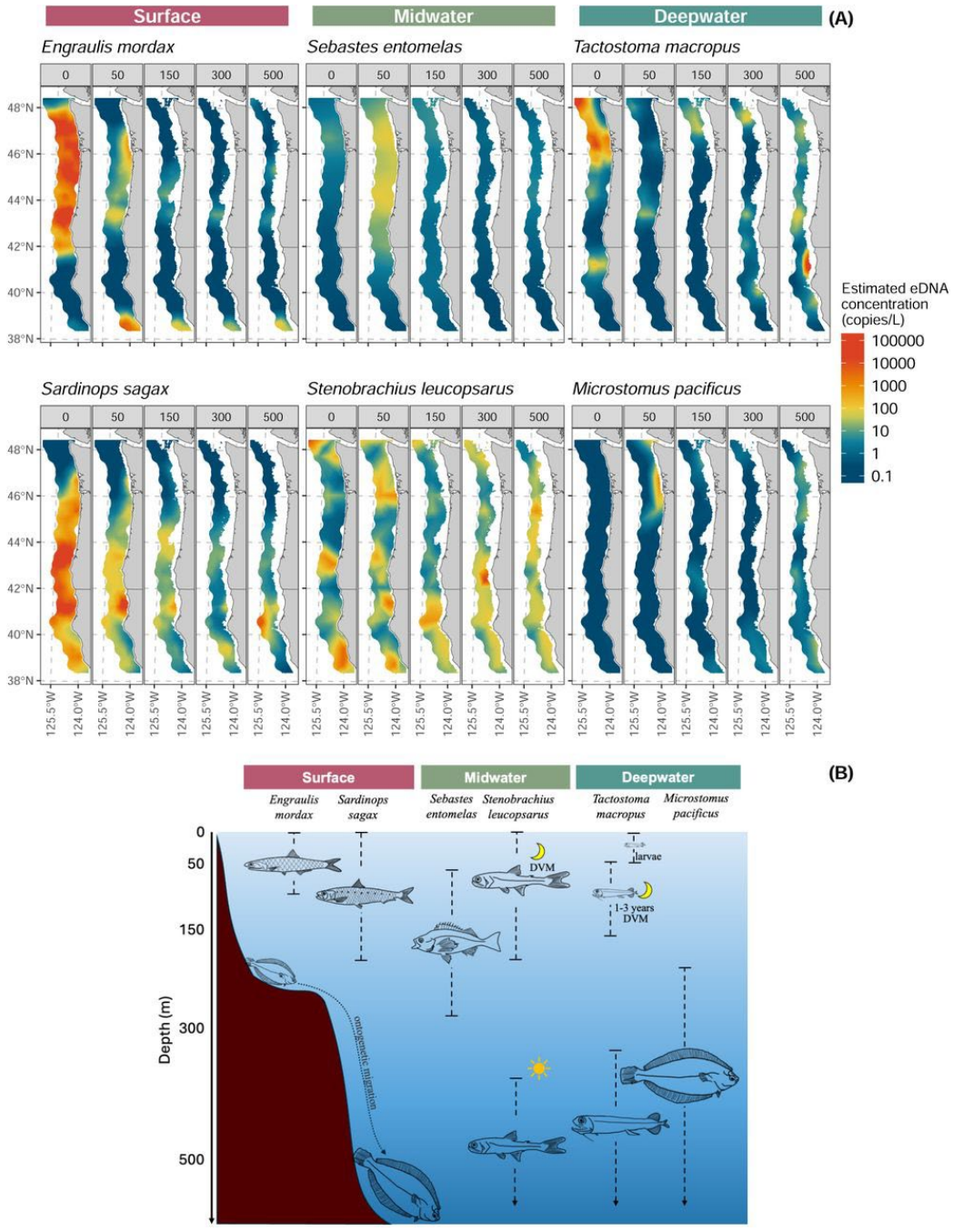

Figure 3.3. Estimated species eDNA concentrations for six selected species from samples collected across 0, 50, 150, 300, and 500 m in 2019 (A), and depth distribution of those species from the literature (B). From Guri et al. (*In Press*).

Unfortunately, the limitation of the qPCR+metabarcoding approach is that the model relies on scaling the abundance of all species off of the value observed for a single focal species. This is fine if the focal species is present and detected in all samples, but leads to problems if the focal taxon is missing from qPCR or metabarcoding observations. Hake is abundant along the West Coast and present in a majority of our observations (approximately 80-85% of water samples contain measurable concentrations of hake), making this a relatively modest problem. However, it may be a more prominent problem in other systems or for other metabarcoding markers that detect a different suite of species. This problem can partially be overcome by adding additional species with qPCR data (e.g., for 2021 hake, sardine, or anchovy occur in more than 90% of individual samples), or by applying more complicated statistical modeling approaches. At present, methods for integrating qPCR and metabarcoding remain in development.

Replicated Metabarcoding

Replicated metabarcoding involves conducting multiple technical PCR replicates during amplicon sequencing to address the stochastic noise and species-specific amplification biases inherent in the PCR process (Shelton et al. 2025, In Press). Recent approaches have demonstrated that estimates of DNA concentrations can be derived from metabarcoding alone by making use of the mean-variance relationship of relative read proportions among technical replicates (Shelton et al. In Press). In brief, low concentration samples have high variability among replicates while high concentration samples have low variability. These methods have been tested with simulated and experimentally constructed DNA communities, but are just beginning to be applied to field samples.

This approach could potentially provide DNA concentration estimates for tens of species simultaneously without the need for a single species DNA concentration reference. Of course, using technical replicate variability to infer concentration requires that technical replicate observations are available for each observation. Furthermore, qPCR observations will generally provide higher precision estimates of DNA concentration than replicated metabarcoding (Shelton et al. In Press). This motivates the development of additional approaches that can link replicated metabarcoding and qPCR observations as complementary data types.

A final component of replicated metabarcoding is that the approach provides a formal framework for connecting observations from multiple metabarcoding markers applied to the same sample. This should enable linking, for example, data from a general fish marker to a marker that can differentiate the taxa within a specific group (e.g., the genus

Sebastes). This property of replicated metabarcoding opens new opportunities for measuring marine ecosystems. Methods using multi-marker information to quantify eDNA concentration are under active development.

Joint Models for eDNA and traditional surveys.

We do not have scope to describe the range of models that are beginning to be developed to integrate traditional and eDNA observations into a single model. We note two examples here simply as entry points to the literature. Keller et al. (2022) developed an integrated occupancy model for crab in Puget Sound based on baited trap and eDNA observations. Guri et al. (2024) combined ddPCR, metabarcoding, and trawl observations to understand fish abundances in Norwegian fjords.

Stock assessment applications to species beyond hake

Widow rockfish and other midwater rockfish

Traditional groundfish surveys, such as the WCGBTS, may inconsistently quantify semi-pelagic species like widow rockfish (*Sebastes entomelas*) or yellowtail rockfish (*Sebastes flavidus)* because bottom trawl gear infrequently encounters midwater aggregations. This creates severe data sparsity and conflicting age-composition signals between bottom surveys and commercial fleets (Barnes et al. 2025, Taylor et al. 2025). The most recent widow rockfish assessment showed that 1) both survey and fishery dependent CPUE indices were generally uninformative, and that 2) data sources conflicted with respect to the age composition and natural mortality of the stock. Additional indices of abundance independent of the bottom trawl surveys may help resolve the conflicting information between these data streams, and could help address questions around hyperstability that have been raised in other west coast stock assessments (Cope et al. 2021). Because expanding ship-based trawl surveys to specifically monitor midwater rockfish is logistically and financially impractical, environmental DNA (eDNA) sampling offers a cost-effective, non-invasive tool that integrates seamlessly into existing survey infrastructure that is currently focused on hake and coastal pelagic species. Using eDNA samples from the midwater hake survey (2019, 2021, and 2023) and IWCPS (2025), we aim to use metabarcoding data to derive abundance indices that help to resolve survey coverage gaps, reconcile conflicting data streams, and improve stock assessment reliability without increasing survey days at sea. Initial eDNA metabarcoding data from our 2019 samples illustrate the concentration of widow rockfish eDNA primarily at 50 m depth, off the coast of Washington and Oregon (see Fig. 3.3).

Coastal pelagic species (CPS)

As shown in Figs 3.1-3.2, qPCR data is currently available for northern anchovy and Pacific sardine from the 2021 and 2023 hake surveys and the 2025 IWCPS survey. Methods similar to those used on hake could be applied to both anchovy and sardine to create comparable eDNA indexes and time-series. Water collection for eDNA on future IWCPS surveys are planned, providing the needed samples for adding to any DNA-based time-series. As qPCR-based eDNA data are not available for other managed CPS species (e.g., Pacific mackerel), development of quantitative indices for additional taxa would necessarily rely on joining qPCR and metabarcoding observations (qPCR+metabarcoding) or using replicated metabarcoding.

Other species and life-stages

Metabarcoding data from the hake and IWCPS surveys contains information on a large number of fish species beyond those listed here. Indices of abundance for more species may be developed using either coupled qPCR and metabarcoding or replicated metabarcoding techniques (see above), similarly to what is discussed for widow rockfish. Indices of abundance based on eDNA data may be useful to inform the assessments of data-limited and data-moderate species that are not reliably sampled by other surveys, for example because they inhabit untrawlable habitats, or for which only information on fishery removals exist. Practically, informative eDNA indices may be difficult to generate for species that are only occasionally encountered by the hake and IWCPS surveys, or that are present in low abundances.

As discussed briefly above, generating eDNA indices of abundance is complicated by the possible presence of DNA from larvae and young-of-the-year fish, which may require excluding samples from locations that are known pre-recruitment habitat (e.g., hake at the surface). The complement to that is that it may be possible to use those same samples to generate recruitment indices, which may then inform stock status as a component of the assessment model or as additional information.

Additional potential uses of eDNA in the PFMC process.

This methodology review document has focused on the use of eDNA data to generate indices of abundance to inform single-species stock assessments. However, eDNA data may potentially inform the PFMC process via additional pathways. A major strength of eDNA approaches is that they can provide information about taxa beyond the focal fishery stocks. There are species that are abundant in the eDNA that are entirely unmentioned by any of the PFMC's Fisheries Management Plans (FMPs; see e.g., *Tactostoma macropus* (a dragonfish) and *Stenobachius leucopsarus* (a myctophid) in Fig. 3.3). Similarly, abundant and important prey species like krill (e.g., *Euphasia*

pacifica) can be detected and quantified using eDNA.The PFMC's Fishery Ecosystem Plan (FEP; PFMC 2022) and Groundfish Fishery Management Plan (GFMP; PFMC 2026) outline several paths for incorporating information about non-target taxa.

First, the GFMP identifies a large number of fish species as Ecosystem Component Stocks (ECS) including mesopelagic fish like myctophids (see section 4.4.4 and Tables 3-3 and 3-4 in PFMC 2026). These species may be captured incidentally in fisheries but there are no established management reference points for ECS. In general, there is very limited information about the abundance, distribution, and trend of most ECS. eDNA provides a possible opening for detecting, quantifying, and therefore providing entirely new information for a suite of ECS.

Second, the FEP discusses how single-species stock assessments may incorporate ecosystem information to support fisheries management (see section 5.1 in PFMC 2022). For a single-species assessment, eDNA observations could provide information about the distribution and/or abundance of important prey (e.g., krill) or guild of prey species (e.g., myctophids) that could inform biological parameters of interest (e.g., productivity or growth) of the assessed species. By broadening the suite of species observed in the ocean, eDNA observations expand the potential relationships among species that can be considered in a single-species assessment context.

Finally, information about new species via eDNA observations will encourage the development of new ecosystem indicators of the California Current Ecosystem. Such ecosystem indicators already play an important role in informing a range of PFMC products from the annual Ecosystem Status Report (e.g., Hunsicker et al. 2026) to risk tables for groundfish and CPS assessments. Risk tables provide an onramp for data that may be considered inadequate to inform abundance, but is of sufficient quality to inform the assessment process. Applications of risk tables were explored in the most recent groundfish assessment cycle to account for ecosystem information in the determination of sigma, a measure of uncertainty in stock status. As such, risk tables may offer a conduit to incorporate trend information from eDNA observations.

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