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ORIGINAL ARTICLE

Comparison of traditional and molecular surveys of fish biodiversity in southern Te Wāhipounamu/Fiordland (Aotearoa/New Zealand)

Paul Czechowski[1,2] | Michel de Lange[3,4] | Michael Heldsinger[5,6] | Anya Kardailsky[1] | Will Rayment[6,7] | Christopher Hepburn[6,7] | Monique Ladds[8] | Michael Knapp[1,7]

[1]Department of Anatomy, University of Otago, Dunedin, New Zealand

[2]Helmholtz-Institut für Metabolismus-, Adipositas- und Gefäßforschung, Helmholtz Zentrum München – Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Leipzig, Germany

[3]Biostatistics Centre, University of Otago, Dunedin, New Zealand

[4]Pacific Edge, Dunedin, New Zealand

[5]RPS Group, West Perth, Western Australia, Australia

[6]Department of Marine Science, University of Otago, Dunedin, New Zealand

[7]Coastal People Southern Skies Centre of Research Excellence, University of Otago, Dunedin, New Zealand

[8]Department of Conservation, Wellington, New Zealand

Correspondence
Paul Czechowski, Helmholtz Institute for Metabolic, Obesity and Vascular Research, Philipp-Rosenthal-Straße 27, 04103 Leipzig, Germany.
Email: paul.czechowski@helmholtz-munich.de and paul.czechowski@gmail.com

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Abstract

Effective management of biodiversity requires regular surveillance of multiple species. Analysis of environmental DNA (eDNA) by metabarcoding holds promise to achieve this relatively easily. However, taxonomy-focused eDNA surveys need suitable molecular reference data, which are often lacking, particularly at the species level and for remote locations. To evaluate the comparability of environmental DNA surveys and traditional surveys in a real-life case study in a marine area of high conservation value, we conducted a biodiversity survey of the fish in remote and pristine Te Wāhipounamu/Fiordland (Aotearoa/New Zealand), incorporating multiple data sources. We compared eDNA-derived species identifications against Baited Remote Underwater Video (BRUV) data collected at the same time and locations as eDNA. We also cross-referenced both eDNA and BRUV data against literature and the Ocean Biodiversity Information System (OBIS), with literature and OBIS data representing a summary of multiple traditional surveying approaches. In total, we found 116 fish species in our study area. Environmental DNA detected 43 species; however, only three of those species overlap with species known from the literature, OBIS, or our BRUV analyses. A total of 61 fish species were known from the region from the literature, while OBIS listed 28 species, and our BRUV analyses picked up 26 species. BRUV data coincided more strongly than eDNA data with literature and OBIS data. Twenty of the 26 species detected by BRUV were known from literature and OBIS. We argue that limitated DNA reference databases are the main cause of this discrepancy, and our results indicate that eDNA of rare and endangered species can be detected if matching reference data were available. Environmental DNA analyses can only identify species present among reference data and with relaxed taxonomic assignment parameters may converge on relatives of detected species if the actually existing species themselves are missing among reference data. However, the high number of species detected by our eDNA analyses confirms that eDNA could be a powerful tool for biodiversity surveys if suitable investments in local reference databases were made.

KEYWORDS
biodiversity, data visualization, environmental DNA, fishes, marine ecology, natural resources conservation

1 | INTRODUCTION

Marine reserve (MR) networks like the ones established in the waters of UNESCO world heritage site Te Wāhipounamu, New Zealand ("Fiordland") conserve biodiversity by stabilizing communities and maintaining food web structure (Wing & Jack, 2013). Effective management of such MR networks requires biodiversity description and surveillance, for example, to avoid overexploitation by fishing (Jack & Wing, 2013), or to avoid damage through influx of non-indigenous species (Cunningham, 2019). Assessment of fish biodiversity is of particular interest due to their sensitivity to most forms of human disturbance, their usefulness at all levels of biological organization, and the favorable benefit-to-cost ratio of fish assessment programs (Harris, 1995).

Analysis of environmental DNA metabarcoding data (eDNA) is a well-established molecular technique for multispecies surveys (Cristescu & Hebert, 2018). Environmental DNA metabarcoding is often advertised across the literature as a method of choice for biodiversity surveys – associated techniques are frequently praised as more cost-efficient than traditional methods (such as baited remote underwater video surveys – BRUV), praised as less dependent on expert taxonomic knowledge, able to be standardized, and able to inform on a broad range of taxa (e.g., Gold et al., 2022; Sigsgaard et al., 2020).

At the same time, reliable low-level taxonomic annotation, particularly at the species level, is a prerequisite for useful biodiversity exploration and natural resource surveillance (Currey et al., 2009; Jack & Wing, 2013). For example, in a southern New Zealand context, *Parapercis colias* (blue cod) is of high commercial interest, but three other of 79 cod species are known from New Zealand (Roberts et al., 2019), so that genus information alone is already ambiguous for determining blue cod presence or absence. Higher level taxonomic classifications (e.g., family and order levels) are even less informative for conservation management, hence yielding species-level data is a very important use case for eDNA surveys.

In the context of eDNA analysis, the desire for reliable low-level taxonomic information translates into the desire for obtaining high-quality 100 bp to 200 bp alignments (Huson et al., 2007) between an unknown, eDNA-derived query sequence, and a taxonomically well-described reference sequence derived from a valid species. However, as taxonomy is constantly revised and species are dynamic entities, even reliable low-level taxonomic reference information can be quickly outdated (Hleap et al., 2021). In our view, the absence of sufficient or reliable reference data can be addressed by relaxation of taxonomy-assigning algorithm parameters to retain sufficient eDNA data for analysis, when the resulting partial drop of the eDNA data's informative quality is sufficiently considered (Czechowski et al., 2021). This means obtaining and carefully inspecting many partially less accurate assignments including false negatives, rather than obtaining fewer accurate assignments, while automating data inspection, and possibly discarding falsely negative information.

Availability of sufficient and reliable reference data for metabarcoding is highly variable depending on taxonomic groups and geographic locations, with fish considered relatively well covered for some regions, such as Europe, in Barcode of Life Data Systems (BOLD) and NCBIs GenBank (Benson et al., 2011; reviewed in Weigand et al., 2019). Substantially fewer reference data are available for fish of southern New Zealand. For example, for six commonly used 12S primer pairs, recognized as well suited for multispecies fish surveys (Weigand et al., 2019; Zhu & Iwasaki, 2023), an average of 36% of northern European fish species are available as reference data, but only 26% of southern New Zealand species (GAPeDNA v1.0.1 web interface, 11-Sep-2021; Marques et al., 2021; also see Table S1; and reviewed by Marques et al., 2021). Where purposefully generated reference data are unavailable, selected publicly available reference data (such as derived from GenBank) are the only option for taxonomic assignments, and useful if taxonomic assignments are verified in the study context (Balvočiūtė & Huson, 2017; Claver et al., 2023).

In this study, we combine data from several sources (literature, online, video, and molecular) to collate a current assessment of the species-level fish biodiversity of Te Wāhipounamu. We evaluate the ability of eDNA data to augment what is known of the local biodiversity whilst comprehensive local reference data are not available. Finally, we show that local reference data are needed to address ecological questions in Te Wāhipounamu. We demonstrate that—while useful—future eDNA surveys of the region would substantially benefit from efforts yielding comprehensive molecular reference data for the taxonomic assignment of eDNA. Besides providing a high-resolution survey of the fish biodiversity of the Te Wāhipounamu region, we hope our study will be a helpful reference for readers interested in propelling efforts to generate molecular reference data for eDNA biodiversity surveys. Often funding for such purely descriptive efforts, including sample collection, accurate taxonomic identification of samples, and sequencing of suitable markers, is challenging to obtain. We hope this study can contribute to highlighting to potential funders the necessity of such efforts for fully utilizing the potential of eDNA as a tool for biodiversity surveys.

2 | METHODS

In our study, we aimed to observe Actinopterygii (ray-finned fishes) and Chondrichthyes (cartilaginous fishes) species in one MR, two commercial exclusion zones (all "MR"), and corresponding control areas in southern Te Wāhipounamu, New Zealand (west coast, approximately from −44.3 to −46.25 Southern latitude; Figure 1a). We

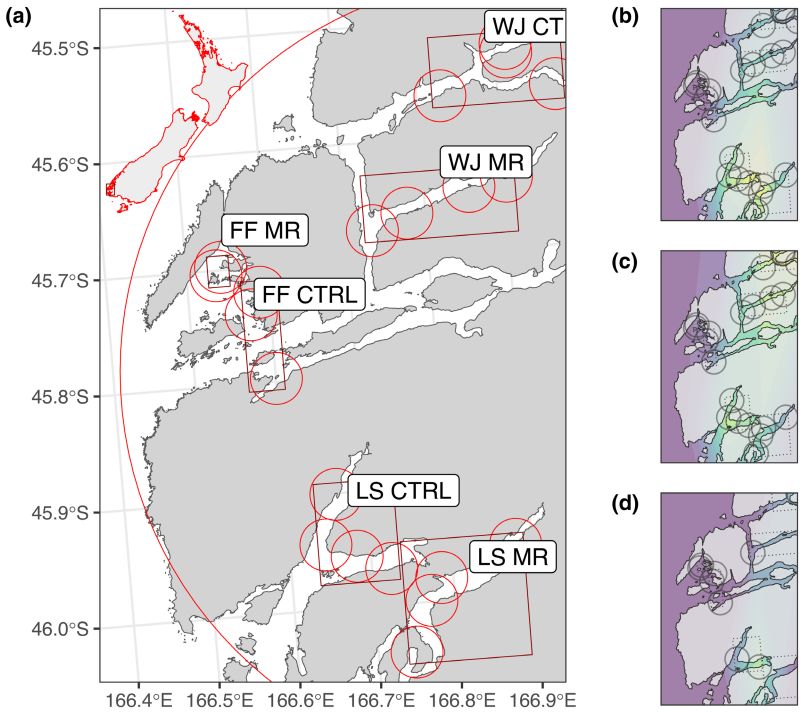

FIGURE 1 Field work area, description, sites, and data coverage for eDNA, BRUV, and OBIS data. Precise sampling locations are obfuscated to aid conservation. (a) We obtained biodiversity information from baited remote underwater video (BRUV) footage and environmental DNA (eDNA) data from 21 field work sites across three sampling regions (highlighted by rectangles)—Five Fingers (FF), Long Sound (LS), and Wet Jacket (WJ). In each region we collected samples inside marine reserves/commercial exclusion zones (MR) and outside in control areas (CTRL). To obtain additional biodiversity information, we queried the Ocean Biodiversity Information System (OBIS – https://obis.org/) for records within a 2.5 km radius of each field work site (small red circles) for the purpose of community structure analysis. Furthermore, we obtained OBIS records for the entire sampling region (large circle) to extend our species list alongside species mentioned across various literature sources (Table 1; Table S5). (b) Environmental DNA (eDNA), and (c) BRUV data in a spatial context, lighter color indicates a higher density of distinct species observations (corresponding to numerical values in Figure 2). (d) Species data for all field work sites could not be obtained from OBIS, necessitating the exclusion of this data in the statistical analyses of regional biodiversity data. Graph created using R package *ggplot2 (3.3.5)*.

obtained and analyzed eDNA and BRUV data, as well as electronic records proximate to the field work area, from the Ocean Biodiversity Information System (OBIS) (Ausubel, 1999). Furthermore, we assembled a list of ray-finned and cartilaginous fishes observed in Te Wāhipounamu from literature. For our analyses, all observations were formalized using NCBI taxonomy (Federhen, 2012), including trivial names, and limited to classes Actinopterygii and Chondrichthyes, while assignments to other taxa were also inspected.

Species observations, obtained using various methods, were extracted from six literature sources, including one meta-analysis (Table S2). Species observations deposited in OBIS were downloaded for a 38-km radius around all field work sites (center point W 166.89°, S -45.80°), as well as for smaller areas surrounding individual field work sites (2.5 km radius; Figure 1a, d).

For a more detailed description of field and laboratory work please refer to the Supporting Information. In summary, for collecting eDNA and filming BRUV we visited three locations in southern Te Wāhipounamu (Moana Uta/Wet Jacket Arm, Taumoana/Five Fingers, and Te Tapuwae a Hua/Long Sound; henceforth WJ MR, FF MR, and LS MR), and accompanying control areas outside those MRs (henceforth WJ CTRL, FF CTRL, and LS CTRL), from 12.–22. December 2019 (Figure 1a). Within each sampling location, at randomized sites, we collected eDNA (mean depth 14.05 m, med.: 15, sd.: 1.4 m), and subsequently deployed BRUV assemblies (mean depth 15.6 m, med.: 16, sd.: 2.6 m). We considered data from 21 sites (FF: 2 FF MR and 3 FF CTRL, WJ: 4 WJ MR and 4 WJ CTRL, and LS: 4 LS MR and 4 LS CTRL). We collected two 900-mL water samples with eDNA at each site, filtered them alongside negative controls, then sealed and stored them until further processing. BRUV footage was obtained for 1 h and analyzed by eye with local taxonomic keys.

Environmental DNA was isolated in a PCR-free facility alongside extraction and cross-contamination controls (Supporting Information: four species of tropical freshwater fish). After in silico PCR to test the suitability of our primer pairs (Figure S2), we amplified our extracts with two well-established and widely used 12S primer pairs. Primer pair "MiFish-U" (Miya et al., 2015) (see Table S1 for primer comparison), was used to target Actinopterygii. Chondrichthyes were targeted with slightly altered primer pair derivatives ("Elas02"; Taberlet et al., 2018a, 2018b). Our single-step PCRs were cycled 45 times, with low annealing temperatures of 45°C ("MiFish-U") or 40°C ("Elas02") necessitated by the long sequencing adapters attached to the amplicons. Amplified eDNA was

then pooled, visualized, purified, combined equimolarly, diluted to 4.5 pmol, and sequenced on an Illumina MiSeq (Illumina, San Diego, US-CA; kit v2, 300 cycles, single-ended).

We defined Amplicon Sequence Variants (ASVs; Callahan et al., 2017) from eDNA after demultiplexing with Cutadapt v3.0 (Martin, 2011), using Qiime2 2020–08 (Bolyen et al., 2019) and DADA2 1.10.0 (Callahan et al., 2016). To yield high-quality sequence data, we did not allow any mismatches, nor Expected Errors (Edgar & Flyvbjerg, 2015) during demultiplexing. Taxonomic annotation of denoised data was obtained using Blast 2.10.0+ (Camacho et al., 2009), recently established as state-of the art for species-level taxonomic assignments when compared to other contemporary algorithms (Hleap et al., 2021). Lacking local reference data, we used a self-curated copy of the NCBI nucleotide collection (Benson et al., 2011; version September 2022), as an overall aggregate of many 12S sequences, for example, including sequences submitted as part of the Meta-Fish-Lib software (Collins et al., 2021), and safeguarding us from misassignments due to a limited search space (Gold et al., 2022). To curate our NCBI-derived reference data, we excluded 20,008,447 environmental samples, thus minimizing taxonomic misassignments to low-quality data (Claver et al., 2023). To yield a maximum of taxonomically annotated ASVs, we chose relaxed taxonomic assignment parameters in combination with an e-value to retain only the most significant alignments. We required a minimum identity of 75% among all alignments and kept five high-scoring pairs for each eDNA query, each of which needed a minimum coverage of 95% to be retained. We set the acceptable e-value to 10^{-10}. We then removed data contained in negative controls, alongside ASVs covered by fewer than 15 reads, by subtraction from the sample data (see Figures S3, S4). As species-level assignments we retained the best high-scoring alignment of each query-reference pair based on the bit score (also see Hleap et al., 2021, and Figure S2 for relationship between bit score and query length).

To assemble a fish species list of southern Te Wāhipounamu, we combined species observations from literature, OBIS, BRUV, and eDNA in one list, and checked all eDNA-derived taxonomic assignments using a comprehensive list of all New Zealand fish (Roberts et al., 2020).

To evaluate eDNA taxonomic assignments obtained without local reference data, while keeping in mind potential misassignments after using relaxed taxonomic assignment parameters, we initially compared our taxonomic assignments to those obtained with MEGAN's (6.24.21) Least Common Ancestor (LCA) algorithm (Huson et al., 2007, 2016) and the same BLAST output files, expecting more, but partially less reliable assignments of our assignment method, minding that also MEGAN can yield false annotations if limited reference data are available (Garrido-Sanz et al., 2022; Somervuo et al., 2017). Unlike many other studies, we also inspected alignment qualities, expecting them to be highly variable. As part of this inspection, we analyzed gap count and query coverage of eDNA reference alignment in relation to observed native or non-native status of the resulting eDNA species observations. Specifically, we fitted a logistic regression model with the response variable of observing a species known from New Zealand or not (True vs. False). We used query coverage (in percent) and gap counts (integers) of alignments as predictors, and individual observation as trials. Assuming missing local reference data, we expected a low gap count and high query coverages to result in higher propensity to observe species not known from New Zealand. Furthermore, we compared species accumulation curves of eDNA and BRUV data, expecting them to be both plateauing and exhaustive, indicating eDNA sampling to be sufficient. Furthermore, we analyzed divergence of species assignments by data source using Euler diagrams, expecting a high divergence for eDNA data from all other data sources along lowering taxonomic hierarchy levels.

3 | RESULTS

Twenty-one field work sites, each with several samples, (Figure 1a) yielded eDNA and BRUV data (Figure 1b, c). Matching local OBIS data could only be obtained for nine field work sites (Figure 1a, small circles, namely LS CNTRL, FF, WJ shown in Figure 1d). Prior to filtering, and including PCR and extraction controls we obtained 3,877,007 sequences across 125 samples and 2139 ASV's (436 Eukaryota, and 1703 Bacteria, Viruses or undefined sequences). The cleaned eDNA data contained 167,203 unique sequences across 43 samples and 98 fish ASV's, which subsequently resolved to 43 species. Within our works' spatial constraints (Figure 1), we obtained a total of 116 fish species (105 Actinopteri, 10 Chondrichthyes, and 1 Myxini), with 61 records from literature, 28 from OBIS, and 26 from BRUV (Figure 2 and Table 1). Among eDNA we recovered 43 species assignments using our taxonomic assignment approach, and ten of those also using MEGAN's LCA algorithm (see Table S4). Out of 26 species detected with BRUV, twenty (see Figure 2) were contained in the literature or in OBIS (77%). Of 43 species assignments detected with eDNA, two (5%) were contained in Fiordland-specific literature or OBIS (*Thyrsites*

FIGURE 2 Distinct species observations across data sources and field work locations. Observation types: BRUV—Observations from baited remote underwater surveys; eDNA—environmental DNA observations; OBIS—data retrieved from the Ocean Biodiversity Information System (https://obis.org/) for the area surrounding field work sites (large circle in Figure 1); PUBL—Fiordland fish species collated from multiple literature records as summarized by Inglis et al. (2008). Sampling Locations: FF—Five Fingers area; LS—Long Sound area; WJ—Wet Jacket area; MR—marine reserve or commercial exclusion zone; CTRL—neither marine reserve nor commercial exclusion zone. Species list: Order follows Table 1, species not listed as New Zealand Species in Roberts et al. (2020) are marked with an asterisk (*) Species detected by our chosen assignment method also detected by MEGAN's LCA (Huson et al., 2007) are highlighted with triple asterisks (***). Graph created using R package *ggplot2 (3.3.5)*.

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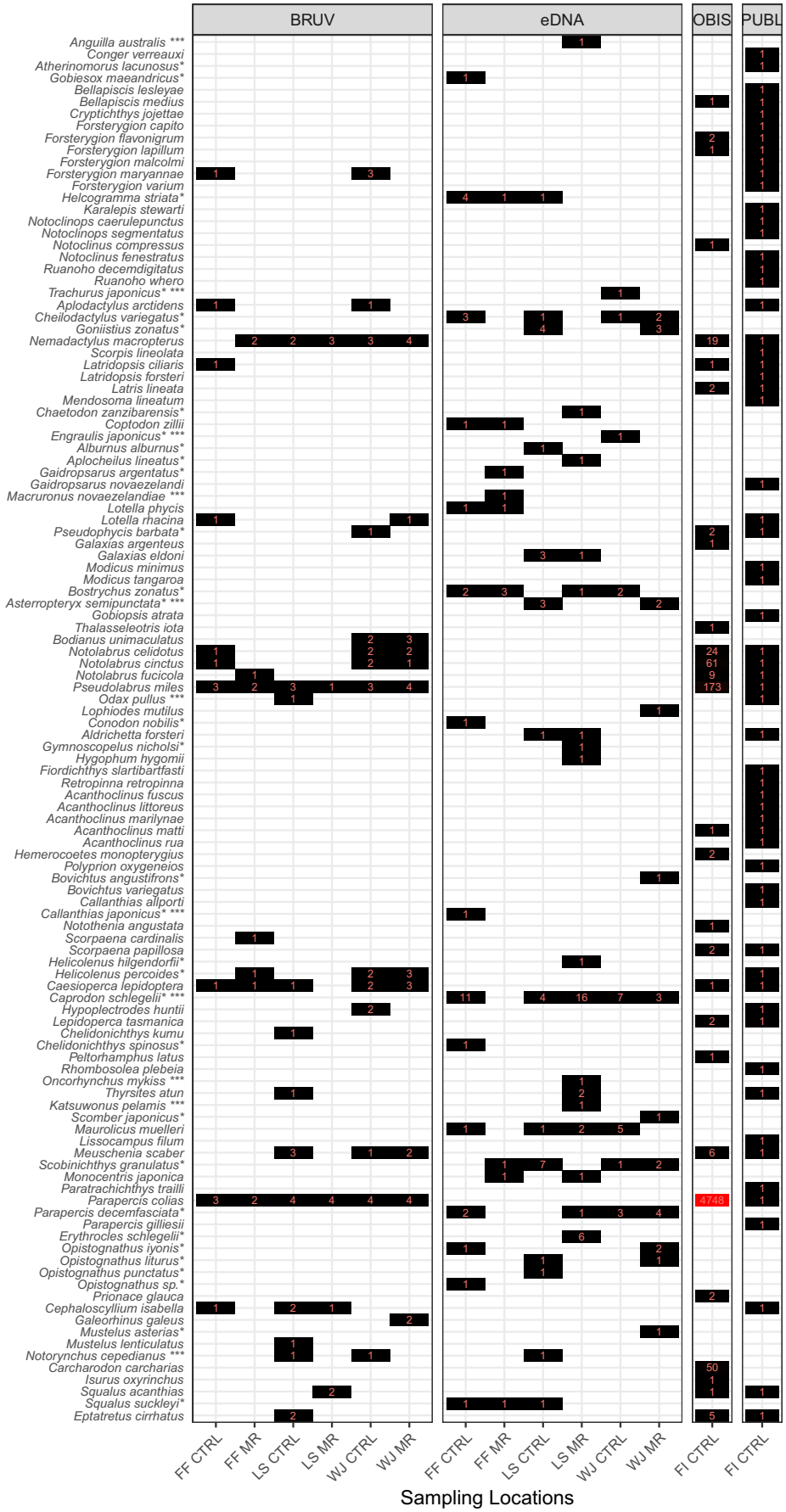

atun, snoek, barracouta, and *Aldrichetta forsteri*, yellow-eye mullet; see Figure 2, Table 1, Table S5). Of the 43 species, seven had perfect alignments (see Table 1), namely *Anguilla australis* (Australian shortfin eel), *Alburnus alburnus* (bleak, not in Roberts et al., 2020), *Macruronus novaezelandiae* (hoki), *Aldrichetta forsteri* (yellow-eye mullet), *Oncorhynchus mykiss* (rainbow trout), *Scomber japonicus* (chub mackerel, not in Roberts et al., 2020), *Mustelus asterias* (starry smooth-hound, not in Roberts et al., 2020), and *Squalus suckleyi* (Puget Sound dogfish, not in Roberts et al., 2020). Of those, two were also discovered with LCA (*O. mykiss* and *A. australis*, Table 1).

Notable fish species detected only using eDNA included *A. australis* (short-finned eel), and *Macruronus novaezelandiae* (hoki) both with perfect alignments, also detected with LCA, and known from New Zealand, but not from Te Wāhipounamu. Noteworthy was an assignment to non-native species (and genus) *Asterropteryx semipunctata* (Starry goby), using both eDNA taxonomic assignment approaches, with 81.9%–82.5% query coverage, and six gaps. The five species only seen on BRUV were *Bodianus unimaculatus* (red pigfish), *Chelidonichthys kumu* (bluefin gurnard), *Galeorhinus galeus* (tope shark), *Mustelus lenticulatus* (spotted estuary smooth-hound), *Scorpaena cardinalis* (red rock cod), species which at present do not appear to have 12S data available on Genbank. Noteworthy was also *Notorynchus cepedianus* (broadnose sevengill shark) seen on BRUV and by both eDNA taxonomic assignment approaches, but not listed in Te Wāhipounamu literature. Interestingly, using eDNA, we obtained perfect assignments (including LCA) for *Arctocephalus forsteri* (New Zealand fur seal), *Balaenoptera* (rorqual whales; highest bit score for *Balaenoptera musculus*, blue whale), and *Tursiops truncatus* (bottlenose dolphin) (see Figure S6). Notable species seen on BRUV, not being Actinopterygii nor Chondrichthyes, included *Jasus edwardsii* (southern rock lobster), *Macroctopus maorum* (*Māori Octopus*), and *Eptatretus cirrhatus* (broadgilled hagfish, tuere), the latter also detected among eDNA.

The ten species assigned both by our eDNA BLAST top hit assignment and LCA were *Caprodon schlegelii* (sunrise perch, not recorded in New Zealand, 0–2 gaps, up to 98.2% coverage), *Katsuwonus pelamis* (skipjack tuna, 0 gaps, 95.9% coverage), *M. novaezelandiae*, *A. australis*, *O. mykiss* (all three as above with perfect alignments), *A. semipunctata* (as above, imperfect alignment), *N. cepedianus* (as seen on BRUV, 0 gaps, 98.4% coverage), *Callanthias japonicus* (yellowsail red bass, not recorded in New Zealand, 1 gap, 95.2% coverage), *Trachurus japonicus* (Japanese jack mackerel, not recorded in New Zealand, 0 gaps, 99.4% coverage), and *Engraulis japonicus* (Japanese anchovy, 0 gaps, 98.8% query coverage; see Table 1). Of the 40 species observed with eDNA, including those with questionable alignments, and neither seen in BRUV nor Fiordland literature, nor OBIS, 10 (25%) appear known from somewhere in New Zealand (Roberts et al., 2020). Among MEGAN's LCA-assigned taxa there were five neither seen in BRUV nor Fiordland literature, and none of those had been observed in New Zealand.

Alignment qualities among eDNA BLAST top-hit taxonomic assignments varied across taxa (see Supporting Information). Logistic regression of alignment qualities across our 156 non-unique eDNA observations estimated the odds ratio for query coverage to 0.75 (95% CU 0.67–0.82) and the odds ratio for gap count to 0.55 (95% CI 0.41–0.70) for observing non-New Zealand species, indicating that indeed alignment qualities were improved in cases where reference data was available from outside New Zealand. Plateauing species accumulation curves suggested exhaustive sampling for BRUV and eDNA (Figures S5,S7). Concordance of taxonomic information between the four data sources diverged with lowering taxonomic levels, most pronounced for eDNA (Figure 3).

4 | DISCUSSION

To date the fish diversity of Te Wāhipounamu has been described based on a diverse range of mostly visual methods (Grange, 1985; Inglis et al., 2008; Mladenov, 2001; Roberts, 2005; Roberts et al., 2020), possibly owed to the fact that eDNA-based surveys are picking up pace at differ speeds around the globe (Capurso et al., 2023; Kelly et al., 2023), and the rather slow recognition that existing specimen and taxonomic expertise needs to be integrated to realize purposeful eDNA biodiversity surveys (de Santana et al., 2021). Furthermore, in New Zealand and elsewhere indigenous interests may not necessarily align with open access publication of genomic data. Here, agreements that reconcile scientific and community interests have to be found before local eDNA databases can be established.

We unite visual observations with the results of concurrent eDNA and BRUV surveys and information from OBIS (Ausubel, 1999). Without purposefully generated reference data for eDNA at hand for the surveyed region, we used a comprehensive public source of reference information, receiving data from many other initiatives, including 12S sequences (Collins et al., 2021; Pruesse et al., 2007), a currently frequently used fish primer set (e.g., see a recent evaluation in Zhu & Iwasaki, 2023), and relaxed taxonomic assignment parameters, to obtain the highest possible yield in identified eDNA species while minimizing missing assignments due to a limited search space (Gold et al., 2022). We did so at the cost of obtaining many less accurate eDNA assignments, which we inspected carefully, also due to shortcomings of taxonomic assignment algorithms (Garrido-Sanz et al., 2022; Somervuo et al., 2017). Our taxonomic assignments derived from eDNA are likely also influenced by transport and diffusion phenomena of suspended genetic material in the water column, when compared to other observation methods—marine eDNA can be transported over distances ranging to tens of kilometers and can persist for up to 2 weeks at low temperatures, with transport and diffusion playing a role in detectability (Andruszkiewicz et al., 2019; McCartin et al., 2022).

We present a current picture of the fish biodiversity in Te Wāhipounamu and add to a range of recent studies combining eDNA and video footage to survey the biodiversity of coastal marine environments, some of which, like us, solely relying on NCBI reference data (e.g., Cheng et al., 2023; Cole et al., 2021; Jeunen

TABLE 1 Details on taxonomic observations across data sources, and alignment qualities of eDNA assignments.

Class	Order	Family	Genus	Species	Common name	Algn. Covrg.	Algn. Gaps	Algn. Bitsc
Actinopteri	Anguilliformes	Anguillidae	*Anguilla*	*Anguilla australis***	Australian shortfin eel	100%	0	300
		Congridae	*Conger*	*Conger verreauxi*	conger eel			
	Atheriniformes	Atherinidae	*Atherinomorus*	*Atherinomorus lacunosus**	wide-banded hardyhead silverside			
	Blenniiformes	Gobiesocidae	*Gobiesox*	*Gobiesox maeandricus**	northern clingfish	79.3%	2	300
		Tripterygiidae	*Bellapiscis*	*Bellapiscis lesleyae*	mottled twister			
				Bellapiscis medius	twister			
			Cryptichthys	*Cryptichthys jojettae*	cryptic triplefin			
			Forsterygion	*Forsterygion capito*				
				Forsterygion flavonigrum	yellow-and-black triplefin			
				Forsterygion lapillum	common triplefin			
				Forsterygion malcolmi	mottled triplefin			
				Forsterygion maryannae	oblique-swimming triplefin			
				Forsterygion varium	striped triplefin			
			Helcogramma	*Helcogramma striata**	tropical striped triplefin	88.2–92.3%	1	300
			Karalepis	*Karalepis stewarti*	scaly-headed triplefin			
			Notoclinops	*Notoclinops caerulepunctus*	blue dot triplefin			
				Notoclinops segmentatus	blue-eyed triplefin			
			Notoclinus	*Notoclinus compressus*	brown topknot			
				Notoclinus fenestratus	New Zealand topknot			
			Ruanoho	*Ruanoho decemdigitatus*	longfinned triplefin			
				Ruanoho whero	spectacled triplefin			
	Carangiformes	Carangidae	*Trachurus*	*Trachurus japonicus*,***	Japanese jack mackerel	99.4%	0	300
	Centrarchiformes	Aplodactylidae	*Aplodactylus*	*Aplodactylus arctidens*	marblefish			
		Cheilodactylidae	*Cheilodactylus*	*Cheilodactylus variegatus**	Peruvian morwong	97.6–98.8%	0	300
			Goniistius	*Goniistius zonatus**	blackbarred morwong	97.6–98.2%	0	300
			Nemadactylus	*Nemadactylus macropterus*	tarakihi			
		Kyphosidae	*Scorpis*	*Scorpis lineolata*	silver sweep			
		Latridae	*Latridopsis*	*Latridopsis ciliaris*	blue moki			
				Latridopsis forsteri	bastard trumpeter			
			Latris	*Latris lineata*	striped trumpeter			
			Mendosoma	*Mendosoma lineatum*	telescope fish			

(Continues)

TABLE 1 (Continued)

Class	Order	Family	Genus	Species	Common name	Algn. Covrg.	Algn. Gaps	Algn. Bitsc
	Chaetodontiformes	Chaetodontidae	*Chaetodon*	*Chaetodon zanzibarensis**	Zanzibar butterflyfish	81.5%	4	340
	Cichliformes	Cichlidae	*Coptodon*	*Coptodon zillii*	redbelly tilapia	90.6%	3	300
	Clupeiformes	Engraulidae	*Engraulis*	*Engraulis japonicus**,***	Japanese anchovy	98.8%	0	300
	Cypriniformes	Leuciscidae	*Alburnus*	*Alburnus alburnus**	bleak	100%	0	310
	Cyprinodontiformes	Aplocheilidae	*Aplocheilus*	*Aplocheilus lineatus**	striped panchax	81.4%	4	340
	Gadiformes	Gaidropsaridae	*Gaidropsarus*	*Gaidropsarus argentatus**	Arctic rockling	90.1%	1	300
				Gaidropsarus novaezealandiae	New Zealand rockling			
		Merlucciidae	*Macruronus*	*Macruronus novaezelandiae****	hoki, blue grenadier	100%	0	300
		Moridae	*Lotella*	*Lotella phycis*	Beardie	95.9%	0	300
				Lotella rhacina	rock cod			
			Pseudophycis	*Pseudophycis barbata**	southern bastard codling			
	Galaxiiformes	Galaxiidae	*Galaxias*	*Galaxias argenteus*	giant kōkopu			
				Galaxias eldoni	Eldon's galaxias	98.2%	0	300
	Gobiesociformes	Gobiesocidae	*Modicus*	*Modicus minimus*	small clingfish			
				Modicus tangaroa	eyespot clingfish			
	Gobiiformes	Eleotridae	*Bostrychus*	*Bostrychus zonatus**	barred gudgeon	85.4–86%	5	300
		Gobiidae	*Asterropteryx*	*Asterropteryx semipunctata**,***	starry goby	81.9–82.5%	6	300
			Gobiopsis	*Gobiopsis atrata*	New Zealand black goby			
		Thalasseleotrididae	*Thalasseleotris*	*Thalasseleotris iota*	New Zealand pygmy sleeper			
	Labriformes	Labridae	*Bodianus*	*Bodianus unimaculatus*	red pigfish			
			Notolabrus	*Notolabrus celidotus*	New Zealand spotty			
				Notolabrus cinctus	girdled wrasse			
				Notolabrus fucicola	yellow-saddled wrasse			
			Pseudolabrus	*Pseudolabrus miles*	Scarlet wrasse			
		Odacidae	*Odax*	*Odax pullus****	greenbone			
	Lophiiformes	Lophiidae	*Lophiodes*	*Lophiodes mutilus*	smooth goosefish	86.9%	1	290
	Lutjaniformes	Haemulidae	*Conodon*	*Conodon nobilis**	barred grunt	86.5%	3	300
	Mugiliformes	Mugilidae	*Aldrichetta*	*Aldrichetta forsteri*	yellow-eye mullet	96–100%	0	300–350
	Myctophiformes	Myctophidae	*Gymnoscopelus*	*Gymnoscopelus nicholsi**	Nichol's lanternfish	79.8%	1	350
			Hygophum	*Hygophum hygomii*	Bermuda lantern fish	78.7%	3	340
	Ophidiiformes	Bythitidae	*Fiordichthys*	*Fiordichthys slartibartfasti*	Fiordland brotula			

TABLE 1 (Continued)

Class	Order	Family	Genus	Species	Common name	Algn. Covrg.	Algn. Gaps	Algn. Bitsc
	Osmeriformes	Retropinnidae	*Retropinna*	*Retropinna retropinna*	cucumberfish			
	Ovalentaria	Plesiopidae	*Acanthoclinus*	*Acanthoclinus fuscus*	olive rockfish			
				Acanthoclinus littoreus	New Zealand rockfish			
				Acanthoclinus marilynae	Stout rockfish			
				Acanthoclinus matti	New Zealand longfin			
				Acanthoclinus rua	little rockfish			
	Pempheriformes	Percophidae	*Hemerocoetes*	*Hemerocoetes monopterygius*	opalfish			
		Polyprionidae	*Polyprion*	*Polyprion oxygeneios*	hāpuku			
	Perciformes	Bovichtidae	*Bovichtus*	*Bovichtus angustifrons**	horny thornfish	95.3%	0	300
				Bovichtus variegatus	thornfish			
		Callanthiidae	*Callanthias*	*Callanthias allporti*	splendid sea perch			
				Callanthias japonicus,***	yellowsail red bass	95.2%	1	290
		Nototheniidae	*Notothenia*	*Notothenia angustata*	Maori chief			
		Scorpaenidae	*Scorpaena*	*Scorpaena cardinalis*	red rock cod			
				Scorpaena papillosa	red scorpionfish			
		Sebastidae	*Helicolenus*	*Helicolenus hilgendorfii**	Hilgendorf's saucord	95.4%	0	340
				*Helicolenus percoides**	red gurnard perch			
		Serranidae	*Caesioperca*	*Caesioperca lepidoptera*	butterfly perch			
			Caprodon	*Caprodon schlegelii*,***	sunrise perch	90.6–98.2%	0–2	290–340
			Hypoplectrodes	*Hypoplectrodes huntii*	redbanded perch			
			Lepidoperca	*Lepidoperca tasmanica*	Tasmanian perch			
		Triglidae	*Chelidonichthys*	*Chelidonichthys kumu*	bluefin gurnard			
				*Chelidonichthys spinosus**	red gurnard	99.4%	0	300
	Pleuronectiformes	Rhombosoleidae	*Peltorhamphus*	*Peltorhamphus latus*	speckled sole			
			Rhombosolea	*Rhombosolea plebeia*	New Zealand flounder			
	Salmoniformes	Salmonidae	*Oncorhynchus*	*Oncorhynchus mykiss****	rainbow trout	100%	0	300
	Scombriformes	Gempylidae	*Thyrsites*	*Thyrsites atun*	Snoek, barracouta	99.4%	0	300
		Scombridae	*Katsuwonus*	*Katsuwonus pelamis****	skipjack tuna	95.9%	0	340
			Scomber	*Scomber japonicus**	chub mackerel	100%	0	300
	Stomiiformes	Sternoptychidae	*Maurolicus*	*Maurolicus muelleri*	pennant pearlside	86.3–99.4%	0–10	270–300
	Syngnathiformes	Syngnathidae	*Lissocampus*	*Lissocampus filum*	shortsnout pipefish			

(Continues)

TABLE 1 (Continued)

Class	Order	Family	Genus	Species	Common name	Algn. Covrg.	Algn. Gaps	Algn. Bitsc
	Tetraodontiformes	Monacanthidae	*Meuschenia*	*Meuschenia scaber*	velvet leatherjacket			
			Scobinichthys	*Scobinichthys granulatus**	rough leatherjacket	98.8–99.4%	0	300
	Trachichthyiformes	Monocentridae	*Monocentris*	*Monocentris japonica*	Japanese pineapplefish	94.4–97.6%	2–3	300–340
		Trachichthyidae	*Paratrachichthys*	*Paratrachichthys trailli*	sandpaper fish			
	Uranoscopiformes	Pinguipedidae	*Parapercis*	*Parapercis colias*	New Zealand blue cod			
				*Parapercis decemfasciata**		80.9%	1	300
				Parapercis gilliesii	yellow weaver			
	undefined	Emmelichthyidae	*Erythrocles*	*Erythrocles schlegelii**	Japanese rubyfish	87.2–87.8%	4	340
		Opistognathidae	*Opistognathus*	*Opistognathus iyonis**	well-building jawfish	89.9–90.5%	2–3	290–300
				*Opistognathus liturus**	seto-amadai	89.3–90.5%	2	290
				*Opistognathus punctatus**	finespotted jawfish	81.7%	5	300
				Opistognathus sp.*	jawfish	86.3%	0	290
Chondrichthyes	Carcharhiniformes	Carcharhinidae	*Prionace*	*Prionace glauca*	blue shark			
		Scyliorhinidae	*Cephaloscyllium*	*Cephaloscyllium isabella*	draughtsboard shark			
		Triakidae	*Galeorhinus*	*Galeorhinus galeus*	tope shark			
			Mustelus	*Mustelus asterias**	starry smooth-hound	100%	0	320
				Mustelus lenticulatus	spotted estuary smooth-hound			
	Hexanchiformes	Hexanchidae	*Notorynchus*	*Notorynchus cepedianus****	broadnose sevengill shark	98.4%	0	320
	Lamniformes	Alopiidae	*Carcharodon*	*Carcharodon carcharias*	great white shark			
			Isurus	*Isurus oxyrinchus*	shortfin mako shark			
	Squaliformes	Squalidae	*Squalus*	*Squalus acanthias*	spiny dogfish			
				*Squalus suckleyi**	Puget Sound dogfish	100%	0	320
Myxini	Myxiniformes	Myxinidae	*Eptatretus*	*Eptatretus cirrhatus*	broadgilled hagfish			

Note: Taxonomic hierarchies conform with NCBI taxonomy where available, thus allowing analysis in relation to environmental DNA data, and are sorted alphabetically—the resulting species order is identical to Figure 2. Taxa not listed as New Zealand species by Roberts et al. (2020) are highlighted with asterisk (*). Species detected by our chosen assignment method also detected by MEGAN's LCA (Huson et al., 2007) are highlighted with triple asterisks (***) Trivial names are indicated where available. For all taxonomic assignments also yield from eDNA we provide the alignment coverage, alignment gaps, and bit scores for the on average 170 bp sequence reads. Since identical species were assigned to multiple Amplicon Sequence Variants (ASVs; Callahan et al., 2017) in some instances, ranges are provided for alignment coverages, gap counts, and bit scores for species-specific alignments.

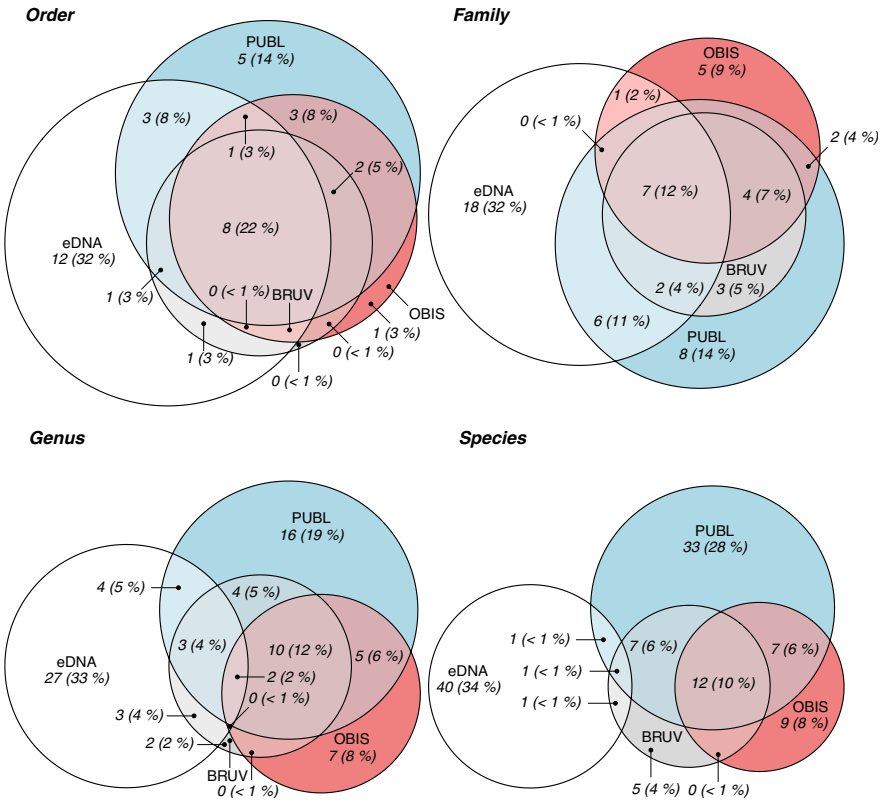

FIGURE 3 Concordance of taxonomic information across four evaluated data sources of Fiordland fish biodiversity expressed through Euler diagrams. Biodiversity data (Table 1; Table S2) is summarized at four different taxonomic levels, shown are unique observation counts at each level, as well as the corresponding percentage of those counts in comparison to all data. Circle sizes are proportional to observation count. Observation types: BRUV (gray)—Observations from baited remote underwater surveys; eDNA (white)—environmental DNA observations; OBIS (red)—data retrieved from the Ocean Biodiversity Information System (https://obis.org/) for the area surrounding field work sites (large circle in Figure 1); PUBL (blue)—Summarized (Inglis et al., 2008) Fiordland fish species collated from multiple literature records. Graph created using R package *eulerr (6.1.0)*.

et al., 2020), others readily using purposefully generated reference data (e.g., Gold et al., 2023; Stoeckle et al., 2020). Unsurprisingly, we show that eDNA is most useful in detecting species if reference data are available (Gold et al., 2023; Stoeckle et al., 2020; reviewed by Taberlet et al., 2018b), and that the detrimental effect of missing reference data is pronounced in remote locations, and when attempting surveys at the species level. Without those matching reference data to assign ASVs, we show (comparable to Andrés et al., 2023; Czechowski et al., 2021), that eDNA can still help describe the extent of "hidden" biodiversity, here of local water samples from Fiordland, even with incomplete reference data.

Arguably, any detected effect of lacking reference data may have been less pronounced by using another, or multiple primer pairs. For example, some studies suggest that the MiFish primer set provides poor taxonomic resolution and a low success rate in species recovery (Jackman et al., 2021), especially for endemic species (Duhamet et al., 2023) while other studies highlight its usefulness in ecosystem conservation strategies, enhanced taxonomic resolution, and efficient fish biodiversity monitoring (Miya et al., 2015; Schroeter et al., 2020; Zhu & Iwasaki, 2023). Furthermore, our primer evaluations with the more recently released software GAPeDNA (Marques et al., 2021) (released after our experiments were completed) show that, for example, the "Fish 16S" primer set (McInnes et al., 2017) would have covered 249 instead of the 119 New Zealand marine fish species covered by our MiFish 12S data (Table S1). However, the overall conclusion remains. Of the over 1294 known New Zealand marine fish species, molecular reference data of any kind are available only for 489 species in southern New Zealand, no available primer pairs have sufficient reference data, and the employed 12S marker is among the most popular for fish species assignment (Claver et al., 2023).

How credible are eDNA-derived species assignments with currently available reference data in combination with the currently used primer set? Overall, they do not seem very credible. For instance, *Alburnus alburnus* is a freshwater species, so a perfect alignment to data from Te Wāhipounamu is puzzling, as there are no known native carp species in New Zealand (Brumley, 1991). Yet, based on sequence similarity this observation can be easily related to *Scomber* (mackerel). But the observed perfect alignment to *Scomber japonicus* is also awkward, with this species not having been observed in New Zealand. Consequently, we believe both observations of *Alburnus* and *Scomber* belonging to either *Katsuwonus pelamis* (skipjack tuna; known from New Zealand) or *Thyrsites atun* (snoek, barracouta; known from the region), despite the former not receiving a perfect alignment in our reference data. The perfect alignment for *O. mykiss* (rainbow trout) is puzzling, as this species is known only from freshwater. For this reason, after inspecting related assignments, we also do not believe a somewhat more plausible assignment to endangered *Galaxias eldoni* to be credible (Eldon's galaxias, freshwater only). Yet, juveniles of threatened species *Galaxias argenteus* (Giant kōkopu; IUCN, 2014) spend time at sea before migrating to fresh water, hence we believe to have found DNA traces of this species. Lastly, some of our shark identifications remain questionable, assignments to *Mustelus asterias* (starry smooth hound) and *Squalus suckleyi* (Puget Sound dogfish) are likely misclassifications of local *Mustelus* and *Squalus* species. We hence believe only the

assignments of *M. novaezelandiae* (hoki) and *A. forsteri* (yellow-eye mullet) to be credible, as these species are known from New Zealand waters. Clearly, species-level identification using eDNA is difficult, even with perfect alignments. We suggest that future marine eDNA surveys in the area should be conducted using more than one marker to be dependable, but with comprehensive reference data for all markers.

Concerning our most reliable taxonomic assignments, we could find no mention of *A. australis* (short-finned eel), and *M. novaezelandiae* (Hoki) in the Te Wāhipounamu-*specific literature; consequently, available reference data of these taxa ought to be added to local sequence data repositories. On the contrary, we found literature mentions of Gobiopsis atrata* (Ayling & Cox, 1982), and recently described *Thalasseleotris iota* (New Zealand pygmy sleeper; Hoese & Roberts, 2005) for Te Wāhipounamu, and we suspect that our imperfect assignment of *Asterropteryx semipunctata* (Starry goby)—itself known from the northern coasts of Australia (Allen et al., 1997) and elsewhere—stems from one of these two species (for a recent multi-locus phylogeny see Gierl et al., 2022). Taxa only detected by BRUV should be added to eDNA sequences collections as well (*B. unimaculatus*, *C. kumu*, *G. galeus*, *M. lenticulatus*, *S. cardinalis*). *Notorynchus cepedianus* (broadnose sevengill shark) and *Eptatretus cirrhatus* (broadgilled hagfish) seen on BRUV and by eDNA, both ought to be added to the list of local species, with reference data available.

Our primers detected marine mammals, which are gnathostomes (jawed vertebrates) just as well as cartilaginous and ray-finned fishes (Meyer & Zardoya, 2003). Detection of those taxa and a slime eel indicate that MiFish primers (Miya et al., 2015) amplify a region conserved across many gnathostomes. We show that prominent species, of high interest to conservation efforts (including whales and bottlenose dolphins; e.g., Currey et al., 2009; Zhang et al., 2023) can be detected with eDNA in Te Wāhipounamu (Figure S6), and reassuringly we observed bottlenose dolphins during field work.

As outlined by Hleap et al. (2021), achieving "exact" taxonomic assignments on the species level is challenging due to the dynamic conceptual and genetic nature of the species concept. Not only will species always have an undiscovered genetic diversity, but reference data annotations must be frequently updated. Accordingly, we demonstrate that even perfect alignments, obtained using two different taxonomic assignment approaches, do not always yield correct assignments (e.g., *A. albunus*, *O. mykiss*), and that imperfect alignments in some instances yield more conceivable taxonomic assignments (e.g., *K. pelamis*). Hence, we here only consider taxonomic assignments to *M. novaezelandiae*, *A. australis*, *N. cepedianus*, and *E. cirrhatus* reliable, as they were observed by two taxonomic assignment approaches, and are known from the region.

Logistic regression indicates taxonomic assignments and alignment qualities to be most impeded by missing reference data for New Zealand and Te Wāhipounamu, and less so by DNA decay (Sassoubre et al., 2016) or other observation biases. This may explain why lacking query coverage was so clearly associated with observing species not from New Zealand. Likewise, increasing gap count at constant query coverage supports a lack of local reference data availability.

Compared to eDNA data, species identification from BRUV data appeared more precise but less exhaustive, with a significantly lower number of species detected data (26). This lower species count is likely a result of not all fish species being attracted to the bait used, and transport and diffusion phenomena of eDNA in the water column (Andruszkiewicz et al., 2019; McCartin et al., 2022).

5 | SUMMARY AND CONCLUSIONS

Our study provides a comparison of environmental DNA and more traditional marine biodiversity survey tools. We show that eDNA analysis is a highly sensitive tool with strong potential for biodiversity surveys, which is, however, still limited by the availability of local reference data, particularly in more remote regions of the world. We hope that by demonstrating the extend of divergence between eDNA and more traditional tools in a real-life case study of a remote region of high conservation interest, we further strengthen the case to fund the establishment of local reference databases, also to benefit the UNESCO Word Heritage Site Te Wāhipounamu.

AUTHOR CONTRIBUTIONS

Authorship assignment followed the Contributor Roles Taxonomy (https://casrai.org/credit). Conceptualization: MK, ML, and PC; Data Curation and Formal Analysis: PC, MH, and MdL; Funding Acquisition: MK and ML; Investigation: PC, AK, MH, ML, and MdL; Methodology: PC, MH, and MK; Project Administration: MK, ML, and PC; Resources: CH, WR, and MH; Software: PC; Supervision: MK and PC; Validation: MdL, WP, and CH; Visualization: PC and MdL; Writing—Original Draft: PC; Writing—Review & Editing: all authors.

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CONFLICT OF INTEREST STATEMENT

The authors do not declare any conflicts of interest.

DATA AVAILABILITY AND BENEFIT-SHARING STATEMENT

The researchers who participated in this eDNA survey and analyses were included as co-authors, data have been shared with all co-authors prior to publication, and research data and findings are being made publicly available to enhance our collective global capacity to mitigate the harms of biodiversity loss. Data associated with this

publication are permanently stored online: Czechowski et al. (2021). Comparison of traditional and molecular surveys of fish biodiversity in southern Te Wāhipounamu / Fiordland (Aotearoa / New Zealand). Zenodo. https://doi.org/10.5281/zenodo.4638297 (always linking to latest version).

ORCID

Paul Czechowski https://orcid.org/0000-0001-7894-4042

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SUPPORTING INFORMATION

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