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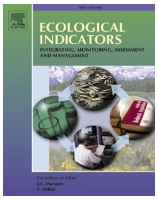

Original Articles

Comparison of environmental DNA and SCUBA diving methods to survey keystone rockfish species on the Central Coast of British Columbia, Canada

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ABSTRACT

The rocky reefs of British Columbia's (BC) coast are a productive ecosystem, home to 38 rockfish species (Genus: *Sebastes*) that are culturally and economically important. Quantitatively assessing rockfish populations is vital to support conservation and stock assessment needs. Self-contained underwater breathing apparatus (SCUBA) diving surveys are a commonly used monitoring method in BC. However, this resource-intensive approach is challenging, particularly for cryptic or deeper species. Herein, we compared environmental DNA (eDNA) detection methods with SCUBA diving surveys to capture overall rockfish biodiversity. We employed two eDNA methods: 1) a targeted quantitative real-time polymerase chain reaction (qPCR) approach to monitor species of particular importance to First Nations collaborators and decision makers, and 2) a metabarcoding approach for assessing community composition using the previously published MiSebastes assay. Both approaches are confounded by the little DNA sequence divergence among species and high sequence variation within species. Overcoming these challenges using a whole mitochondrial approach with the *mtGrasp* and *unikseq* pipelines, we generated highly useful eDNA tools. We found that eDNA methods were highly comparable to dive surveys, as both methods indicated a similar ecological reality, including species detections and distributions. Though there are certain species that cannot be distinguished by the MiSebastes assay, eDNA metabarcoding still detected more rockfish species overall. Both eDNA methods show potential for use alongside conventional methods for scalable incorporation into community-based monitoring programs.

1. Introduction

Biodiversity is fundamental to the wellbeing of all life. Despite ongoing efforts, the world is losing biodiversity at an unprecedented rate due to climate change and anthropogenic development (Galli, Wackernagel, Iha, and Lazarus, 2014; Perrings, Folke, and Mäler, 1992). The global implications of these losses are catastrophic and drastic improvements are needed in conservation and biomonitoring methods to track these changes (Ma, 2023).

Rockfish populations of the genus *Sebastes* have been declining over the past decades, due to poorly regulated commercial and recreational fisheries, alongside rapid climate change (Frid, McGreer, Preez, Blaine, and Norgard, 2021; Haggarty, Shurin, and Yamanaka, 2016). They are a diverse, yet closely related group of fish that are mainly distributed throughout temperate regions of the Pacific Ocean. Globally there are over 120 species within the genus, with a variety of life histories and phenotypes (Love, Yoklavich, and Thorsteinson, 2002). Rockfish are long-lived, with certain species living over 200 years (Kolora et al.,

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2021). They grow slowly, require many years to reach sexual maturity, and often remain within restricted home territories (Love et al., 2002). These factors, in conjunction with high risk of barotrauma due to their deep sea habitats, make them very sensitive to population collapse, and loss of genetic diversity via overharvesting (Johansson, Banks, Glunt, Hassel-Finnegan, and Buonaccorsi, 2008; Kolora et al., 2021; Yu and Kim, 2018).

Rockfish have been a culturally important food fish for First Nations in the Pacific Northwest for millennia, and they are considered a "flagship" species meaning that their conservation confers protection to associated habitats and species (Frid et al., 2021). In British Columbia, Canada there are 38 species that inhabit a range of habitats from the ocean surface to depths well over 1,000 m depending upon the species. To address declines in population size in the province Rockfish Conservation Areas (RCAs) have been established starting in 2002 (Fisheries and Oceans Canada, 2006).

Currently, First Nations of the Central Coast of British Columbia including the Kitasoo/Xai'xais Nation are using fishery-independent methods like SCUBA dive surveys or towed video surveys to monitor federally placed RCAs and areas of interest within their territories such as the newly established Gitdisdzu Lugyeks (Kitasu Bay) Indigenous Protected and Conserved Area (IPCA)) (Frid et al., 2021; Kitasoo/X'ais x'ais Stewardship Authority, 2022). Dive surveys are a globally well-established method to determine marine biodiversity (Bravo et al., 2023; Edgar et al., 2020; Robinson et al., 2023; Vieira et al., 2020). They not only provide species identification and abundance estimates, but also highly detailed associated observations on behaviour, water currents and visibility, and habitat characteristics like complexity and bottom composition. These are vital factors tied closely to biological productivity and therefore can provide a basis for good recommendations on the placement of protected areas. Despite their advantages, dive surveys are logistically challenging, prone to misidentification of species, and time and resource intensive. They require highly trained individuals who can reliably identify species, and who can handle adverse conditions that come with working in the field. In addition, SCUBA divers are depth-constrained, and due to the deep living range of rockfish, these methods can simply not access some key rockfish habitats (Marliave, Conway, Gibbs, Lamb, and Gibbs, 2009).

A new approach that is increasingly utilized in biomonitoring is the detection and classification of environmental DNA (eDNA). This refers to DNA that can be isolated from environmental samples such as water (Ficetola, Miaud, Pompanon, and Taberlet, 2008; Thomsen and Willerslev, 2015). Organisms shed DNA-containing skin, feces, gametes, etc. into their environments. By detecting these biological traces through eDNA testing, one can investigate biodiversity and monitor species of interest through a molecular lens. When used alongside conventional methods, eDNA can greatly increase the scope of monitoring efforts due to its relatively lower costs and ease of sampling(Gold, Sprague, Kushner, Zerecero Marin, and E., 2021; He et al., 2023) Furthermore, eDNA is minimally invasive to the organisms of interest, does not rely on time-constrained visual observation, and does not require extensive training for sample collection (Robinson et al., 2023).

There are different eDNA approaches that can be used based on the ecological questions being posed (Acharya-Patel, Allison, and Helbing, 2021). Within the present study we employed two eDNA methods: 1) a targeted approach to monitor rockfish species of particular importance, and 2) a metabarcoding approach to assess community composition.

Targeted eDNA methods use species-specific assays that have been specially designed to detect gene sequences unique to the target species from environmental samples (Acharya-Patel et al., 2021; Langlois, Allison, Bergman, To, and Helbing, 2021). The most common targeted eDNA analytical method employs quantitative real-time polymerase chain reaction (qPCR) assays that, when properly validated (Abbott et al., 2023; Langlois et al., 2021), are powerful tools to identify species of interest. Using a recently developed whole mitochondrial genome (mitogenome) screening approach called *unikseq* (Allison et al., 2023; Langlois et al., 2021) we validated targeted qPCR eDNA assays for three species: *Sebastes maliger* (Quillback rockfish; (Allison et al., 2023)) *Sebastes paucispinis* (Bocaccio rockfish) and *Sebastes ruberrimus* (Yelloweye rockfish). The species were chosen because they are particularly culturally, ecologically, and economically important (Frid and Mooers, 2009). Moreover, all three species are listed as threatened by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (COSEWIC, 2009, 2013, 2020). *S. ruberrimus* is also listed as a species of special concern under the Canadian Species at Risk Act (SARA) (Fisheries and Oceans Canada, 2018).

While targeted eDNA assays are particularly suited for reproducible, cost-effective, standardized evaluation of environmental samples, metabarcoding methods can provide valuable insight into community analysis. Metabarcoding assays rely on a hypervariable "barcode" region within the mitochondrial genome that is flanked by two shorter regions (20 – 30 bp) that are relatively conserved between species (Miya, Gotoh, and Sado, 2020). These barcode regions are amplified and sequenced with high throughput sequencing methods, then bioinformatically matched with known species sequences from a reference database (Gehri, Larson, Gruenthal, Sard, and Shi, 2021; Gold et al., 2021; Reidy et al., 2022).

The barcode currently most used for fish is the MiFish primer pair that amplifies a short fragment of the mitochondrially-encoded *12S* genic region (*mt-rnr1*) of the mitochondrial genome (Miya et al., 2020). Despite the broad utility of the MiFish assay (Kawato et al., 2021; Miya et al., 2020; Reidy et al., 2022), it cannot differentiate between most rockfish species. This is because the *Sebastes* genus underwent a rapid divergence within the last million years, and *mt-rnr1* remains highly conserved in this genus (Hyde and Vetter, 2007; Vaux et al., 2019). Consequently, a different MiSebastes metabarcoding primer pair was designed using the mitochondrially-encoded *cytochrome b* genic region (*mt-cyb*), specifically for rockfish species along the coast of North America (Min, Barber, and Gold, 2021). Since this assay performed well when tested on an aquarium population of California rockfishes (Min et al., 2021), we used it in the present study.

If eDNA methods are to be integrated into largescale monitoring, there is a need for more systematic evaluation of both eDNA assay choice for target communities (Min et al., 2021; Zhang, Zhao, Yao, and Gilbert, 2020), and whether eDNA is the appropriate biomonitoring tool for each scenario (J. A. Darling, 2020). The recent evolutionary history of *Sebastes* has resulted in a monophyletic group of many closely related species that overlap geographically. This means that there is high interspecific conservation within gene sequences (Hyde and Vetter, 2007; Love et al., 2002). At the same time, *Sebastes* species show considerable site fidelity as adults, and they are susceptible to regional isolation due to ocean currents or geographical barriers. This has resulted in distinct populations of rockfish species with high intraspecific variability (Hyde and Vetter, 2007; Love et al., 2002). This combination of high interspecific conservation and intraspecific variability makes *Sebastes* a challenging group to study with any genomics based methods including eDNA, especially since it is well known that the more overlap there is between these two factors, the less effective DNA barcoding becomes (Meyer and Paulay, 2005). Thus, the overall goal of this work is to investigate the potential of targeted and metabarcoding based eDNA methods and compare them with conventional SCUBA diving surveys in their abilities to capture rockfish species of interest and overall rockfish biodiversity.

2. eDNA assay design and validation

2.1. Targeted eDNA assay generation

Targeted eDNA assays were designed using *unikseq* (Allison et al., 2023) and validated using an established workflow described previously (Allison et al., 2023; Langlois et al., 2021). Reporting and performance characteristics were compliant with two Canadian national eDNA

Table 1
Target assay sequences, molecular characteristics, and calculated performance parameters.

Target Species		***Sebastes maliger***	***Sebastes paucispinis***	***Sebastes ruberrimus***
eDNA qPCR Assay name		eSEMA3	eSEPA9	eSERU5
Target Gene		*mt-d-loop*	*mt-nd5*	*mt-d-loop*
Optimal T_a (°C)		51.8	55.4	54.4
Amplicon Size (bp)		85	115	75
Assay Component Sequences (5′ → 3′)	**Forward Primer**	CGAAGGTATTACATAAAGCA	CCAGTAAACAATACAAAACTAC	CTACCAGTCCACTCATAA
	Reverse Primer	GAGTGTTTGTGTTAGGTCTTA	CCTAGAATTAAATTCAGCTTG	TGGGATTTCATATTCTACTTG
	Probe	CCAACAATCATTTATAAGGACTGAGCGAAT	TACCCACCATTTCTCCAACATGCTA	AACATAAGATATACGCCCATCAACCC
	Amplicon	CGAAGGTATTACATAAAGCAAAATAATAAGACCAACAATCATTTATAAGGACTGAGCGAATATTTAAGACCTAACACAAACACTC	CCAGTAAACAATACAAAACTACACCTAATCTAATTACCCACCATTTCTCCAACATGCTAGGCTTTTTCCCCTCAATCATCCACCGATTAACCCCCAAGCTGAATTTAATTCTAGG	CTACCAGTCCACTCATAAAAACATAAGATATACGCCCATCAACCCCCGGTACCGCAAGTAGAATATGAAATCCCA
Continuous Data (C_t > 95 %)	**Slope**	−3.5083	−3.404	−4.0059
	% Efficiency	93	97	78
	Y-Intercept	40.028	36.206	41.248
	R^2 Value	0.9998	0.9997	0.9977
Binomial Data (Copy #/rxn)	**LOD (95 % CI)**	1 (0.7–1.7)	0.2 (0.2–0.4)	0.2 (0.2–0.4)
	LOQ (95 % CI)	3.9 (2.8–6.5)	0.9 (0.6–1.6)	0.9 (0.7–1.6)
	LOB	0	0	0

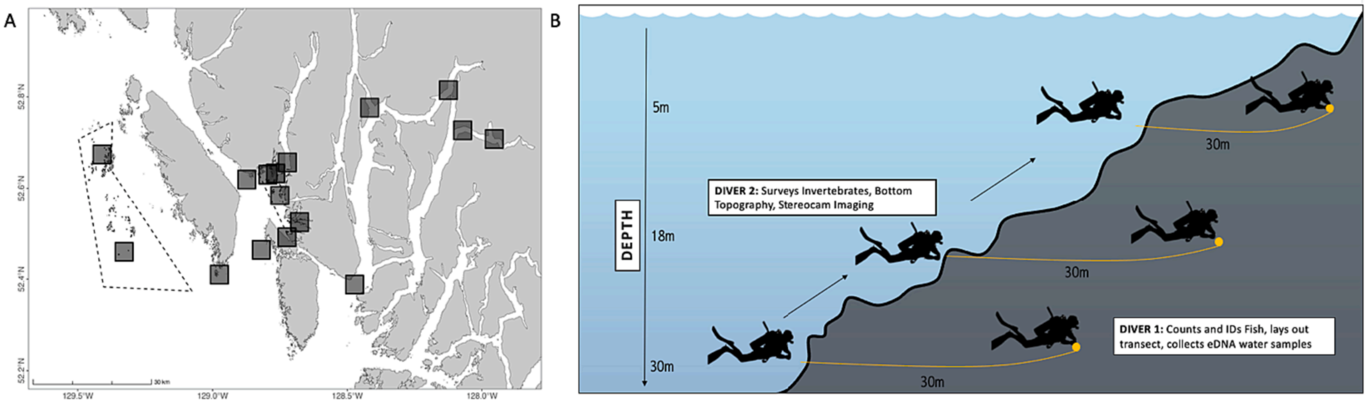

Fig. 1. Field survey information. A) Map of survey stations, exact location masked on a scale of 16 km^2 B) Graphical depiction of field protocols. Each dive buddy pair descends to 30 m. The first diver rolls out a transect counting, identifying, and sizing rockfish. At the end of the transect the first diver then collects three water samples for eDNA analysis. The second diver records important substrate features, invertebrate life, and does opportunistic stereocam imaging of rockfish. This process is then repeated at 18 m and 5 m depths. All dives are done in accordance with CSA standards and using DCIEM Tables.

standards (Abbott et al., 2023; Gagné et al., 2021). To design the *Sebastes maliger* (Quillback rockfish), *Sebastes paucispinis* (Bocaccio rockfish) and *Sebastes ruberrimus* (Yelloweye rockfish) targeted qPCR assays, all available full mitochondrial genome sequences for species within the *Sebastes* genus as well as the closely related *Sebastolobus* genus were accessed from the National Center for Biotechnology Information (NCBI) GenBank (Sayers et al., 2022). However, since there were not many available, additional *Sebastes* sequences were graciously supplied by Dr. Gregory Owens (Kolora et al., 2021). We also obtained voucher tissue DNA specimens of key *Sebastes* species that reflect haplotype variation in British Columbia and performed whole genome shotgun sequencing followed by mitogenome sequence assembly using *mtGrasp* version 0.1.0 (Yang and Coombe, 2023). The species that were sequenced included chosen target species, species that were most likely to co-occur, and relevant species for which there was no sequence data available. The full lists of all species mitogenomes and accession numbers used in the present study are in Table S1.

The *unikseq* (v1.0.0) parameters used for each assay are shown in Table S2, and the output sequences for eSEPA9 and eSERU5 are included in Supplementary Files S1-S2. The relevant information for eSEMA3 can be found in (Allison et al., 2023). To ensure that the candidate assay primer and probe sequences would not amplify DNA from other potentially interfering taxa, they were cross-referenced against the NCBI non-redundant (nr) sequence database and visualized against co-occurring rockfish species (Figs. S1–S3). They were then tested against tissue derived gDNA of target and non-target species (Table S3) (Allison et al., 2023).

Once promising assay candidates had been designed (Table 1) assay sensitivity was determined via standard curve construction with gBlocks® synthetic DNA obtained from Integrated DNA Technologies (Coralville, Iowa, United States) following the methodology described previously (Hobbs, Round, Allison, and Helbing, 2019). Using the gBlocks® data, standard curves were created to establish a relationship between C_t values and the initial DNA copy number. The eLowQuant (Lesperance, Allison, Bergman, Hocking, and Helbing, 2021) method was used to generate the limit of blank (LOB), limit of detection (LOD), and limit of quantitation (LOQ) (Table 1).

For the final validation step each assay was tested on a verified positive environmental sample (Abbott et al., 2023). We used water samples from the Pacific Canada Pavilion tank (PacCan) and the Bella Bella tank at the Vancouver Aquarium for all our assay candidates. These recirculating tanks are home to several local *Sebastes* species and the exact content of each tank is known (Table S4).

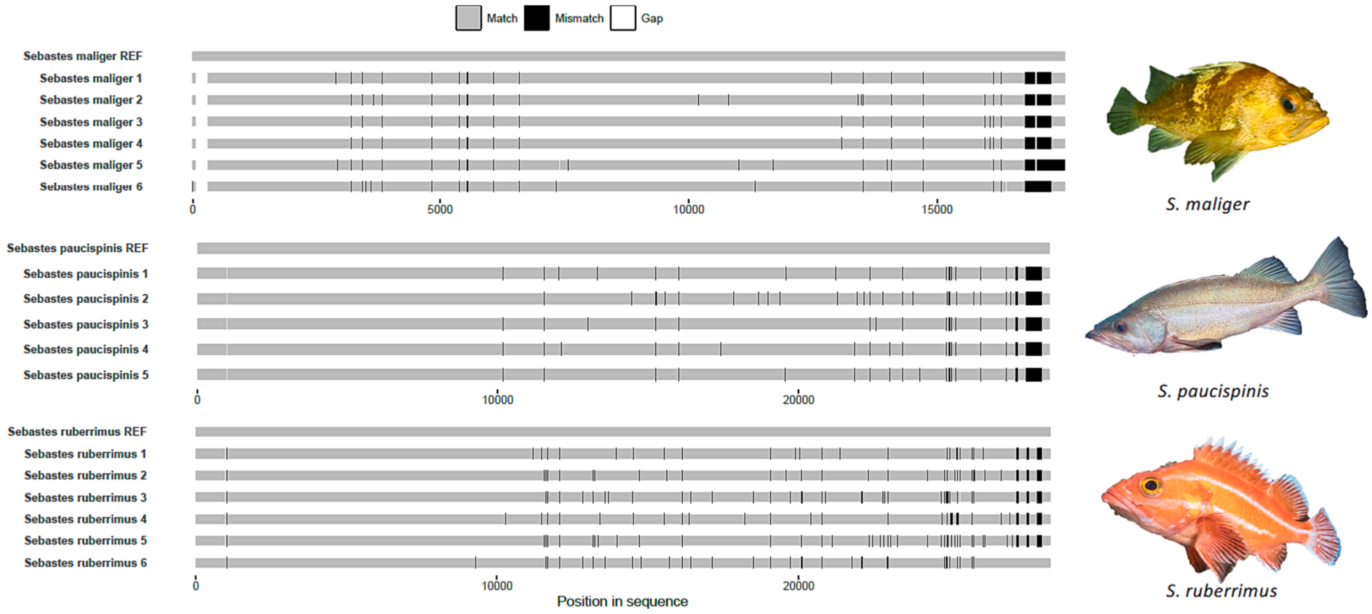

Fig. 2. Whole mitochondrial genome alignments (~16,000 bp) of multiple individuals of each target species. Grey indicates bp conservation between individuals and black lines indicate sequence differences. The first row is the reference sequence that was used for assay design. In all three target species, there are sequence differences between individuals across the entire mitochondrial genome. This indicates high intraspecific variability.

2.2. *Metabarcoding assay validation and assessment*

Though the MiSebastes assay targets a mitochondrial region with much higher variability than the MiFish target region, there are still some redundancies between amplicons of very closely related rockfish species. We assessed these assays *in silico* for their ability to amplify and distinguish rockfish species in British Columbia using available sequence data as a proxy for environmental samples. First, we compiled the MiSebastes amplicon sequences from all rockfish species for which entire mitochondrial sequences were available. From each amplicon we aligned the primer sequences and identified all mismatches within the primer regions. We then aligned the entire amplicon DNA sequence regions to identify identical sequences across multiple *Sebastes* species.

3. Field surveys

3.1. *Field sample collection*

Data collection for the present study did not require permits from federal agencies. The data collected were either from seawater samples or observational. The Kitasoo/Xai'xais First Nations hold Indigenous rights to their own territories, and all seawater samples were collected with the permission and logistical support of the Kitasoo/Xai'xais Stewardship Authority.

In September of 2021 and 2022, 16 stations were cumulatively visited in the Kitasoo/Xai' xais Territory along the central coast of British Columbia Canada (Fig. 1). At each station visual data on rockfish and their habitats were collected as per the protocol developed by the Central Coast Indigenous Resource Alliance (CCIRA) (McGreer et al., 2020). This survey design uses three 30 m transects parallel to the shoreline shore, starting from the deepest at ~ 30 m, then at ~ 18 m, and finally at ~ 5 m below the surface. On each transect divers were responsible for identifying, counting, and size estimation of fish, as well as characterizing habitat complexity and invertebrate diversity (Fig. 1). This protocol was modified with the addition of eDNA sample collection at each transect for a total of nine eDNA samples per station.

Water samples were collected using 1 L Kendall Kangaroo Joey™ bags (Medtronic, Brampton, ON, Canada). These bags are sterile and flexible, which makes them amenable to water collection while diving. At each transect the diver collecting eDNA would open the Kangaroo bag and swish the bag back and forth multiple times until it filled completely. Three 1 L water samples per transect for a total of nine samples per dive were collected. On the last day of each trip, the divers' drysuits were soaked in a tote of freshwater and three 1L suit control samples were taken to account for any potential contamination from the divers' suits. All water samples collected were stored in a cooler with ice packs until filtration through a 0.45 μm mixed nitrocellulose filter (Thermo Fisher Scientific Inc., Ottawa, ON, Canada) later that day. A 500 mL negative filtration control was also prepared by filtering bottled water in this manner every day filtering was done. Filters were stored at −20 °C until DNA could be extracted. One sample collected at South Aristozabal floated away and thus was not included in the analyses (Table S5).

3.2. *Sample preparation and integrity testing*

DNA was extracted from filters using the DNeasy Blood and Tissue kit (Qiagen Inc., Mississauga, ON, Canada; Cat# 69506) as described previously (Helbing and Hobbs, 2019; Hobbs et al., 2019). All extractions were performed in a laminar flow hood wiped with 10 % bleach (v/v) solution and 70 % ethanol (v/v) solution prior to use. For qPCR analysis one quarter of each filter was extracted. For metabarcoding analysis, two quarters of each filter were extracted, pooled, and concentrated to 50 μL. All eluates were stored at −20 °C until analysis could be done.

Before testing the samples with rockfish specific approaches, the integrity of DNA in each sample was assessed using the qPCR based IntegritE-DNA^{TM} test (Hobbs et al., 2019). This test ensures that the eDNA samples are viable and no inhibitors were present that would affect PCR analysis. Any samples that failed this test (average qPCR C_t values > 27) were cleaned with a OneStep PCR Inhibitor Removal Kit (Zymo Research, Irvine, CA, USA, Cat # D60303) and retested. Only samples that passed this test were further tested and sequenced (Table S6).

3.3. *eDNA assay testing - qPCR*

Each eDNA sample from the field surveys was tested with the eSEMA3, eSEPA9, and eSERU5 assays with eight technical replicates

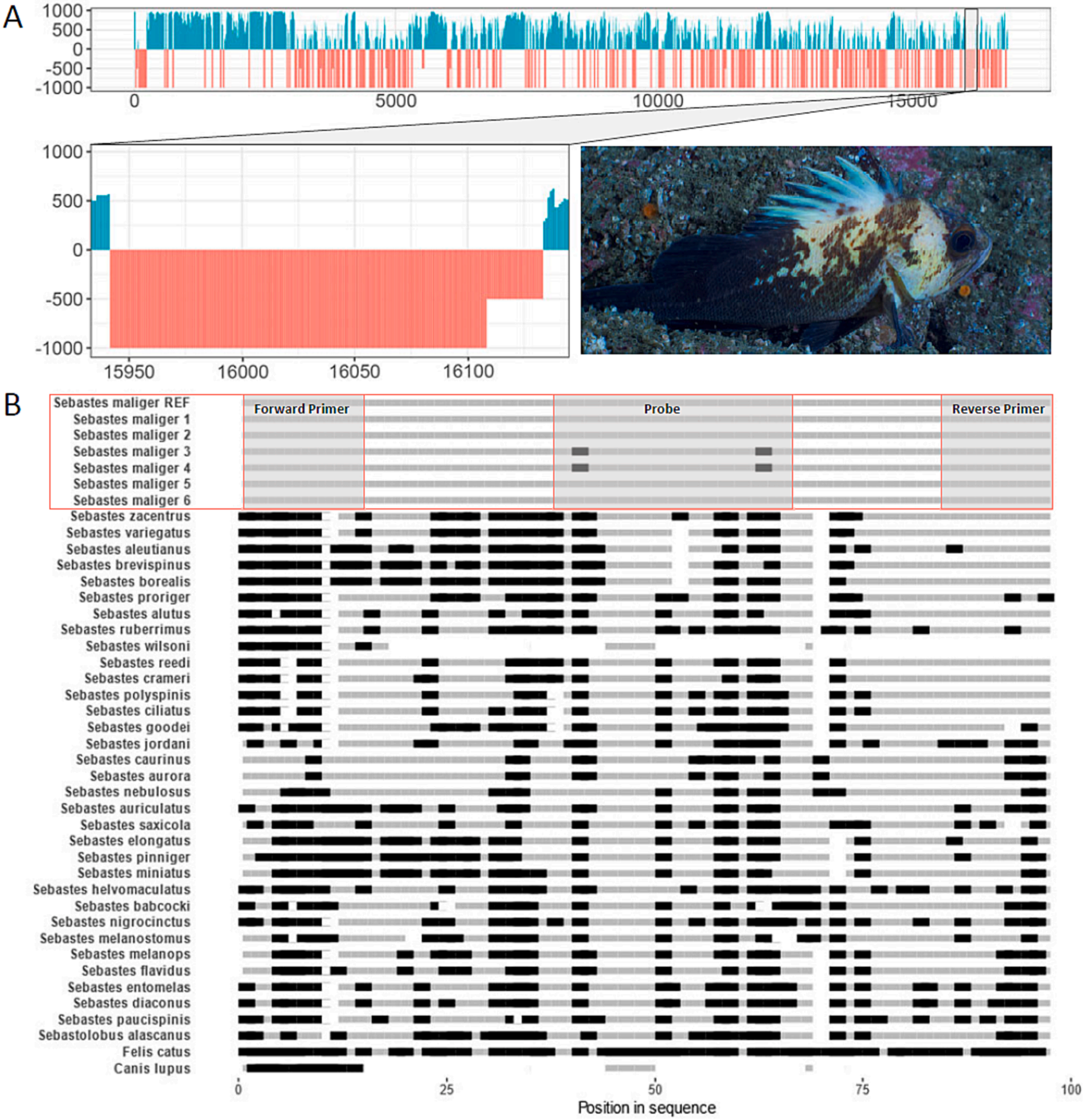

Fig. 3. *Sebastes maliger* target assay design visualization. A) *Unikseq* output butterfly plot. The blue regions indicate high conservation between rockfish species, and the pink regions indicate regions of higher variability that are more amenable for assay design. The inset shows the *unikseq* output region that the assay was designed from. B) eSEMA3 amplicon alignment using multiple individuals of the target species, all other BC rockfish species for which there is sequence data available, and human, cat, and dog sequences. Grey indicates bp conservation between individuals and black lines indicate sequence differences. The first row is the reference sequence that was used for assay design. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

each using the QIAcuity Probe PCR kit from QIAGEN Inc on Bio-Rad CFX96 Real-Time PCR Detection Systems (Bio-Rad Laboratories (Canada) Ltd, Mississauga, Ontario). DNA amplification thermocycler conditions were an initial activation step of 2 min at 95 °C followed by 50 cycles of 15 s denaturation at 95 °C, 30 s annealing at 64 °C, and 45 s extensions at 72 °C. Each plate was run with eight NTCs and two positive controls comprised of synthetic DNA at a concentration of 20 copies/reaction. If any negative controls amplified, or if positive controls failed to amplify, the entire plate was re-run. These qPCR results were converted into an estimate of DNA copy number/L.

Water sample replicates were grouped within each transect. and each transect was treated as a sample to enable comparison with the dive data. If the mean estimated DNA copy number/L across field replicates exceeded the calculated lower confidence interval of the assay's LOD (Lesperance et al., 2021), the transect was designated as a positive detection.

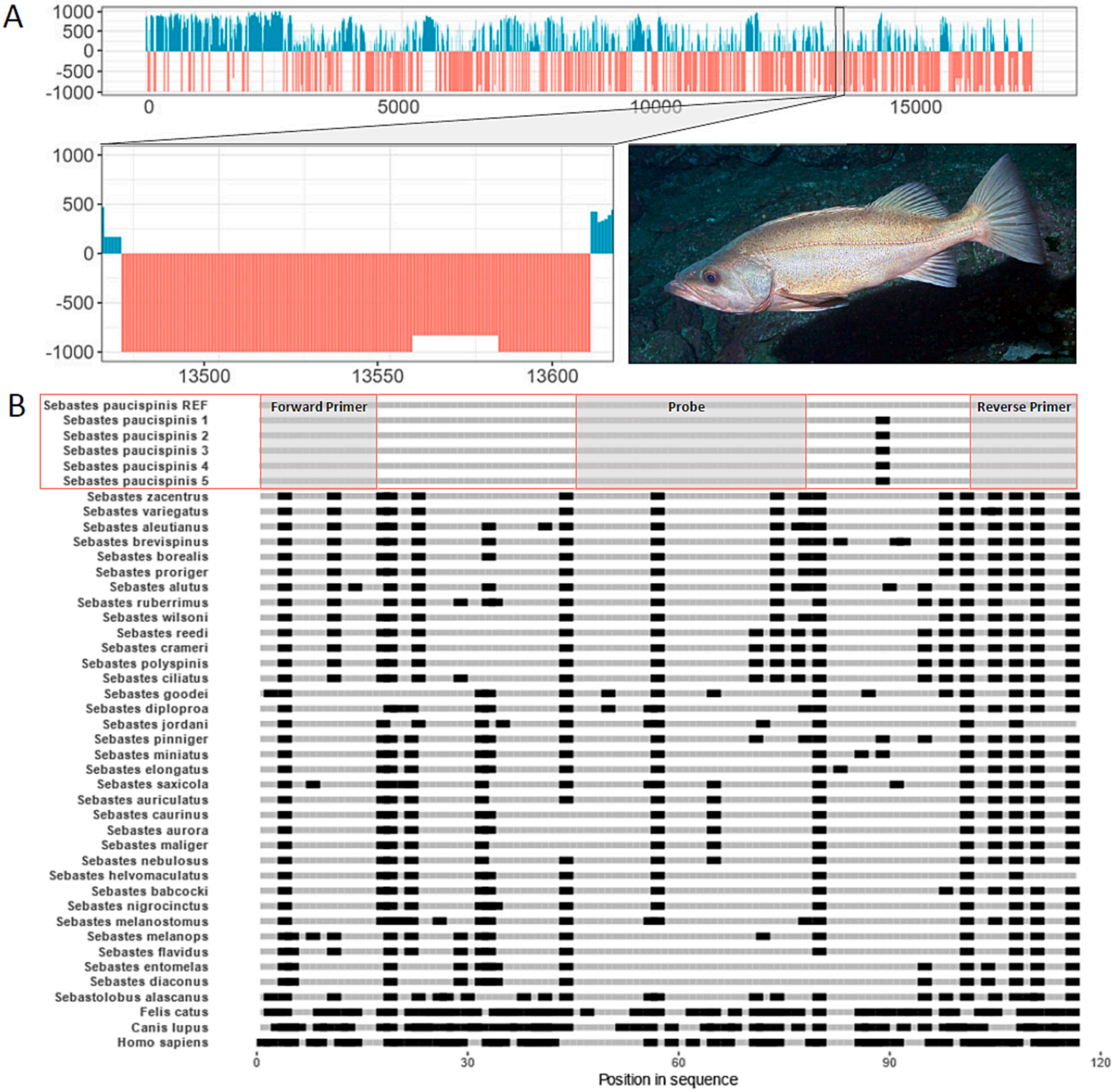

Fig. 4. *Sebastes paucispinis* target assay design visualization. A) *Unikseq* output butterfly plot of the relevant regions for the design of the eSEPA9 targeted qPCR assay. B) eSEPA9 amplicon alignment. There is no intraspecific variability in any of the key assay regions. See the Fig. 3 legend for more details.

3.4. *eDNA assay testing - metabarcoding*

Library preparation for the MiSebastes (Min et al., 2021) metabarcoding assay was comprised of a commonly used two-step polymerase chain reaction (Bourlat, Haenel, Finnman, and Leray, 2016; Reidy et al., 2022). The first PCR step is used to amplify target regions. Each sample was run in triplicate with a 25 μL reaction volume comprised of 5.5 μL sterile Nuclease-Free water, 0.6 μL of both the fwd and reverse MiSebastes primers (10 μM), 3.75 μL BSA (NEB), 12.5 μL 2X Taq (Froggabio), and 2 μL template. For the first PCR, DNA amplification conditions were an initial activation step of 5 min at 95 °C, followed by 45 cycles of 30 s denaturation at 95 °C, 30 s annealing at 52 °C, and 45 s extensions at 72 °C, with a final extension of 5 min at 72 °C.

The triplicate PCR replicates were pooled and a SPRI bead (Beckman Coulter, Indianapolis, IN, USA) cleanup step (using 0.8X beads) was performed to remove excess primers and non-specific fragments. The SPRI beads cleanup followed the manufacturer's protocol, and samples were resuspended with 25 μL of sterile nuclease free water. For both assays, a negative DNase-free water control sample was included in all PCR reactions. The second PCR step was used to attach Illumina adapters (Illumina, San Diego, USA) with unique identifiers to the amplicons for sequencing. This PCR was carried out with 25 μL reaction volume, containing 5 μL of sterile Nuclease-Free water, 2.5 μL of each index (Nextera i7 and i5), 12.5 μL of 2X Taq (Froggabio), and 2.5 μL template (first PCR product). Different combinations of indices (i5 and i7) were used to assign a unique identification to each sample.

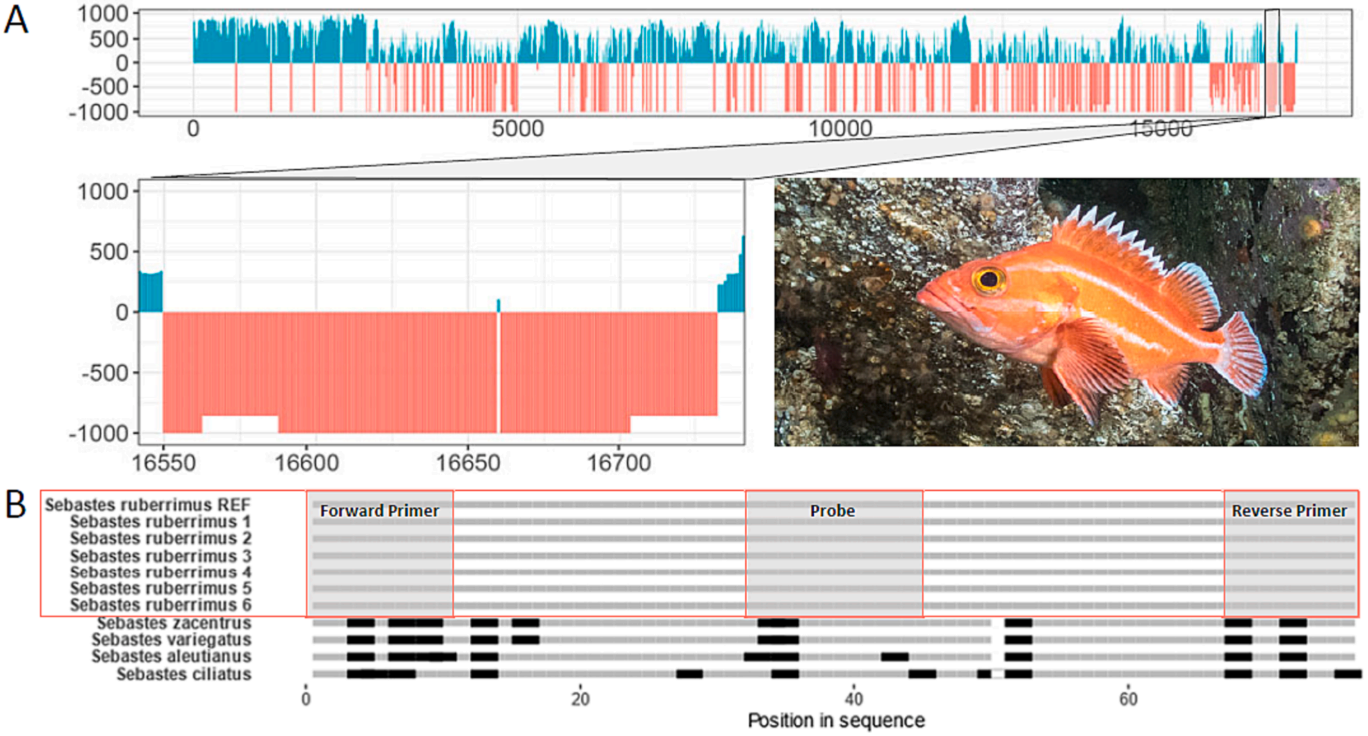

Fig. 5. *Sebastes ruberrimus* target assay design visualization. A) *Unikseq* output butterfly plot of the relevant regions for the design of the eSERU5 targeted qPCR assay. B) eSERU5 amplicon alignment. Note that the eSERU5 region is out of range to align with most other species as well as human, cat, and dog sequences. *In situ* validation confirmed that species of concern were not amplified by this assay. There is no intraspecific variability in any of the key assay regions. See the Fig. 3 legend for more details.

For the second PCR, DNA amplification thermocycler conditions were an initial denaturation for 3 min at 95 °C, followed by eight to ten cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 55 °C, and extension for 30 s of 72 °C, with a final extension for 5 min at 72 °C. Another SPRI beads (Beckman Coulter, Indianapolis, IN, USA) cleanup was carried out (using 0.8X beads) and samples were resuspended with 25 µL of nuclease-free water. DNA was quantified with the Quant_IT dsDNA High Sensitivity Kit (Thermo Fisher Scientific Inc., Ottawa, ON, Canada), and then all samples were pooled so that there were 40 ng of DNA per sample. The quality of the library pool was accessed in the BioAnalyzer (Agilent) using the High Sensitivity DNA Analysis Kit and quantified using the NEBNext Library Quant Kit for Illumina. Prepared libraries were sequenced with the Illumina MiSeq at the Hakai Institute genomics facility (Reidy et al., 2022). The 2021 and 2022 samples were run separately with the Illumina V2 300 cycle kit and results were pooled.

3.5. Bioinformatics

The paired-end metabarcoding reads were processed using the dada2 library v1.22.0 in R v4.1.3 (Callahan et al., 2016), and cutadapt v2.10 to trim primers (Martin, 2011). After learning error rates, dereplication, sample inference, and read merging with dada2 using default parameters, we removed bimeras, amplicon sequence variants (ASVs) present in only a single sample with lower than 0.001 relative abundance, and two samples with fewer than 150 sequence reads present. Taxonomic assignment was carried out using a custom library of amplicons created from in-housesequence data and those from Dr. Greg Owens (Kolora et al., 2021), as well as a BLASTn search of the NCBI nucleotide database. The Galaxy Tool LCA was used to determine last common ancestor (LCA) taxonomy strings for each ASV (Beentjes et al., 2019). The top scoring blast hit was applied without using LCA if it had ≥ 98 % similarity with the query, otherwise, LCA was employed to determine a consensus taxonomy.

Once the taxonomy classifications were verified, the Vegan package (R v3.6.3) was used to generate rarefaction curves (Oksanen et al., 2017). These curves indicate whether sequencing depth was adequate to capture species representation and thus more reliable metabarcoding results. Then, the Decontam package (R v4.1.3) (Davis, Proctor, Holmes, Relman, and Callahan, 2018) was used with its suggested aggressive parameters to remove reads that were potentially contaminants based on the prevalence of certain ASVs detected from the diving suit control data and field blanks.

3.6. Rockfish diversity comparisons

Using the Vegan package (R v3.6.3), alpha diversity estimates, measured as species richness were compared between MiSebastes and dive survey data, and between depths using the non-parametric Wilcoxon signed-rank test (Oksanen et al., 2017). Alpha diversity refers to the number of different species within a community, and is a key metric within biodiversity studies (Wilson and Shmida, 1984).

For each species detected by dive survey and by either eDNA method, a matrix was made indicating whether each transect was designated as a positive or negative detection. From these matrices the percentage of eDNA only detections, dive only detections, positive detection for both methods, and negative detection for both methods were calculated. At the station level, if there was at least one depth where the target species was detected, the station was designated as a positive detection. To quantitatively assess the difference between station designations via diving and eDNA methods, we performed the Fisher's exact test (Fisher, 1958). *Phyloseq* (R v3.6.3) (McMurdie and Holmes, 2013) was used to generate taxonomic barplots for both the metabarcoding and dive data, aggregated by depth and location for visualization.

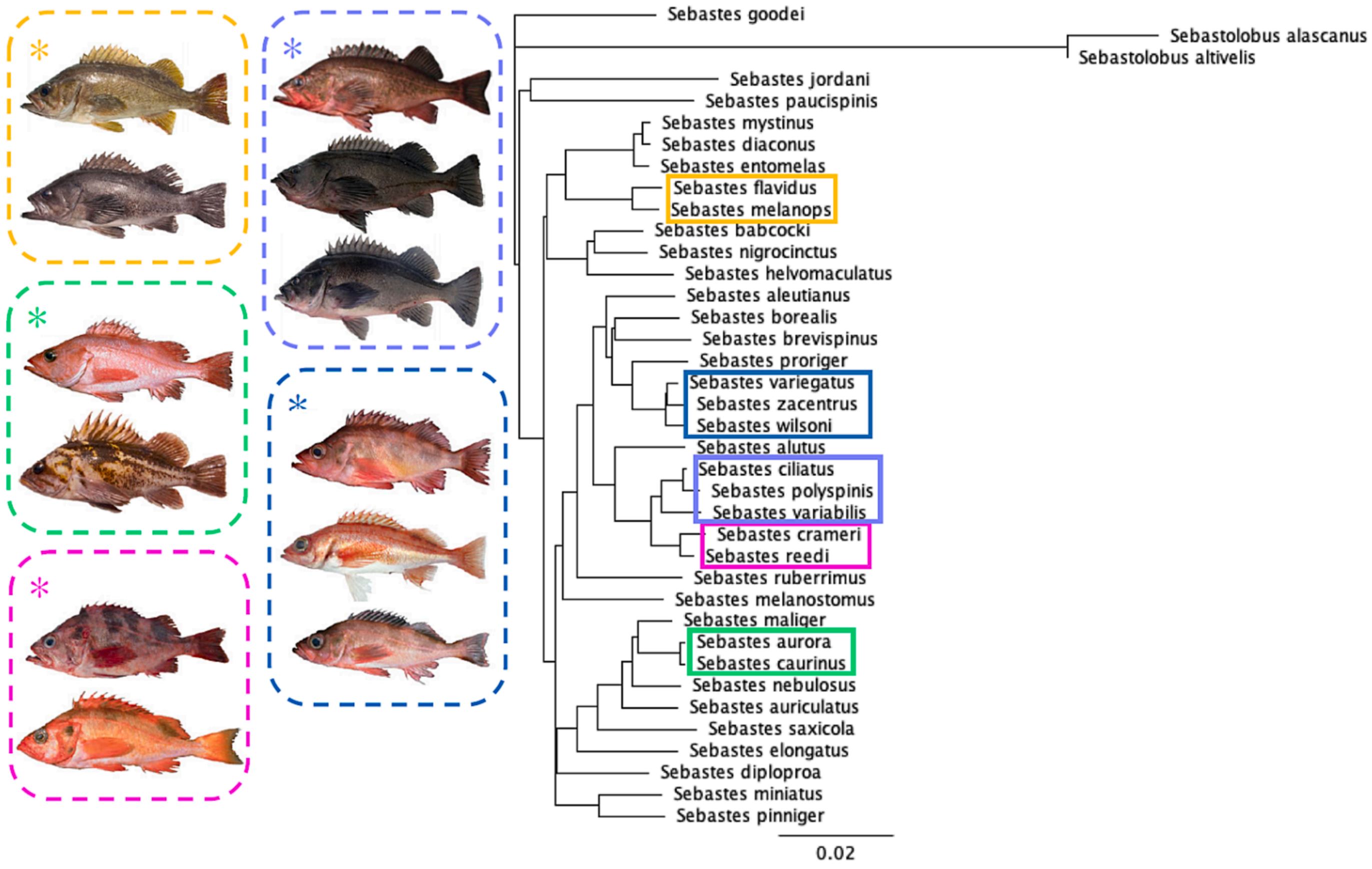

Fig. 6. Neighbour-joining tree generated from entire reference mitogenomes of BC rockfish with indicated indiscernible groupings for the MiSebastes metabarcoding assay. Based off the available sequence data, the fish in each box have the identical sequences at the target amplicon and thus cannot be identified to species level. For the present study, they are thus treated as species complexes. The remainder of species can be discerned at the species level.

4. Results and discussion

4.1. eDNA assay validation results

4.1.1. eDNA assay performance - qPCR

Within the context of the present study, the full mitochondrial genomes of most rockfish species that are known to exist on the Pacific coast of North America were accessible (Kolora et al., 2021). To ensure that potential intraspecific variation was captured, five to six voucher specimens of each target species were sequenced (Fig. 2), as well as other common species in British Columbia, from our area of study (Table S7). Although eSEPA9 and eSEMA3 show intraspecific variability in the assay region, probe and primer binding efficiency is not affected (Figs. 3 and 4). This was confirmed through assay testing with tissue samples from the sequenced individuals. eSERU5 does not show any intraspecific variability in the assay region (Fig. 5). However, the eSERU5 amplification efficiency at 78 % (Table 1) is slightly lower than the 80 % recommended by the new Canadian national standard on performance criteria for the analysis of eDNA by targeted qPCR (Abbott et al., 2023). However, given the considerable constraints for the design of this assay, it was the best possible we could do at this time.

The reference sequences originally used to design these assays were from animals in California. Including the voucher specimens from British Columbia in the assay design and validation procedure greatly strengthened our assay design capability to encompass common haplotypes occurring in our area of study. eSEMA3, eSEPA9, and eSERU5 all performed very well when tested on Vancouver Aquarium samples (Table S8), indicating that the assays would work well applied to field samples from British Columbia since the aquarium fish were collected locally. However, it is likely that all our assays will perform better in certain regions based on the dominant haplotypes present.

4.1.2. eDNA assay performance – metabarcoding

For the MiSebastes assay, ten common primer mismatches in the forward primer region were found that were represented in the available rockfish sequences (Table S9). From the 80 globally distributed species for which an entire mitogenome was available, 63 species had a unique MiSebastes amplicon, and 17 species shared an identical MiSebastes amplicon with at least one other species (Table S10). This analysis demonstrated that there are certain rockfish species in BC that lack genetic variation at the MiSebastes region, and thus there are some species in BC that must be treated as complexes with this metabarcoding assay. Using the Tamura-Nei model on Geneious Prime, Neighbour-Joining phylogenetic trees for the British Columbia species were assembled from reference mitogenomes. This analysis confirmed that the indiscernible species complexes were also phylogenetic clades (Fig. 6). It should be noted that there could be haplotypes within species for which sequence information is unknown that could allow species level identification in certain geographical regions.

To assess this phenomenon quantitatively, the custom library of amplicons for MiSebastes was queried with the BLAST tool (Altschul et al., 1997). Using the top hit for each species, the error percentage (16 %), the average E-value (4.18E-100) and the average bit score (350) were generated. The error percentage refers to the proportion of top hits were assigned to the incorrect species. In this case, these hits were in fact unidentifiable species as all “incorrect hits” occurred for species within the previously identified species complexes. The barcode gap from the custom library was calculated using the MACER package (R v3.6.3) (Young, Gill, Gillis, and Hanner, 2021). Forty-one percent of species did not have adequate sequence coverage for the assessment of barcode gap,

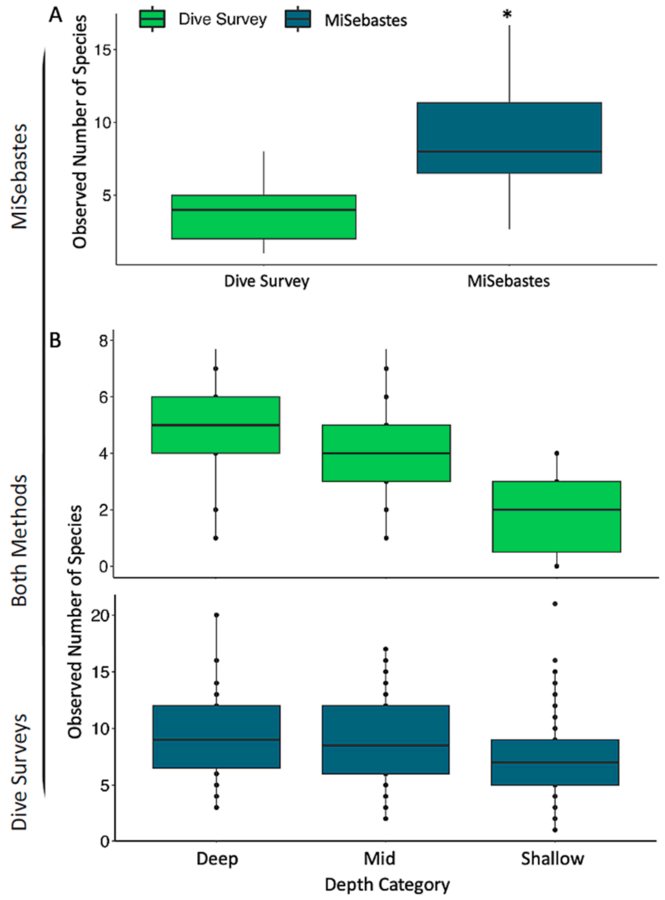

Fig. 7. Alpha diversity estimates via observed number of species A) between detection methods (Paired-Wilcoxon Test: Observed p = 3.6e-11), and B) between depths. There is a consistent trend of higher rockfish diversity of at greater depths. No significant differences between depths for dive data and MiSebastes metabarcoding results using a Paired Wilcoxon Test.

38 % of species did have a barcode gap, and 20 % did not. The species without a gap were within identified species complexes. These analyses confirm that except for the species complexes, most BC rockfishes (at least 24 of 38 based on available sequence data) can be identified to species level using the MiSebastes assay (Fig. 6).

4.2. Field survey results

4.2.1. Metabarcoding molecular analyses

The MiSebastes amplicon sequencing produced 16,987,427 reads for downstream analyses, of which 6,304,907 reads were removed after quality filtering. Of the 10,682,520 reads (mean reads/sample = 67,185 from 1,798 ASVs) remaining, 1,416,204 unassigned reads and 64,056 non-*Sebastes* reads were removed, leaving 9,202,260 reads (mean reads/sample = 57,875 from 287 ASVs). The taxonomic classifications from these 287 ASVs were compared between the custom library and NCBI, and 224 ASVs (98.7 %) had identical taxonomic assignments between the two reference databases. The annotation mismatches between the custom database and NCBI occurred for various reasons: The NCBI BLASTn search assigned 12 ASVs to species that were not within the geographical range of the present study and could assign one ASV only to the genus level. For these ASVs,taxonomic classification from the custom library was performed. Twenty-six ASVs were assigned with a higher percent identity from the NCBI BLASTN search than with the custom library. This is likely because the *cytb* region (mitochondrial gene targeted by the MiSebastes assay) has high coverage on NCBI and likely covers more haplotypes than are present in the custom library.

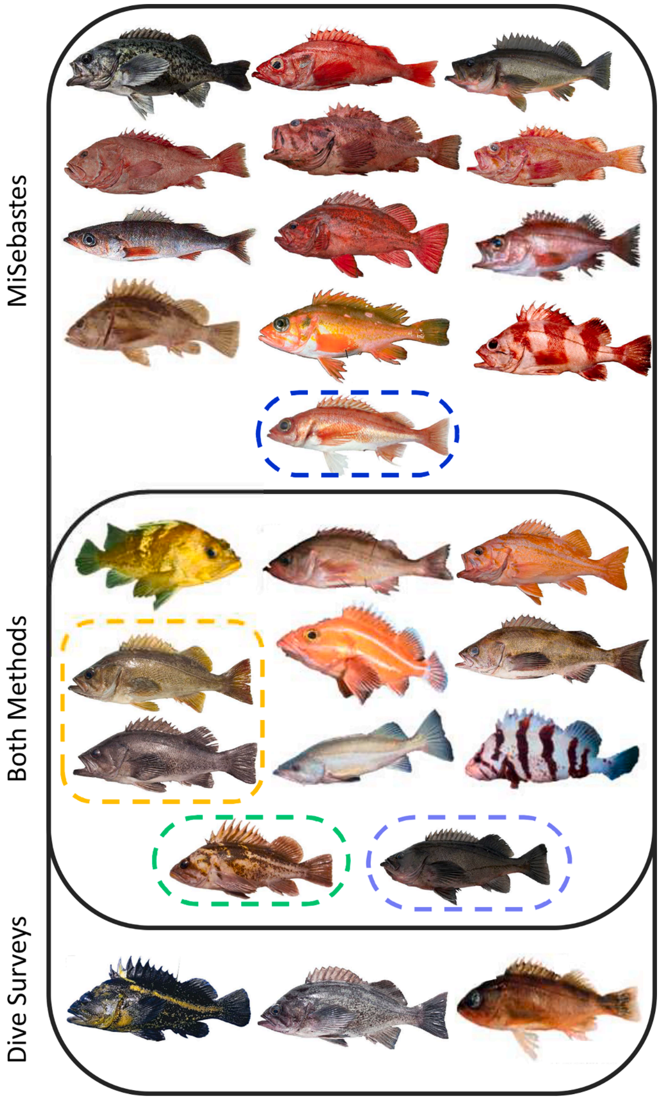

Fig. 8. Overall detection of rockfish species. Colored boxes indicate species complexes corresponding to those identified in Fig. 6.

Finally, 20 of the mismatched ASVs were within the known species complexes that could not be discerned by MiSebastes (Fig. 6) resulting in equivalent blast scores to multiple different species. Those ASVs were therefore assigned to a species-complex rather than a single species. All rarefaction curves generated reached a plateau, confirming that there was adequate sequencing depth for species representation (Fig. S4). The MiSebastes assay did detect a few rockfish reads in the field blanks, however these should be accounted for using the decontamination pipeline which removed 2 ASVs from ecological analysis (MiSebastes ASV# 299, 958) (Figs. S5, S6).

4.2.2. SCUBA diving and eDNA results comparison

Overall, the MiSebastes assay detected significantly higher alpha diversity than dive surveys in the observed number of species (Fig. 7; Paired-Wilcoxon Test: p = 3.6e-11) It is also clear that there is more rockfish diversity on the deeper transects. This is consistent with what is known of rockfish ecology.

Across all samples, both diver and metabarcoding-based detection methods identified eleven species (*S. melanops, S. flavidus, S. ruberrimus, S. maliger, S. caurinus, S. nigrocinctus, S. proriger, S. entomelas, S. paucispinis, S. ciliatus and S. pinniger*) (Fig. 8). Three of the identified species are complexes. MiSebastes cannot confidently distinguish between

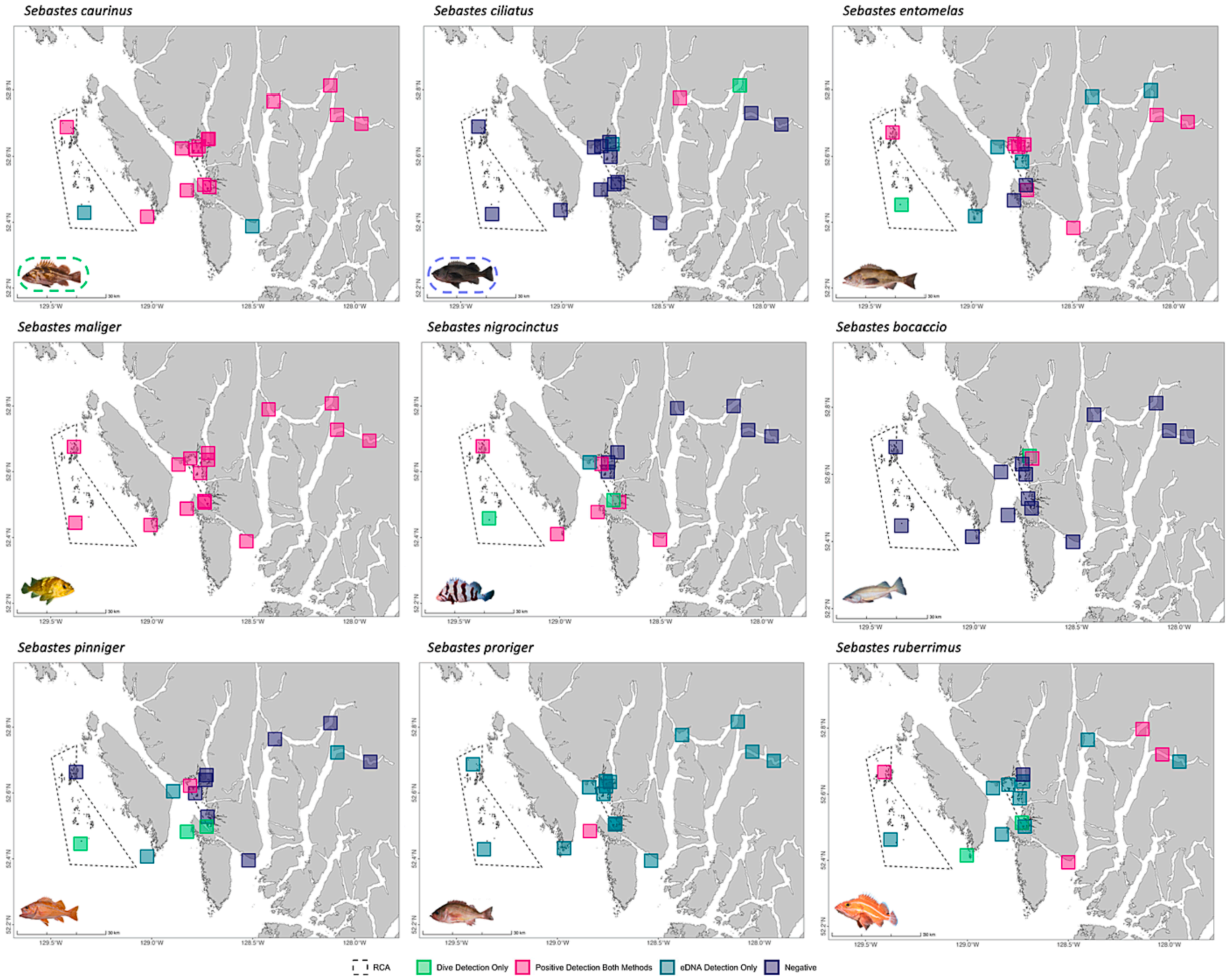

Fig. 9. Each map depicts comparisons between detection by the MiSebastes assay and by dive survey. *S. caurinus,* and *S.ciliatus* are depicted as the more likely to occur species of their species complexes. The *S. melannops_flavidus* complex is not depicted because both species are equally likely to occur.

S. melanops and *S. flavidus; S. ciliatus, S. polyspinis* and *S. variabilis;* and *S. aurora* and *S. caurinus.* Since *S. aurora* generally occurs at depths greater than 80 m and is less common than *S. caurinus*, these classifications are likely the latter species. Though the MiSebastes assay cannot distinguish *S. ciliatus* from *S. polyspinis* and *S. variabilis,* and it is also hard to visually discriminate between these species. Thirteen additional species were detected solely by metabarcoding using the MiSebastes assay; *S. auriculatus, S. aleutianus, S. jordani, S. miniatus, S. alutus, S. helvomaculatus, S. babcocki, S. brevispinis, S. borealis, S. mystinus, S. melanostictus, S. diploproa,* and the *S. zacentrus, S. wilsoni,* and *S. variegatus* species complex. There were also three species detected by diving alone: *S. emphaeus, S. nebulosus* and *S. diaconus. S. emphaeus* has very little genetic information available, so it may have been detected but not properly annotated. It is interesting that MiSebastes failed to detect *S. nebulosus* as there are no known primer mismatches for that species, and it was present at a number of sites surveyed. It is possible that the dominant haplotype in the region may not be known. It is likely that divers misidentified *S. mystinus* as *S.diaconus.* These two species are so difficult to visually distinguish that they were not formally classified as separate species until 2015 (Vaux et al., 2019). The MiSebastes amplicon sequence for these two species, however, is distinct. Therefore, the fact that divers did not detect *S. mystinus* and the MiSebastes assay did not detect *S. diaconus* suggests it was S. *mystinus* that the divers observed since it is more reliable to distinguish the two species genetically than visually.

Focusing on species detection at the station level considerable agreement was observed between diving and the MiSebastes metabarcoding assay (Fig. 9). Using the Fisher's exact test we determined that for all species except *S.ruberrimus* ($p = 0.029$), and *S. proriger* ($p < 0.00001$), there were no significant differences between station designations between diving and eDNA methods (*S.pinniger* ($p = 1$), *S.caurinus* ($p = 0.48$), *S. nigrocinctus* ($p = 1$), *S.ciliatus* ($p = 1$), *S. entomelas* ($p = 0.25$), *S.maliger* ($p = 1$), *S. paucispinis* ($p = 1$)). Two noteworthy observations that aptly demonstrate the synthesis of a common ecological reality between methods include the clear lack of *S. nigrocinctus,* and *S. pinniger* in the fjords and the general ubiquity of *S. caurinus* and *S. maliger.* The fact that *S. proriger* eDNA seems ubiquitous but the species is rarely observed, is very interesting, and could be due to many different reasons such as a high amplification efficiency of this species by the MiSebastes assay or the species simply being missed or misidentified by divers.

To compare detection agreement between methods at the transect level, the overall efficacy of each eDNA method in detecting the target species were calculated and reported as the percentage of transects where detections were eDNA only, dive only, or both negative or both positive (Fig. 10A; Table S11). There was agreement between diving and

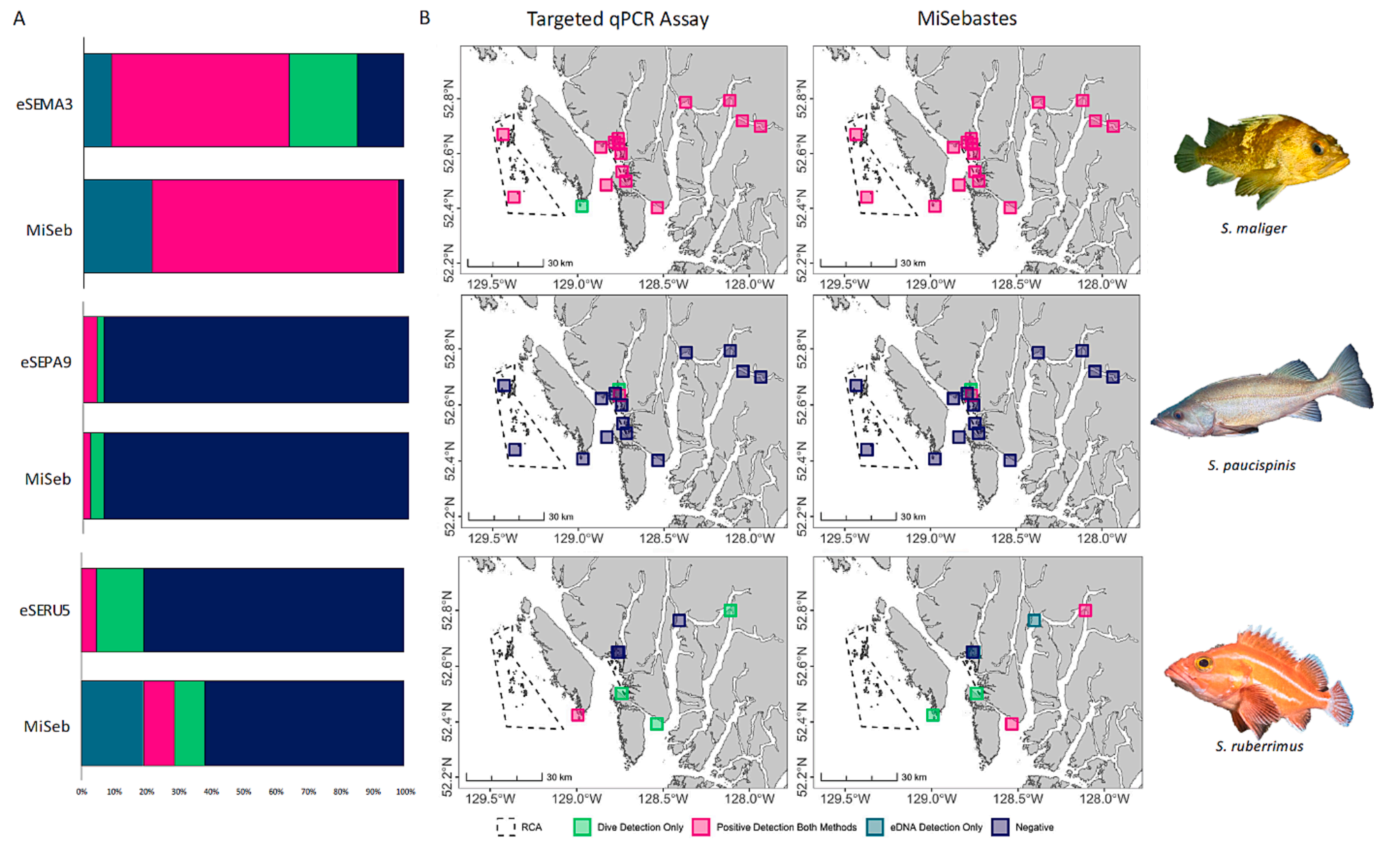

Fig. 10. *Sebastes* target species detection comparison between dive surveys and eDNA methods. A) Depicts comparisons as percentage agreement between methods at the transect level, B) Depicts comparisons at the station level. For *S. ruberrimus*, only the 2021 data results are shown due to likely contamination of the 2022 samples (see text for details).

eDNA methods for the majority of transects across target species. At the transect level for both *S. maliger* and *S. ruberrimus* there are more dive only detections of target species in comparison with the qPCR results, and there are generally more eDNA only detections in comparison with metabarcoding results. The high stringency of the qPCR assays results in the higher likelihood of false negative results, while lower stringency of metabarcoding results in higher likelihoods of false positive results. It is difficult to discern which method is closer to true species distribution.

Closer examination of the target species indicates there were no significant differences between detection via diving or qPCR assay for *S. maliger* (eSEMA3; $p = 1$)*, S. paucispinis* (eSEPA9; $p = 1$) or *S.ruberrimus* (eSERU5; $p = 0.48$; Fisher's exact test) (Fig. 10B). However, there was a surprising number of *S. ruberrimus* positive detections by both eDNA methods in the samples collected in 2022 even though this species generally does not occur at shallow depths (Fisher's exact test; eSERU5; $p = 0.015$, MiSebastes; $p = 0.0023$). There was no *S. ruberrimus* DNA detected in the field controls using eSERU5 assay. Though these signals may reflect true eDNA transported from deeper depths through natural upwelling, it is most likely due to fishing activities that were occurring during the time of sampling as rockfish were being pulled up through the water column. Unfortunately, the likely contamination of *S. ruberrimus* DNA confounds our results for this species. We therefore evaluated the 2021 data for *S. ruberrimus* separate from the 2022 out of caution and found excellent concordance between analytical methods (eSERU5; $p = 0.27$, MiSebastes; $p = 1$; Fisher's exact test). The above observations are a testament to the extreme sensitivity of targeted qPCR assays, and a subsequent field study focused on this species is warranted.

It should also be noted that *S. maliger* and *S. ruberrimus* were detected in low concentrations in the dive suit controls in both 2021 and 2022, but *S. paucispinis* was not. This indicates that despite efforts to minimize contamination, it is very challenging to control all sources of contamination in the field when using eDNA methods. Regardless, these three species are of conservation concern and these assays will undoubtedly be a useful tool for managers working with rockfish.

Although there was considerable overlap in the species detected by both methods, this does not extend to the estimated proportion of each species within the community (Fig. 11, S7). The top five species detected by the MiSebastes metabarcoding assay by read proportion and species prevalence were the *S. flavidus_melanops* species complex (48 % of total reads; 97 % prevalence across samples), *S. ruberrimus* (22 %; 55 %), *S. maliger* (10 %; 82 %), the *S. caurinus_aurora* species complex (7 %; 48 %), and *S. auriculatus* (2 %; 37 %). Whereas the top five species detected by divers as a proportion of the total number of fish seen were *S. entomelas* (69 % of all fish seen), *S. flavidus* (18 %), *S. maliger* (4 %), *S. paucispinis* (3 %), and *S. caurinus* (1.5 %) and, by species prevalence, *S. maliger* (71 %), *S. flavidus* (60 %), *S. caurinus* (50 %), *S. nebulosus* (44 %), and *S. entomelas* (35 %). The other species detected can be found in Table 2.

Many factors can influence why relative species abundances are not always consistent between methods. However, primer bias is a major factor for consideration. This is a molecular bias whereby PCR assays can preferentially bind and amplify certain species based off genetic sequence characteristics, thereby not reflecting true community composition. This is a well-known concept in metabarcoding and is a reason why we cannot yet rely on eDNA metabarcoding data to infer relative abundance (Acharya-Patel et al., 2021; Beng and Corlett, 2020; Kelly, Shelton, and Gallego, 2019; Shelton et al., 2023). Species detection using eDNA metabarcoding, on the other hand, is quite reliable.

Thus, it is quite compelling that eDNA methods were able to detect many species that divers were not. A few common species found in the diveable depth range (*S. auriculatus, miniatus,* and *brevispinis*) were simply not observed by divers at the study stations as they were out of

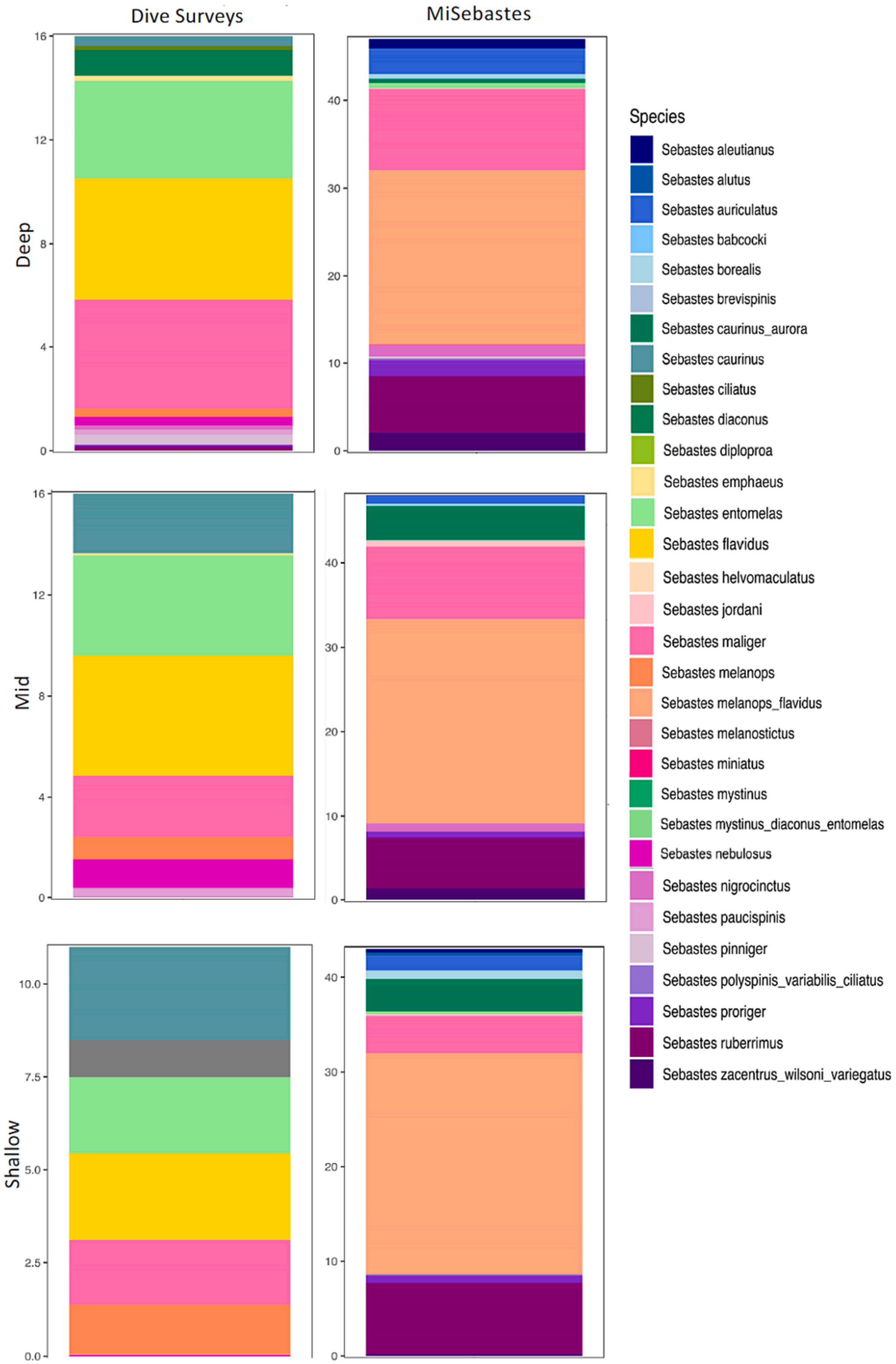

Fig. 11. Visualization of the amplicon sequencing results per sample aggregated by depth. The colors show the relative proportion of rockfish species detected at each depth by MiSebastes metabarcoding and dive surveys. Note that MiSebastes cannot discern between certain species as indicated in the legend on the right.

the visual range of the transect or concealed. Additional species detected by eDNA are known to be cryptic and have typical depth ranges starting near the deeper dive transects of the present study (*S. borealis* (24–1200 m), *S. helvomaculatus* (25–549 m), *S. zacentrus* (25–450 m) and *S. wilsoni* (30–2704 m)). Finally, there are several species that generally live much deeper in the water column (*S. diploproa* (90–80 m), *S. babcocki* (49–625 m), *S. alutus* (90–825 m), *S. jordani* (91–491 m), *S. aleutianus* (150–450 m), *S. melanostictus* (84–1000 m), and *S. variegatus* (70–560 m))(Froese, Capuli, Garilao, and Pauly, 2023; "Washington Department of Fish and Wildlife," 2023).

These detections bring up the difficult questions regarding the ecology of eDNA (Barnes and Turner, 2015; Harrison, Sunday, and

Table 2
Sebastes species detected by diving and eDNA metabarcoding methods. The data are presented as relative abundance and as species prevalence.

Detection Method	Species Detected	Proportion of total individuals seen or of total reads (%)	Proportion of positive detections across all eDNA samples or transects
Dive Survey	*Sebastes entomelas*	68.75	35.42
	Sebastes flavidus	17.82	60.42
	Sebastes maliger	4.02	70.83
	Sebastes paucispinis	2.88	6.25
	Sebastes caurinus	1.42	50
	Sebastes melanops	1.32	16.67
	Sebastes diaconus	1.04	4.17
	Sebastes nebulosus	0.81	43.75
	Sebastes emphaeus	0.74	14.58
	Sebastes pinniger	0.43	8.33
	Sebastes ruberrimus	0.31	14.58
	Sebastes nigrocinctus	0.24	16.67
	Sebastes caurinus_maliger_auriculatus †	0.08	2.08
	Sebastes ciliatus	0.05	4.17
	Sebastes melanops_flavidus†	0.04	2.08
	Sebastes proriger	0.03	2.08
MiSebastes	*Sebastes melanops_flavidus*‡	48.25	97.4
	Sebastes ruberrimus	21.62	55.19
	Sebastes maliger	10.45	81.82
	Sebastes caurinus_aurora‡	6.94	48.05
	Sebastes auriculatus	3.11	37.01
	Sebastes zacentrus_wilsoni_variegatus‡	1.99	27.92
	Sebastes aleutianus	1.83	12.99
	Sebastes nigrocinctus	1.71	16.23
	Sebastes borealis	1.68	10.39
	Sebastes proriger	1.35	24.68
	Sebastes entomelas	0.3	19.48
	Sebastes pinniger	0.27	5.19
	Sebastes jordani	0.2	11.69
	Sebastes polyspinis_variabilis_ciliatus‡	0.1	3.25
	Sebastes miniatus	0.07	1.3
	Sebastes alutus	0.03	0.65
	Sebastes helvomaculatus	0.03	1.3
	Sebastes babcocki	0.03	0.65
	Sebastes brevispinis	0.03	3.25
	Sebastes mystinus	0.01	0.65
	Sebastes diploproa	0.01	0.65
	Sebastes paucispinis	0	0.65
	Sebastes melanostictus	0	1.95

†Cannot discern visually as juveniles.
‡Cannot genetically differentiate.

Rogers, 2019). The few studies that have attempted to address the complex fate of eDNA particles within the marine environment paint an intricate picture. Some studies found that eDNA detections will reflect the local community despite water movement caused by tides (Kelly, Gallego, and Jacobs-Palmer, 2018), and can discern communities separated by physical barriers like a kelp forest on a scale of only 60 m (Port et al., 2016). Other studies that have simulated eDNA particle movement through larger oceanographic processes estimate that eDNA could be detected 40 km away from the source on a timescale of four days (Andruszkiewicz et al., 2019). This uncertainty has important implications on the confidence of the detection of improbable species in an environmental sample. While the current strength on each dive performed in the present study was recorded, this information is not useful without the context of *in situ* measurements such as upwelling, mixing, and stratification. Despite these limitations, eDNA surveying methods are still useful as divers are unable to survey the very deep rockfish habitats, and the methodology is far less invasive than commonly used long line survey methods with high rates of mortality. eDNA surveys from deeper water samples using these assays will be extremely informative and less invasive.

5. Conclusion

The genus *Sebastes* is of particular interest to many stakeholders and decision-making groups in the Pacific Northwest due to its cultural, ecological, and economic importance. However, the combination of high intraspecific variability and high interspecific conservation makes this genus particularly difficult to genetically distinguish between species, while also making it easy to miss haplotypes of individual species (Hyde and Vetter, 2007; Johansson et al., 2008; Kolora et al., 2021; Love et al., 2002; Vaux et al., 2019; Yu and Kim, 2018). Fundamentally, eDNA methods rely on genetic sequence differences to discern species of interest from complex environmental samples. The integration of eDNA into larger scale projects necessitates a greater effort on the part of the eDNA scientist or practitioner to pre-emptively investigate the sequence availability, coverage, and quality that exists for the group of species being studied. To address intraspecific variability, more sequence data from different regions needs to be integrated into the assay or validation pipeline. We suggest that assays should also be tested on tissue-derived DNA from voucher specimens from the region where the assay is going to be applied. Indeed, this is encouraged by the Canadian national eDNA standard (Abbott et al., 2023; Gagné et al., 2021; Helbing and Hobbs, 2019; Lesperance et al., 2021). More sequence data will also help with resolving interspecific conservation. When the commonly used metabarcoding regions like *mt-co1* and *mt-rnr1* (Kawato et al., 2021; Miya et al., 2020; Reidy et al., 2022; Robinson et al., 2023) are conserved within a group of interest – as they are within *Sebastes* – using other regions like the *mt-cytb* region used in the MiSebastes metabarcoding assay described herein can be very helpful. However, as demonstrated in Fig. 6, there are still important species like *S. flavidus* and *S. melanops* that cannot be discerned. Though outside the scope of this work, the *unikseq* (Allison et al., 2023) pipeline can also be used to find useful barcode regions as it systematically parses through entire mitochondrial genomes to find areas that are amenable to assay design. Thus, as sequencing costs continue to decline, and eDNA applications continue to increase, the argument for sequencing whole mitochondrial genomes – ideally multiple voucher specimens per species - is clear.

The purpose of the present work was to compare SCUBA diving methods and eDNA methods in their capabilities to assess rockfish biodiversity. Even though the MiSebastes assay cannot discern all the rockfish species in British Columbia, it can discern the majority, and the present work indicates which species must be treated as species complexes. Similarly, while diving there are some species that are very difficult to visually distinguish. This highlights the fact that all detection methods – even those that are well established – have limitations, but they still generate highly useful biodiversity data for decision makers (J. A. Darling, 2020; J.A. Darling, Jerde, and Sepulveda, 2021).

It is important to remember that eDNA research is highly interdisciplinary. A field eDNA study carried out in the marine environment exists at the intersection of ecology, biochemistry, biology, oceanography, genomics, and bioinformatics. As eDNA studies expand in scale and scope, integrating these different forms of data will allow us to gain a clearer picture of biodiversity and species distribution. Overall, both SCUBA surveys and eDNA methods were comparable in their ability to assess rockfish diversity; however, it is also clear that each method had its own strengths and weaknesses. While dive surveys often miss species that tend to hide or have a depth range deeper than the survey depths, they can provide important ecological information like species abundance, size distribution, invertebrate biodiversity, and habitat complexity. These parameters are vital when making meaningful fisheries or protection-based management decisions. On the other hand, eDNA methods detected more species overall, particularly those that are unusual or live deeper. eDNA sampling is also much more scalable and, because of the ease of sampling, can easily be integrated into community-based monitoring programs. Using multiple approaches together will undoubtedly improve surveying capacity and understanding of areas important for rockfish biodiversity.

CRediT authorship contribution statement

Neha Acharya-Patel: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Emma Groenwold:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis. **Matthew A. Lemay:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, Formal analysis. **Rute Clemente-Carvalho:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Evan Morien:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Sarah Dudas:** Writing – review & editing, Conceptualization. **Emily Rubidge:** Writing – review & editing, Conceptualization. **Cecilia Lingyu Yang:** Writing – review & editing, Methodology, Formal analysis. **Lauren Coombe:** Writing – review & editing, Methodology, Formal analysis. **René L. Warren:** Writing – review & editing, Methodology, Formal analysis. **Alejandro Frid:** Writing – review & editing, Investigation, Conceptualization. **Inanc Birol:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Formal analysis. **Caren C. Helbing:** Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw sequence reads and metadata are deposited in the SRA (Bioproject ID #: PRJNA1028663). Additional data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.111830.

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