1   Supplementary information*

2   # Environmental DNA needs reference data for taxonomy-based

3   # conservation policy – A case study from Te Wahipounamu,

4   # Aotearoa / New Zealand

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6   October 22, 2021

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*Please also consult the supplementary online materials at https://doi.org/10.5281/zenodo.4638297 for tables, figures, code, and data.

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1   Materials and methods

Data collation and analysis

All analyses were conducted in R (R Core Development Team, 2019) and Megan (Huson et al., 2016) of increasing versions. Packages used for analysis in R included *curl, data.table, decontam, dplyr, flextable, future.apply, ggpubr, grid, gridExtra, indicspecies, janitor, knitr, magick, magrittr, officer, openxlsx, phyloseq, primerTree, readxl, reshape2, robis, sf, sjPlot, sp, taxize, taxonomizr, tidyr, tidyverse, vegan* and *xaringan* (Auguie, 2017; Bache and Wickham, 2020; Bengtsson, 2021; B. Callahan and Davis, 2021; Chamberlain et al., 2020; Dowle and Srinivasan, 2021; Firke, 2021; Gohel, 2021a; Gohel, 2021b; Hester, 2020; Kassambara, 2020; Lüdecke, 2021; McMurdie et al., 2021; Oksanen et al., 2015; Ooms, 2021a; Ooms, 2021b; Pebesma, 2021; Pebesma and Bivand, 2021; Provoost and Bosch, 2021; Schauberger and Walker, 2021; Sherrill-Mix, 2021; Wickham, 2020; Wickham, 2021a; Wickham, 2021b; Wickham and Bryan, 2019; Wickham, François, et al., 2021; Xie, 2021a; Xie, 2021b).
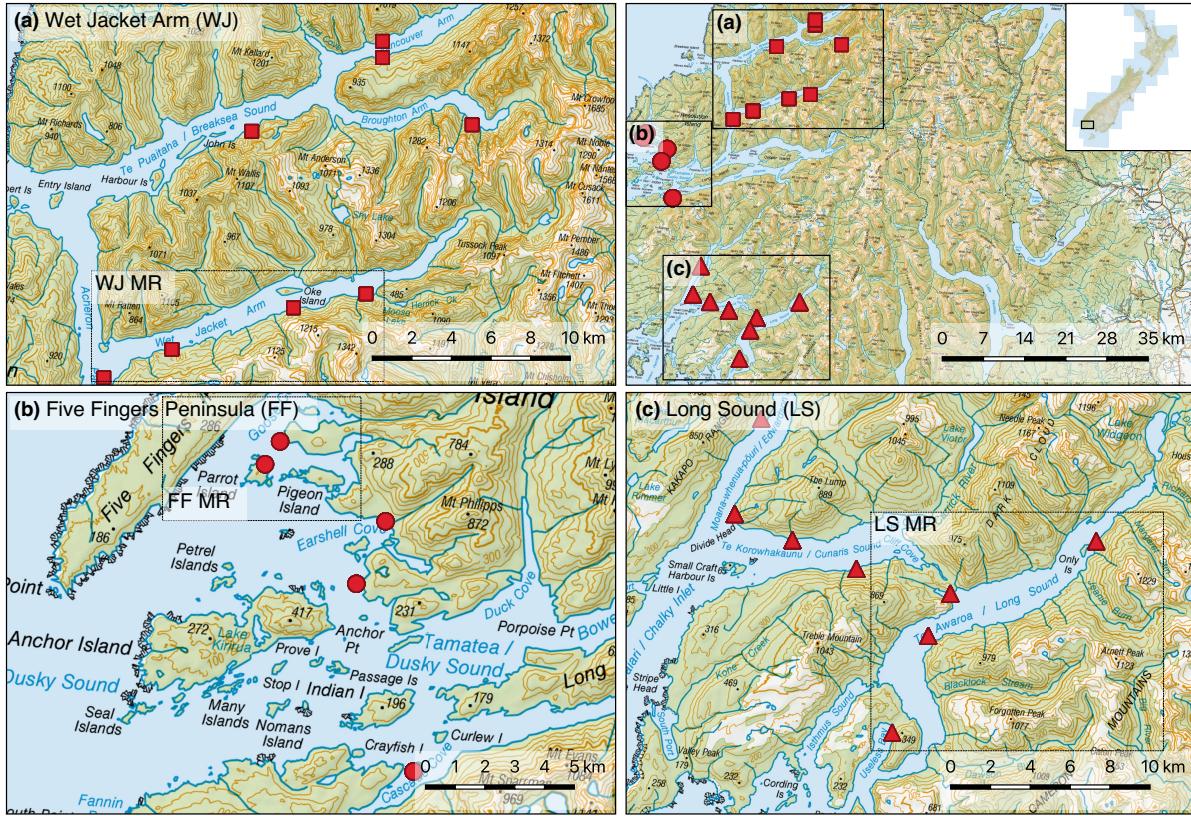
Figure 1: Sampling locations for BRUV and eDNA data collection in Te Wahipounamu, New Zealand. We analyzed species observations from 21 locations. Within each of the three sampling regions (WJ, FF, and LS), samples collected inside MRs are delineated by hatched rectangles, control samples are outside of rectangles. Base layer sourced from the LINZ Data Service licensed for reuse under CC BY 4.0.

Field work

To obtain eDNA at each sampling location (Fig.1), duplicates of 900 ml water portions were collected using a bleached Niskin Bottle, passed through separate 0.22 µm Sterivex columns (Merck, US-NJ) using sterile disposable 50 ml syringes, akin to Jeunen et al. (2020). To control contamination, negative controls were obtained after every second sample by passing molecular grade distilled water through third columns. After filtering, columns were filled with 5 ml Longmire's solution, sealed, and stored at 4°C for 21 days and -20°C subsequently (Majaneva et al., 2018).

BRUV footage was obtained for one hour using two waterproof cameras, recording at 1080p resolution, at 60 frames per second, 40 cm apart, at a converging angle of 8°, aided by two 300 lm lights. We filmed a permeable plastic container suspended 1.2 m below the cameras, carrying 500g bait *(Sardinops sagax)*, akin to (Jeunen et al., 2020). Video footage were analyses by eye, taxonomic observations were formalized for further analysis using the NCBI taxonomy database (Federhen, 2012) and R package *taxonomizr* (Sherrill-Mix, 2021).

(a) Mifish U: ray-finned fish, tetrapods and lungfish.

(b) Elas02: cartilaginous and ray-finned fish, tetrapods, and lungfish
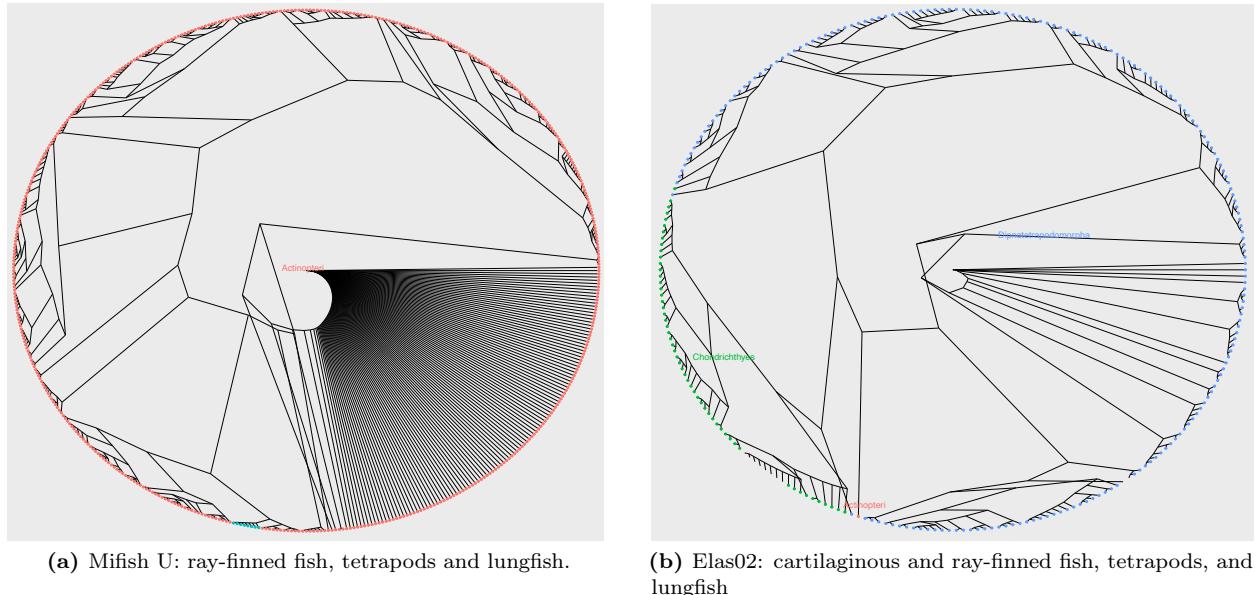
Figure 2: *In silico* PCR of used primer pairs: Likely amplification specificity of Mifish U and Elas02.

Primer selection and *in silico* PCR

Primer selection was verified using GAPeDNA v.1.0.1 (https://shiny.cefe.cnrs.fr/GAPeDNA/; Marques et al. 2021) and Scite (Nicholson et al., 2021). We assed our primer selection by initially evaluating online information provided by GAPeDNA with regards to reference data coverage for marine fish in the southern New Zealand province for all primer pairs listed. Subsequently, we retrieved the number of publications referencing the work introducing the primers.

  In silico PCR of primers "MiFish-U" (Miya et al., 2015) and "Elas02" (Taberlet et al., 2018) was performed with R package *primerTree* (Hester, 2020) with default parameters (500 alignments, three possible mismatches).

Environmental DNA data processing

Amplicon Sequence Variants (ASV's *sensu* B. J. Callahan, McMurdie, and Holmes 2017) were generated from raw sequence data including use of Qiime v2 2020-08 (Bolyen et al., 2019). Initially, sequence quality was checked with FastQC (Andrews, 2010) as called by MultiQC (Ewels et al., 2016). We then used Cutadapt v3.0 (Martin, 2011) to deconvolute samples, while not allowing for any mismatches, and choosing an Expected Error value (Edgar and Flyvbjerg, 2015) of zero, to obtain high quality data.

  Deconvoluted data were imported into Qiime and denoised (Rosen et al., 2012) using the DADA2 algorithm (v1.10.0; B. J. Callahan, McMurdie, Rosen, et al. 2016). Taxonomic annotation of denoised sequence data was obtained using Blast 2.10.0+ (Camacho et al., 2009) and a recent copy (April 2020) of the NCBI nucleotide collection (Benson et al., 2011) excluding environmental samples, while requiring a minimum identity 75%, a minimum query coverage of 95% and an e-value of 10^{-10}, retaining the first high-scoring alignment of each query-subject pair based on Bit-score (see Fig. 3 on the next page for raw data summary). To mitigate impact
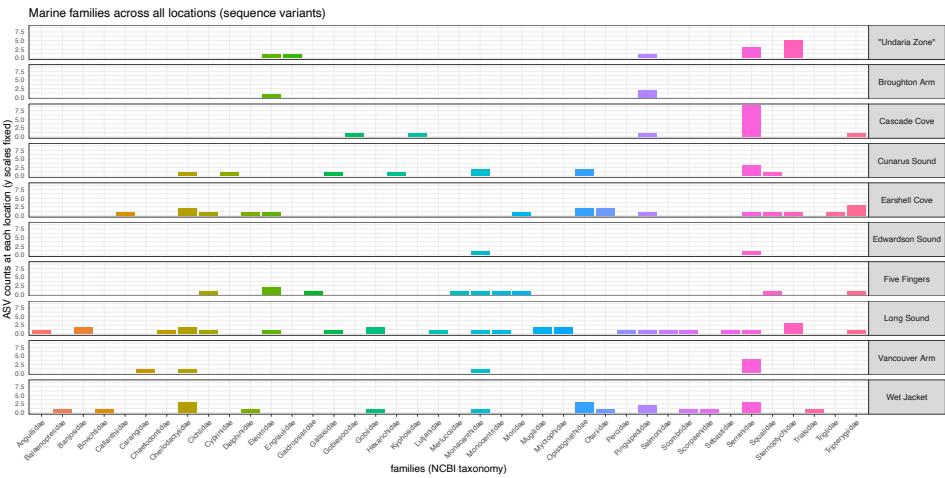
Figure 3: Taxonomic assignments for all sample types among raw data, prior to filtering. `blank`: field water controls, `eDNA`: study observations, `nctrl-pcr`: PCR negative controls, `nctrl-xtr`: Extraction negative controls, `pcntrl-blnd`: positive controls.

of minor cross-contamination or tag jumping in our data (Schnell et al., 2015), as detected using our positive controls, we analyzed and subtracted negative control data from all samples before statistical analysis, which included using R package *decontam* (B. Callahan and Davis, 2021), and removing ASVs with covered by less the 15 reads each.

For statistical analysis, taxonomic observations from eDNA were formalized with R packages *taxize* and *taxonomizr* (Chamberlain et al., 2020; Sherrill-Mix, 2021) akin to BRUV, OBIS, and literature data. After inspection of all eDNA and BRUV derived species assignments, eDNA species lists were limited to classes Actinopteri and Chondrichthyes, and all other data was deemed irrelevant for comparison with BRUV observations (crustaceans, mollusks, and hagfish seen on BRUV were absent from eDNA data prior to data filtering, keeping in mind the amplification profile shown in Fig. 2 on the preceding page).

Indicator species analysis

To find species that could be used as ecological indicators of associated ANOSIM-distinguished community or habitat types, we used indicator species analysis (De Cáceres et al., 2010) on the data set used for ANOSIM.

Binomial regression

To further test the credibility of eDNA information test and whether incompletely resolved ("cryptic") eDNA ASVs inflated the number of species observed across multiple locations during ANOSIM, we used binomial regression. The response variable was non-native status of eDNA detected species (CD Roberts et al., 2019). Predictor variables were the number of alignment gaps (integer), and alignment query coverage (percentage). The number of trials was defined using by numbers of locations at which each assigned species was seen.

Table 1: Evaluation of primer selection through GAPeDNA v.1.0.1 (https://shiny.cefe.cnrs.fr/GAPeDNA/; Marques et al. 2021). Listed is reference data coverage for marine fish in the southern New Zealand province as provided by the web tool, and citation count of primer references. GAPeDNA lists 489 sequenced species for southern New Zealand (11-Sep-2021). Citation counts obtained 13-Sep-2021. Total distinct citations as estimated by Scite (Nicholson et al., 2021).

Primer region	primer	species sequenced	reference	citations
12S	ACMDB	27% (134)	Bylemans et al. 2018	45
	Ac12S	29% (141)	Evans et al. 2016	229
	Am12S	25% (124)	Evans et al. 2016	229
	-	31% (154)	Kelly et al. 2014	263
	MiFish-U	24% (119)	Miya et al. 2015	378
	Teleo	24% (117)	Valentini et al. 2016	545
16S	-	51% (249)	DiBattista et al. 2017	42
	Ac16S	19% (95)	Evans et al. 2016	229
	Ve16S	36% (177)	Evans et al. 2016	229
	-	29% 144	Kitano et al. 2007	148
	Fish	51% (249)	McInnes et al. 2017	23
	ar / br	21% (101)	Palumbi 1996	*undeterminable*
	-	51% (249)	Shaw et al. 2016	141
COI	coi1	6% (29)	Ivanova et al. 2007	877
	vf1d	16% (80)	Ivanova et al. 2007	877
	F1	10% (48)	Ward et al. 2005	2 327
	F2	6% (30)	Ward et al. 2005	2 327
CytB	-	13% (66)	Kocher et al. 1989	3 536
	-	17% (85)	*M. Miya - undeterminable*	*undeterminable*
	2cb	8% (41)	Thomsen et al. 2012	521
	2de	15% (74)	Thomsen et al. 2012	521
	cb	7% (36)	Thomsen et al. 2012	521
18S	-	2% (11)	*J. McDondald - undeterminable*	*undeterminable*

2 Results

Primer selection and *in silico* PCR

Results of our primer assessment are shown in Table 1. *In silico* analyses found the employed primers to amplify some tetrapods as shown in Fig. 2 on page 4.

Environmental DNA data processing

The denoised, quality-filtered sequenced data consisted of 3 877 007 sequences across 125 samples and 2 139 ASV's (436 Eukaryota, as well as 1 703 non-eukaryote, i.e. Bacteria, Viruses, and undefined taxa; furthermore taxa across 5 super-phyla, 31 phyla, 56 classes, 151 orders, 254 families, 426 genera, and 914 species). Sample mean coverage was 31 016 reads (min.: 1, med.: 2 001, max.: 272 614, standard deviation 54 592), and ASV mean (min, median, max) coverage was 1 812 reads (min.: 1, med.: 18, max.: 803 140, standard deviation 24 270).

Notable contaminants most likely introduced during water filtering (and subsequently removed) included *Homo sapiens* (human, 30 1070 reads), and *Cervus elaphus* (red deer, 19 372 reads). Low abundant ASVs
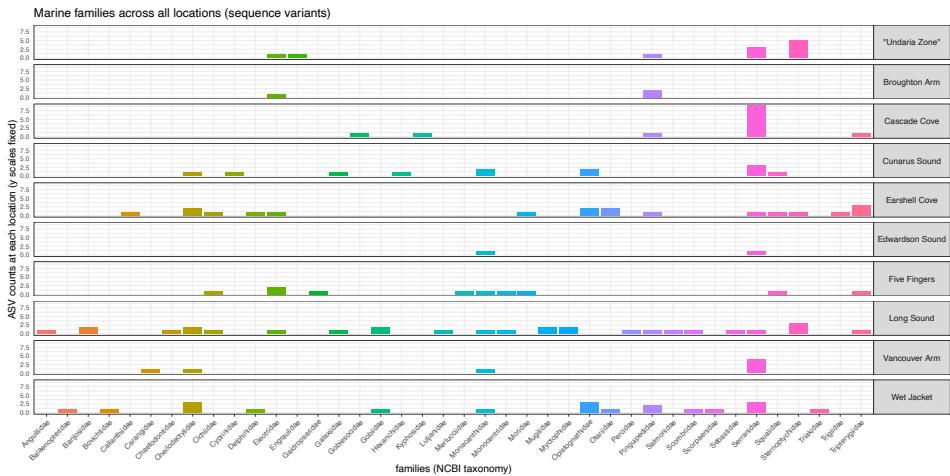
Figure 4: Family level assignments and ASV counts among clean data. For location names refer to Fig. 1 on page 3. For species level assignments refer to Table 4 on page 17. For highly highly detailed information refer to the complete study data provided as supplemental online material.

removed as possible cross-contamination not associated with humans included *Trichosurus vulpecula* (bush-tail possum, 16 reads), and *Sus scrofus* (wild boar, 11 reads). We deem the contaminant profile to be very similar to a study conducted in Stewart island (southern New Zealand) with the same primers (Jeunen et al., 2020).

After completed filtering sequence data contained 157 100 sequences across 43 samples and 96 ASV's (96 Eukaryota; including taxa across one super-phylum, 1 phylum, 3 classes, 28 orders, 40 families, 43 genera, and 47 species; also see Fig. 4. Sample mean coverage was 3 353 reads (min.: 32, med.: 1 504, max.: 49 976, standard deviation was 7 940), and ASV mean coverage was 1 636 reads (min.: 17, med.: 304, max.: 24 182, standard deviation 3 793).

Three (ray-finned) fishes not identifiable from video footage had been removed from the final species observation list. NCBI taxonomy did not contain entries for five video-observed species *(Forsterygion maryannae, Cephaloscyllium isabellum, Hypoplectrodes huntii, Notolabrus cinctus, Eptatretus cirrhatus)*.

Summaries of taxonomic observations

A summary of observations across data sources is listed in Table 4 on page 17. From Grange (1985), Inglis et al. (2008), Mladenov (2001), Clive Roberts (2005), and Wing and Jack (2013) we included into our analysis the species *Acanthoclinus fuscus, Acanthoclinus littoreus, Acanthoclinus marilynae, Acanthoclinus matti, Acanthoclinus rua, Aldrichetta forsteri, Aplidium adamsi, Aplodactylus arctidens, Aplodactylus arctidens, Atherinomorus lacunosus, Bellapiscis lesleyae, Bellapiscis medius, Bovichtus variegatus, Caesioperca lepidoptera, Callanthias allporti, Cephaloscyllium isabellum, Cominella sp., Conger verreauxi, Cryptichthys jojettae, Eptatretus cirrahtus, Fiordichthys slartibartfasti, Forsterygion flavonigrum, Forsterygion lapillum, Forsterygion malcolmi, Forsterygion varium, Gaidropsarus novaezelandi, Modicus minimus, Gobiopsis atrata, Grahamina capito, Helicolenus percoides, Hypoplectrodes huntii, Karalepis stewarti, Latridopsis ciliaris, Latridopsis forsteri, Latris lineata, Lepidoperca tasmanica, Lissocampus filum, Lotella rhacina, Mendosoma lineatum, Modicus tangaroa, Nemadactylus macropterus, Notoclinops caerulepunctus, Notoclinops segmentatus, Notoclinus fenestratus, Notolabrus celidotus, Notolabrus cinctus, Notolabrus fucicola, Forsterygion maryannae,*

Table 2: Observation methods across literature sources and OBIS: Species observations listed in CD Roberts et al. (2019) for all of New Zealand were not used as part of the literature corpus analyses of Te Wahipounamu, but only to confirm native status of eDNA assignments.

Data source	Observation methods
Inglis et al. 2008	literature survey, poison stations and beach seine netting
Grange (1985)	unknown
Mladenov (2001)	unknown
Clive Roberts (2005)	Rotenone ichthyocide, spear gun, fishing with baited lines
Wing and Jack (2013)	diving surveys for conspicuous reef fish
OBIS	6 192 human observations, 62 machine observations, 59 preserved specimen
CD Roberts et al. (2019)*	voucher specimen described by taxonomic ichthyologist, validated species from literature, clear published account

Odax pullus, Parapercis colias, Parapercis gilliesii, Paratrachichthys trailli, Meuschenia scaber, Patiriella regularis, Polyprion oxygeneios, Pseudolabrus miles, Pseudophycis barbata, Retropinna retropinna, Rhombosolea plebeia, Ruanoho decemdigitatus, Ruanoho whero, Scorpaena papillosa, Scorpis lineolata, Squalus acanthias, and *Thyrsites atun.* Observation methods for these species are listed in Table 2.

Notable taxa observed during environmental DNA (eDNA) processing included[1][2]

- in field controls: *Homo sapiens*♠, *Cervus elaphus*♠, *Serranidae* sp., *Poecilia reticulata*♣, *Pictilabrus laticlavius**

- in positive controls: *Poecilia reticulata*♣, *Chromobotia macracanthus**, *Poecilia latipinna*♣, *Poecilia formosa*♣, *Corydoras aeneus**

- in negative controls: *Homo sapiens*♠, *Pictilabrus laticlavius**, *Poecilia latipinna*♣

Actinopteri obtained through eDNA analysis and video footage included:

- in reserves, but not outside: *Anguilla australis, Banjos banjos, Benitochromis finleyi, Bovichtus diacanthus, Chaetodon zanzibarensis, Gaidropsarus argentatus, Gymnoscopelus nicholsi, Helicolenus hilgendorfi, Hygophum hygomii, Katsuwonus pelamis, Lutjanus sanguineus, Macruronus novaezelandiae, Monocentris japonicus, Notolabrus fucicola, Oncorhynchus mykiss, Sander lucioperca, Scomber japonicus, Scorpaena cardinalis, Scorpaena pepo*

- outside reserves, but not inside: *Aplodactylus arctidens, Callanthias japonicus, Chelidonichthys kumu, Chelidonichthys spinosus, Engraulis japonicus, Forsterygion maryannae, Gobiesox maeandricus, Hypoplectrodes huntii, Latridopsis ciliaris, Microcanthus strigatus, Odax pullus, Opistognathus punctatus, Opistognathus sp., Phoxinus sp., Pseudophycis barbata, Thyrsites atun, Trachurus japonicus*

[1] observations ordered by descending read abundance

[2] Taxon lists follow NCBI taxonomy (Federhen, 2012). Genera and species are known in Aotearoa / New Zealand (CD Roberts et al., 2019) unless indicated with an asterisk ("*") or not a "fish" ("♠"), or freshwater taxa ("♣").
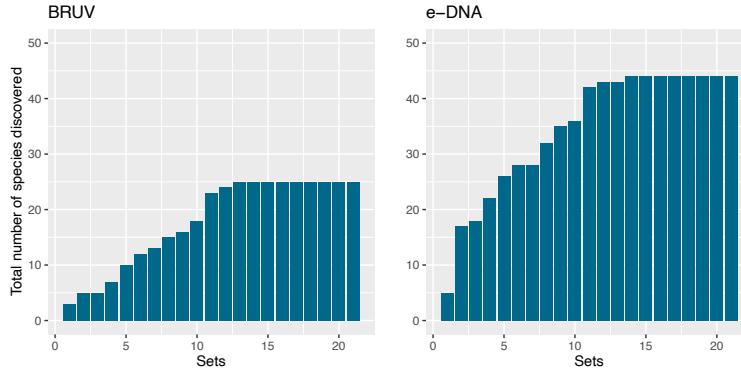
Figure 5: Species accumulation curves for BRUV and eDNA

- both inside and outside marine reserves: *Aldrichetta forsteri, Asterropteryx semipunctata, Bodianus unimaculatus, Bostrychus zonatus, Caesioperca lepidoptera, Caprodon schlegelii, Cheilodactylus variegatus, Cheilodactylus, zonatus, Coptodon zillii, Galaxias sp.* ('southern'), *Helcogramma striata, Helicolenus percoides, Lotella phycis, Lotella rhacina, Maurolicus muelleri, Meuschenia scaber, Nemadactylus macropterus, Notolabrus celidotus, Notolabrus cinctus, Opistognathus iyonis, Opistognathus liturus, Parapercis colias, Parapercis decemfasciata, Pseudolabrus miles, Scobinichthys granulatus*

Species accumulation curves

Species accumulation curves for BRUV and eDNA data are shown in Fig. 5.

DNA sequence alignments

Amplicon sequence variants affected by gaps in alignments for taxonomic assignments included, in part, those belonging to families Callanthiidae, Chaetodontidae, Cichlidae, Eleotridae, Gaidropsaridae, Gobiesocidae, Gobiidae, Kyphosidae, Lutjanidae, Monocentridae, Myctophidae, Opistognathidae, Percidae, Pinguipedidae, Serranidae, Sternoptychidae, and Tripterygiidae.

Amplicon sequence variants affected by imperfect query coverage included, in part, those belonging to families Anguillidae, Banjosidae, Bovichtidae, Callanthiidae, Carangidae, Chaetodontidae, Cheilodactylidae, Cichlidae, Cyprinidae, Eleotridae, Engraulidae, Gaidropsaridae, Galaxiidae, Gobiesocidae, Gobiidae, Hexanchidae, Kyphosidae, Lutjanidae, Merlucciidae, Monacanthidae, Monocentridae, Moridae, Mugilidae, Myctophidae, Opistognathidae, Percidae, Pinguipedidae, Salmonidae, Scombridae, Scorpaenidae, Sebastidae, Serranidae, Squalidae, Sternoptychidae, Triakidae, Triglidae, and Tripterygiidae.

Alignments for *Arctocephalus forsteri* (New Zealand fur seal), *Balaenoptera musculus* (blue whale), and *Tursiops truncatus* (bottlenose dolphin) are shown in Fig. 6 on the following page.

ANOSIM results

ANOSIM results are summarized in Table 3 on page 11.

```
750_12S_single_end_ee3-seq_blast-noenv.xml.rma6
  Taxonomy
    Arctocephalus forsteri [2]
      0daa4a78c7301bf9aeff406aca8b3c5e [length=168, matches=5]
        DATA[length=168]
        Arctocephalus forsteri; score=304.0
            >Arctocephalus forsteri isolate NZFS28 mitochondrion, complete genome gi|1001823670|gb|KT693360.1| acc|KT693360
                    Length = 16568

             Score = 304 bits (336), Expect = 0
             Identities = 168/168 (100%), Gaps = 0/168 (0%)
             Strand = Plus / Plus

            Query:      1 CACCGCGGTCATACGATTAACCCAAACTAACGGGCCCACGGCGTAAAGCGTGTAAAAGATTTATCAACACTAAAGTTAAAATTTAACCAAGCCGTAAAAAGCCACCGTTAATACAAAATA 120
                          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
            Sbjct:    324 CACCGCGGTCATACGATTAACCCAAACTAACGGGCCCACGGCGTAAAGCGTGTAAAAGATTTATCAACACTAAAGTTAAAATTTAACCAAGCCGTAAAAAGCCACCGTTAATACAAAATA 443

            Query:    121 TACTACGAAAGTGACTTTACTACTTCTGATTACACGATAGCTAAGACC 168
                          |||||||||||||||||||||||||||||||||||||||||||||||
            Sbjct:    444 TACTACGAAAGTGACTTTACTACTTCTGATTACACGATAGCTAAGACC 491
        Arctocephalus forsteri; score=304.0
        Arctocephalus forsteri; score=304.0
        Arctocephalus forsteri; score=304.0
        Arctocephalus forsteri; score=304.0
      d1f91a6b1aedda3bcb1923785e70e170 [length=168, matches=5]
        DATA[length=168]
        Arctocephalus forsteri; score=304.0
            >Arctocephalus forsteri isolate NZFS33 mitochondrion, complete genome gi|1001823726|gb|KT693365.1| acc|KT693365
                    Length = 16569

             Score = 304 bits (336), Expect = 0
             Identities = 168/168 (100%), Gaps = 0/168 (0%)
             Strand = Plus / Plus

            Query:      1 CACCGCGGTCATACGATTAACCCAAACTAACGGGCCCACGGCGTAAAGCGTGTAAAAGATTTATTAACACTAAAGTTAAAATTTAACCAAGCCGTAAAAAGCTACCGTTAATACAAAATA 120
                          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
            Sbjct:    324 CACCGCGGTCATACGATTAACCCAAACTAACGGGCCCACGGCGTAAAGCGTGTAAAAGATTTATTAACACTAAAGTTAAAATTTAACCAAGCCGTAAAAAGCTACCGTTAATACAAAATA 443

            Query:    121 TACTACGAAAGTGACTTTACTACTTCTGATTACACGATAGCTAAGACC 168
                          |||||||||||||||||||||||||||||||||||||||||||||||
            Sbjct:    444 TACTACGAAAGTGACTTTACTACTTCTGATTACACGATAGCTAAGACC 491
        Arctocephalus forsteri; score=304.0
        Arctocephalus forsteri; score=304.0
        Arctocephalus forsteri; score=304.0
        Arctocephalus forsteri; score=300.0
```

(a) *Arctocephalus forsteri*

```
750_12S_single_end_ee3-seq_blast-noenv.xml.rma6
  Taxonomy
    Balaenoptera [1]
      220f8674b8542f842cb3f1cbe568c3e4 [length=170, matches=5]
        DATA[length=170]
        Balaenoptera musculus; score=308.0
            >Balaenoptera musculus mitochondrial DNA complete genome gi|414126|emb|X72204.1| acc|X72204
                    Length = 16402

             Score = 308 bits (340), Expect = 0
             Identities = 170/170 (100%), Gaps = 0/170 (0%)
             Strand = Plus / Plus

            Query:      1 CACCGCGGTCATACGATTAACCCAAATTAATAGAAACACGGCGTAAAGAGTGTTAAGGAGTCTCATAGAATAAAGTCAAACCTTAATTAAGCTGTAAAAAGCCATAATTAAAATTAAGCC 120
                          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
            Sbjct:    743 CACCGCGGTCATACGATTAACCCAAATTAATAGAAACACGGCGTAAAGAGTGTTAAGGAGTCTCATAGAATAAAGTCAAACCTTAATTAAGCTGTAAAAAGCCATAATTAAAATTAAGCC 862

            Query:    121 AAACTACGAAAGTGACTTTAATATGATCTGATCACACGACAGCTAAGATC 170
                          ||||||||||||||||||||||||||||||||||||||||||||||||||
            Sbjct:    863 AAACTACGAAAGTGACTTTAATATGATCTGATCACACGACAGCTAAGATC 912
        Balaenoptera musculus; score=303.0
        Balaenoptera borealis; score=285.0
        Balaenoptera omurai; score=285.0
            >Balaenoptera omurai mitochondrial DNA, complete genome, isolate:NSMT-32992 gi|90265620|dbj|AB201257.1| acc|AB201257
                    Length = 16404

             Score = 285 bits (315), Expect = 0
             Identities = 165/170 (97%), Gaps = 0/170 (0%)
             Strand = Plus / Plus

            Query:      1 CACCGCGGTCATACGATTAACCCAAATTAATAGAAACACGGCGTAAAGAGTGTTAAGGAGTCTCATAGAATAAAGTCAAACCTTAATTAAGCTGTAAAAAGCCATAATTAAAATTAAGCC 120
                          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||||||||  ||||||||||||||||||||||  |||||||||||||
            Sbjct:    322 CACCGCGGTCATACGATTAACCCAAATTAATAGAAACACGGCGTAAAGAGTGTTAAGGAATCACATAAAATAAAGTCAAGCCTTAATTAAGCTGTAAAAAGCCATAATTAAGATTAAGCC 441

            Query:    121 AAACTACGAAAGTGACTTTAATATGATCTGATCACACGACAGCTAAGATC 170
                          ||||||||||||||||||||||||||||||||||||||||||||||||||
            Sbjct:    442 AAACTACGAAAGTGACTTTAATATGATCTGATCACACGACAGCTAAGATC 491
        Balaenoptera omurai; score=285.0
```

(b) *Balaenoptera musculus*

```
750_12S_single_end_ee3-seq_blast-noenv.xml.rma6
  Taxonomy
    Tursiops truncatus [1]
      d26313c10cde039a42c32765437db728 [length=171, matches=5]
        DATA[length=171]
        Tursiops truncatus; score=310.0
            >Tursiops truncatus isolate OM_Tt_109 NADH dehydrogenase subunit 6 (ND6) gene, partial cds; tRNA-Glu gene, complete
            sequence; cytochrome b (CYTB) gene, partial cds; D-loop, tRNA-Phe, 12S ribosomal RNA, and tRNA-Val genes, complete
            sequence; and 16S ribosomal RNA gene, partial sequence; mitochondrial gi|1367973882|gb|MG762991.1| acc|MG762991
                    Length = 4440

             Score = 310 bits (342), Expect = 0
             Identities = 171/171 (100%), Gaps = 0/171 (0%)
             Strand = Plus / Plus

            Query:      1 CACCGCGGTCATACGATTGACCCAAACTAATAGACACCCGGCGTAAAGAGTGTCAAAGAACAATATAAAAATAAAGTCAAACCTTAATTAAGCTGTAAAAAGCCATAATTAAAATTAAGT 120
                          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
            Sbjct:   2968 CACCGCGGTCATACGATTGACCCAAACTAATAGACACCCGGCGTAAAGAGTGTCAAAGAACAATATAAAAATAAAGTCAAACCTTAATTAAGCTGTAAAAAGCCATAATTAAAATTAAGT 3087

            Query:    121 TAAACTACGAAAGTAACTTTACCATAAACTGAATACACGACAACTAAGACC 171
                          |||||||||||||||||||||||||||||||||||||||||||||||||||
            Sbjct:   3088 TAAACTACGAAAGTAACTTTACCATAAACTGAATACACGACAACTAAGACC 3138
        Tursiops truncatus; score=310.0
        Tursiops truncatus; score=310.0
        Tursiops truncatus; score=310.0
        Tursiops truncatus; score=310.0
```

(c) *Tursiops truncatus*

Figure 6: Alignment examples (a) for *Arctocephalus forsteri* (New Zealand fur seal), (b) *Balaenoptera musculus* (blue whale), and (c) *Tursiops truncatus* (bottlenose dolphin), as exported from Megan (Huson et al., 2016).

Table 3: Summary of ANOSIM (Clarke, 1993) results. Results are shown for Jaccard (Jaccard, 1912) distances of fish species observations between field work data sets, based on different grouping variables. Sets aggregated by grouping variables were used for permutation testing. Significant values derived from permutation testing within grouping variable (n = 9 999).

Replication over	Tax. level	Location grouping	Obs. method	ANOSIM R	Significance
SET.ID	SPECIES	RESERVE.GROUP.LOCATION	eDNA	0.113618524	0.1401
	GENUS			0.129317111	0.1066
	FAMILY			0.116758242	0.1349
	ORDER			0.090266876	0.1763
	SPECIES	RESERVE.GROUP.INSIDE		-0.075454545	0.8757
	GENUS			-0.042090909	0.7155
	FAMILY			-0.030363636	0.6528
	ORDER			0.003454545	0.4354
	SPECIES	RESERVE.GROUP.LOCATION	BRUV	0.383437991	0.0005
	GENUS			0.410518053	0.0003
	FAMILY			0.410518053	0.0004
	ORDER			0.289246468	0.0039
	SPECIES	RESERVE.GROUP.INSIDE		0.106272727	0.0758
	GENUS			0.075818182	0.1296
	FAMILY			0.095727273	0.0855
	ORDER			0.064727273	0.1503

Indicator species analysis

Indicator species analysis saw *Bodianus unimaculatus* (red hogfish) non-randomly common at WJ CTRL+WJ MR (stat 0.725, *p* 0.0282). On genus level, *Bodianus* (hogfish) appeared non-randomly at WJ CTRL+WJ MR (statistic 0.725, *p* 0.03). At family level, Labridae (wrasses) appeared associated with FF CTRL+FF MR+WJ CTRL+WJ MR (stat 0.725, *p* 0.0385). At order level Perciformes was common at FF MR+WJ CTRL+WJ MR (statistic 0.699, *p* 0.0301).

Binomial regression

Regression analysis is summarized in Fig. 7 on the following page. Model coefficients are summarized in Fig. 8 on the next page.

References

Andrews, S. (2010). FastQC: A quality control tool for high throughput sequence data.

Auguie, B. (2017). *Gridextra: Miscellaneous functions for "grid" graphics.* R package version 2.3. Retrieved from https://CRAN.R-project.org/package=gridExtra

Bache, S. M., & Wickham, H. (2020). *Magrittr: A forward-pipe operator for r.* R package version 2.0.1. Retrieved from https://CRAN.R-project.org/package=magrittr

Bengtsson, H. (2021). *Future.apply: Apply function to elements in parallel using futures.* R package version 1.8.1. Retrieved from https://CRAN.R-project.org/package=future.apply

Benson, D. a., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Sayers, E. W. (2011). GenBank. *Nucleic acids research, 39,* D32–7. ISBN: 03051048. doi:10.1093/nar/gkq1079
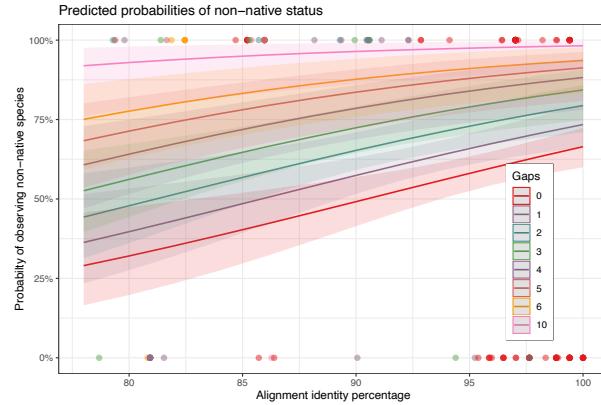
Figure 7: Summary of binomial regression. The response variable was non-native status of eDNA detected species (`TRUE`: eDNA derived species assignment not in CD Roberts et al. (2019), 19 species among 53 ASVs; `FALSE`: 25 species among 39 ASVs), predictor variables were the number of alignment gaps and alignment query coverage (%), the number of trials were defined using by numbers of locations at which each assigned species was seen. Graph created using R package *sjPlot* (2.8.0).
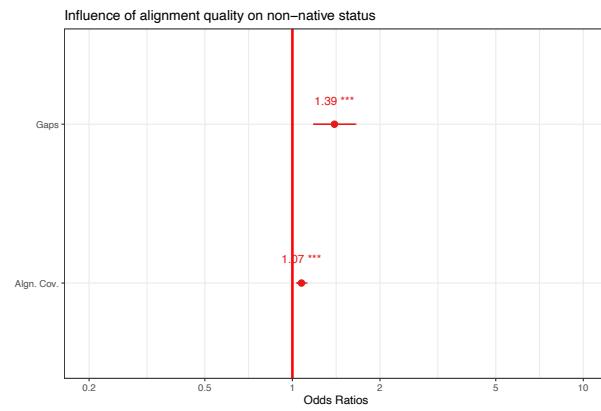

Figure 8: Relationship between non-native status (CD Roberts et al., 2019), gaps, and alignment coverage. The further the odds ratio from 1, the more pronounced the effect.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., . . . Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. doi:10/gf5292

Bylemans, J., Gleeson, D. M., Hardy, C. M., & Furlan, E. (2018). Toward an ecoregion scale evaluation of eDNA metabarcoding primers: A case study for the freshwater fish biodiversity of the murray-darling basin (australia). *Ecology and Evolution*, 1–16. doi:10/gfdrgt

Callahan, B., & Davis, N. M. (2021). *Decontam: Identify contaminants in marker-gene and metagenomics sequencing data*. R package version 1.12.0. Retrieved from https://github.com/benjjneb/decontam

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*(12), 113597. ISBN: 1751-7370 Publisher: Nature Publishing Group. doi:10/gcnwrw

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from illumina amplicon data. *Nature Methods*, *13*(7), 581–583. ISBN: 1548-7105 (Electronic)$\backslash$r1548-7091 (Linking) _eprint: 15334406. doi:10/f84fxp

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: Architecture and applications. *BMC Bioinformatics*, *10*(1), 421. ISBN: 1471210510. doi:10/cnjxgz

Chamberlain, S., Szoecs, E., Foster, Z., & Arendsee, Z. (2020). *Taxize: Taxonomic information from around the web*. R package version 0.9.99. Retrieved from https://CRAN.R-project.org/package=taxize

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Austral Ecology*, *18*(1), 117–143. doi:10.1111/j.1442-9993.1993.tb00438.x

De Cáceres, M., Legendre, P., & Moretti, M. (2010). Improving indicator species analysis by combining groups of sites. *Oikos*, *119*(10), 1674–1684. doi:10.1111/j.1600-0706.2010.18334.x

DiBattista, J. D., Coker, D. J., Sinclair-Taylor, T. H., Stat, M., Berumen, M. L., & Bunce, M. (2017). Assessing the utility of eDNA as a tool to survey reef-fish communities in the Red Sea. *Coral Reefs*, *36*(4), 1245–1252. doi:10.1007/s00338-017-1618-1

Dowle, M., & Srinivasan, A. (2021). *Data.table: Extension of 'data.frame'*. R package version 1.14.0. Retrieved from https://CRAN.R-project.org/package=data.table

Edgar, R. C., & Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics*, *31*(21), 3476–3482. ISBN: 1367-4803. doi:10/gdq95z

Evans, N. T., Olds, B. P., Renshaw, M. A., Turner, C. R., Li, Y., Jerde, C. L., . . . Lodge, D. M. (2016). Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. *Molecular Ecology Resources*, *16*(1), 29–41. ISBN: 1755-098X. doi:10/gg3njr

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047–3048. doi:10/f3s996

Federhen, S. (2012). The NCBI taxonomy database. *Nucleic Acids Research*, *40*, D136–D143. doi:10/c452q3

Firke, S. (2021). *Janitor: Simple tools for examining and cleaning dirty data*. R package version 2.1.0. Retrieved from https://github.com/sfirke/janitor

Gohel, D. (2021a). *Flextable: Functions for tabular reporting*. R package version 0.6.7. Retrieved from https://CRAN.R-project.org/package=flextable

246 Gohel, D. (2021b). *Officer: Manipulation of microsoft word and powerpoint documents*. R package version
247   0.3.19. Retrieved from https://CRAN.R-project.org/package=officer

248 Grange, K. R. (1985). *The intertidal ecology of the soft shores of freshwater basin, milford sound. report*
249   *prepared by the new zealand oceanographic institute for the department of lands and survey*. New Zealand
250   Oceanographic Institute. Wellington, New Zealand.

251 Hester, J. (2020). *Primertree: Visually assessing the specificity and informativeness of primer pairs*. R package
252   version 1.0.5. Retrieved from https://CRAN.R-project.org/package=primerTree

253 Huson, D. H., Beier, S., Flade, I., Gï¿œrska, A., El-Hadidi, M., Mitra, S., . . . Tappu, R. (2016). MEGAN
254   community edition - interactive exploration and analysis of large-scale microbiome sequencing data.
255   *PLOS Computational Biology*, *12*(6), e1004957. doi:10/gfzpks

256 Inglis, G., Post-border Directorate, & MAF Biosecurity New Zealand. (2008). *Milford sound: First baseline*
257   *survey for non-indigenous marine species (research project ZBS2005/19)*. OCLC: 702296120. Wellington,
258   N.Z.: MAF Biosecurity New Zealand. Retrieved August 5, 2021, from http://www.biosecurity.govt.nz/
259   files/pests/salt-freshwater/milford-2008-resurvey-report.pdf

260 Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. N. (2007). Universal primer cocktails for fish
261   DNA barcoding. *Molecular Ecology Notes*, *7*(4), 544–548. doi:10.1111/j.1471-8286.2007.01748.x

262 Jaccard, P. (1912). The distribution of the flora in the alpine zone. *New Phytologist*, *11*(2), 37–50. doi:10/fvhsjd

263 Jeunen, G.-J., Urban, L., Lewis, R., Knapp, M., Lamare, M., Rayment, W., . . . Gemmell, N. (2020). Marine
264   environmental DNA (eDNA) for biodiversity assessments: A one-to-one comparison between eDNA and
265   baited remote underwater video (BRUV) surveys. doi:10.22541/au.160278512.26241559/v1

266 Kassambara, A. (2020). *Ggpubr: Ggplot2 based publication ready plots*. R package version 0.4.0. Retrieved
267   from https://rpkgs.datanovia.com/ggpubr/

268 Kelly, R. P., Port, J. A., Yamahara, K. M., & Crowder, L. B. (2014). Using Environmental DNA to Census
269   Marine Fishes in a Large Mesocosm. *PLOS ONE*, *9*(1), e86175. doi:10.1371/journal.pone.0086175

270 Kitano, T., Umetsu, K., Tian, W., & Osawa, M. (2007). Two universal primer sets for species identification
271   among vertebrates. *International Journal of Legal Medicine*, *121*(5), 423–427. doi:10.1007/s00414-006-
272   0113-y

273 Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pï¿œï¿œbo, S., Villablanca, F. X., & Wilson,
274   A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with
275   conserved primers. *Proceedings of the National Academy of Sciences*, *86*(16), 6196–6200. Publisher:
276   National Academy of Sciences Section: Research Article. doi:10/c2hk4r

277 Lüdecke, D. (2021). *Sjplot: Data visualization for statistics in social science*. R package version 2.8.9. Retrieved
278   from https://strengejacke.github.io/sjPlot/

279 Majaneva, M., Diserud, O. H., Eagle, S. H. C., Bostrï¿œem, E., Hajibabaei, M., & Ekrem, T. (2018). Environ-
280   mental DNA filtration techniques affect recovered biodiversity. *Scientific Reports*, *8*(1), 4682. ISBN:
281   4159801823052. doi:10/gc7tf2

282 Marques, V., Milhau, T., Albouy, C., Dejean, T., Manel, S., Mouillot, D., & Juhel, J.-B. (2021). GAPeDNA:
283   Assessing and mapping global species gaps in genetic databases for eDNA metabarcoding. *Diversity and*
284   *Distributions*. Place: Hoboken Publisher: Wiley WOS:000629615300001. doi:10.1111/ddi.13142

285 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMB-*
286   *net.journal*, *17*(1), 10. _eprint: ISSN 2226-6089. doi:10.14806/ej.17.1.200

14

287 McInnes, J. C., Jarman, S. N., Lea, M.-A., Raymond, B., Deagle, B. E., Phillips, R. A., . . . Alderman,
288   R. (2017). DNA Metabarcoding as a Marine Conservation and Management Tool: A Circumpolar
289   Examination of Fishery Discards in the Diet of Threatened Albatrosses. *Frontiers in Marine Science*, *4*,
290   277. doi:10.3389/fmars.2017.00277

291 McMurdie, P. J., Holmes, S., with contributions from Gregory Jordan, & Chamberlain, S. (2021). *Phyloseq:*
292   *Handling and analysis of high-throughput microbiome census data*. R package version 1.36.0. Retrieved
293   from http://dx.plos.org/10.1371/journal.pone.0061217

294 Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., . . . Iwasaki, W. (2015). MiFish, a set of
295   universal PCR primers for metabarcoding environmental DNA from fishes: Detection of more than 230
296   subtropical marine species. *Royal Society Open Science*, *2*(7), 150088. ISBN: 2054-5703. doi:10/gmcj95

297 Mladenov, P. V. (2001). New zealand fiords: Researching, managing, and conserving a unique ecosystem. *New*
298   *Zealand Journal of Marine and Freshwater Research*, *35*(4), 653–661. doi:10.1080/00288330.2001.9517032

299 Nicholson, J. M., Mordaunt, M., Lopez, P., Uppala, A., Rosati, D., Rodrigues, N. P., . . . Rife, S. C. (2021).
300   *Scite: A smart citation index that displays the context of citations and classifies their intent using deep*
301   *learning*. Cold Spring Harbor Laboratory. doi:10.1101/2021.03.15.435418

302 Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., . . . Wagner, H. (2015).
303   Vegan: Community ecology package. CRAN.

304 Ooms, J. (2021a). *Curl: A modern and flexible web client for r*. R package version 4.3.2. Retrieved from
305   https://CRAN.R-project.org/package=curl

306 Ooms, J. (2021b). *Magick: Advanced graphics and image-processing in r*. R package version 2.7.3. Retrieved
307   from https://CRAN.R-project.org/package=magick

308 Palumbi, S. R. (1996). Nucleic Acids II: The Polymerase Chain Reaction. In *Molecular Systematics* (Second,
309   Vol. 2). Sunderland, Massachusetts: Sinauer Associates.

310 Pebesma, E. (2021). *Sf: Simple features for r*. R package version 1.0-2. Retrieved from https://CRAN.R-
311   project.org/package=sf

312 Pebesma, E., & Bivand, R. (2021). *Sp: Classes and methods for spatial data*. R package version 1.4-5. Retrieved
313   from https://CRAN.R-project.org/package=sp

314 Provoost, P., & Bosch, S. (2021). *Robis: Ocean biodiversity information system (obis) client*. R package version
315   2.6.1. Retrieved from https://github.com/iobis/robis

316 R Core Development Team. (2019). R: A language and environment for statistical computing. R Foundation
317   for Statistical Computing. Retrieved from http://www.r-project.org/.

318 Roberts, C. [CD], Stewart, A., Struthers, C., Barker, J., & Kortet, S. (2019). *Checklist of the fishes of new*
319   *zealand* (No. July). Museum of New Zealand Te Papa Tongarewa. Series: Version 1.1 July 2019. Retrieved
320   from https://collections.tepapa.govt.nz/document/10564

321 Roberts, C. [Clive] (Ed.). (2005). *Regional diversity and biogeography of coastal fishes on the west coast south*
322   *island of new zealand*. Wellington, N.Z: Dept. of Conservation, 250.

323 Rosen, M. J., Callahan, B. J., Fisher, D. S., & Holmes, S. P. (2012). Denoising PCR-amplified metagenome
324   data. *BMC Bioinformatics*, *13*(1). ISBN: 1471-2105 (Electronic)$\backslash$r1471-2105 (Linking).
325   doi:10/gb8vq2

326 Schauberger, P., & Walker, A. (2021). *Openxlsx: Read, write and edit xlsx files*. R package version 4.2.4.
327   Retrieved from https://CRAN.R-project.org/package=openxlsx

Schnell, I. B., Bohmann, K., & Gilbert, M. T. P. (2015). Tag jumps illuminated - reducing sequence-to-sample misidentifications in metabarcoding studies. *Molecular Ecology Resources*, *15*(6), 1289–1303. doi:10.1111/1755-0998.12402

Shaw, J. L. A., Clarke, L. J., Wedderburn, S. D., Barnes, T. C., Weyrich, L. S., & Cooper, A. (2016). Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. *Biological Conservation*, *197*, 131–138. doi:10.1016/j.biocon.2016.03.010

Sherrill-Mix, S. (2021). *Taxonomizr: Functions to work with ncbi accessions and taxonomy*. R package version 0.8.0. Retrieved from https://CRAN.R-project.org/package=taxonomizr

Taberlet, P., Bonin, A., Zinger, L., & Coissac, E. (2018). *Environmental DNA: For biodiversity research and monitoring* (1st ed.). New York, NY: Oxford University Press.

Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E. (2012). Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples. *PLOS ONE*, *7*(8), e41732. doi:10.1371/journal.pone.0041732

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., ... Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*(4), 929–942. doi:10.1111/mec.13428

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1847–1857. doi:10/c6vm58

Wickham, H. (2020). *Reshape2: Flexibly reshape data: A reboot of the reshape package*. R package version 1.4.4. Retrieved from https://github.com/hadley/reshape

Wickham, H. (2021a). *Tidyr: Tidy messy data*. R package version 1.1.3. Retrieved from https://CRAN.R-project.org/package=tidyr

Wickham, H. (2021b). *Tidyverse: Easily install and load the tidyverse*. R package version 1.3.1. Retrieved from https://CRAN.R-project.org/package=tidyverse

Wickham, H., & Bryan, J. (2019). *Readxl: Read excel files*. R package version 1.3.1. Retrieved from https://CRAN.R-project.org/package=readxl

Wickham, H., François, R., Henry, L., & Müller, K. (2021). *Dplyr: A grammar of data manipulation*. R package version 1.0.7. Retrieved from https://CRAN.R-project.org/package=dplyr

Wing, S. R., & Jack, L. (2013). Marine reserve networks conserve biodiversity by stabilizing communities and maintaining food web structure. *Ecosphere*, *4*(11), art135. doi:10.1890/ES13-00257.1

Xie, Y. (2021a). *Knitr: A general-purpose package for dynamic report generation in r*. R package version 1.33. Retrieved from https://yihui.org/knitr/

Xie, Y. (2021b). *Xaringan: Presentation ninja*. R package version 0.22. Retrieved from https://github.com/yihui/xaringan

A   Taxonomic observations

Table 4: Details on taxonomic observations across data sources. Taxonomic hierarchies conform with NCBI taxonomy, where available, and are sorted alphabetically – the resulting species order is identical to Fig. 2 of *main text*. Taxa not listed as New Zealand species in (CD Roberts et al., 2019) are highlighted with Asterisk (*). Trivial names are indicated, where available from NCBI.

Class	Order	Family	Genus	Species	Common name	BRUV	eDNA	OBIS	Literature
Actinopteri	Anguilliformes	Anguillidae	*Anguilla*	*Anguilla australis*	Australian shortfin eel		1		
		Congridae	*Conger*	*Conger verreauxi*	conger eel				TRUE
	Atheriniformes	Atherinidae	*Atherinomorus*	*Atherinomorus lacunosus*	hardyhead silverside				TRUE
	Blenniiformes	Gobiesocidae	*Gobiesox**	*Gobiesox maeandricus**	northern clingfish		1		
		Tripterygiidae	*Bellapiscis*	*Bellapiscis lesleyae*	mottled twister				TRUE
				Bellapiscis medius	twister				TRUE
			Cryptichthys	*Cryptichthys jojettae*					TRUE
			Forsterygion	*Forsterygion capito*	spotted robust triplefin				TRUE
				Forsterygion flavonigrum	yellow-and-black triplefin			TRUE	TRUE
				Forsterygion lapillum	common triplefin				TRUE
				Forsterygion malcolmi					TRUE
				Forsterygion maryannae		4			TRUE
				Forsterygion varium	striped triplefin				TRUE
			*Helcogramma**	*Helcogramma striata**			6		
			Karalepis	*Karalepis stewarti*					TRUE
			Notoclinops	*Notoclinops caerulepunctus*					TRUE
				Notoclinops segmentatus					TRUE
			Notoclinus	*Notoclinus compressus*				TRUE	
				Notoclinus fenestratus					TRUE
			Ruanoho	*Ruanoho decemdigitatus*					TRUE
				Ruanoho whero	spectacled triplefin				TRUE
	Carangiformes	Carangidae	*Trachurus*	*Trachurus japonicus*	Japanese jack mackerel		1		
	Centrarchiformes	Aplodactylidae	*Aplodactylus*	*Aplodactylus arctidens*		2			TRUE
		Cheilodactylidae	*Cheilodactylus*	*Cheilodactylus variegatus*			7		
				Cheilodactylus zonatus	blackbarred morwong		7		
			Nemadactylus	*Nemadactylus macropterus*	tarakihi	14		TRUE	TRUE
		Kyphosidae	*Microcanthus**	*Microcanthus strigatus**	stripey		1		

Class (cont.)	Order (cont.)	Family (cont.)	Genus (cont.)	Species (cont.)	Common name (cont.)	BRUV (cont.)	eDNA (cont.)	OBIS (cont.)	Literature (cont.)
			Scorpis	*Scorpis lineolata*	silver sweep				TRUE
		Latridae	*Latridopsis*	*Latridopsis ciliaris*	blue moki	1		TRUE	TRUE
				Latridopsis forsteri	bastard trumpeter				TRUE
			Latris	*Latris lineata*	striped trumpeter			TRUE	TRUE
			Mendosoma	*Mendosoma lineatum*					TRUE
	Chaetodontiformes	Chaetodontidae	*Chaetodon*	*Chaetodon zanzibarensis*			1		
	Cichliformes	Cichlidae	*Benitochromis**	*Benitochromis finleyi**			1		
			*Coptodon**	*Coptodon zillii**	redbelly tilapia		2		
	Clupeiformes	Engraulidae	*Engraulis**	*Engraulis japonicus**	Japanese anchovy		1		
	Cypriniformes	Cyprinidae	*Phoxinus**	*Phoxinus sp.**			1		
	Gadiformes	Gaidropsaridae	*Gaidropsarus*	*Gaidropsarus argentatus*	Arctic rockling		1		
				Gaidropsarus novaezelandi					TRUE
		Merlucciidae	*Macruronus*	*Macruronus novaezelandiae*	blue grenadier		1		
		Moridae	*Lotella*	*Lotella phycis*			2		
				Lotella rhacina	rock cod	2			TRUE
			Pseudophycis	*Pseudophycis barbata*	southern bastard codling	1		TRUE	TRUE
	Galaxiiformes	Galaxiidae	*Galaxias*	*Galaxias argenteus*				TRUE	
				Galaxias sp.			4		
	Gobiesociformes	Gobiesocidae	*Modicus*	*Modicus minimus*					TRUE
				Modicus tangaroa					TRUE
	Gobiiformes	Eleotridae	*Bostrychus**	*Bostrychus zonatus**	barred gudgeon		8		
		Gobiidae	*Asterropteryx**	*Asterropteryx semipunctata**	starry goby		5		
			Gobiopsis	*Gobiopsis atrata*					TRUE
		Thalasseleotrididae	*Thalasseleotris*	*Thalasseleotris iota*				TRUE	
	Labriformes	Labridae	*Bodianus*	*Bodianus unimaculatus*	red pigfish	5			
			Notolabrus	*Notolabrus celidotus*	New Zealand spotty	5		TRUE	TRUE
				Notolabrus cinctus		4		TRUE	TRUE
				Notolabrus fucicola	yellow-saddled wrasse	1		TRUE	TRUE
			Pseudolabrus	*Pseudolabrus miles*		16		TRUE	TRUE
		Odacidae	*Odax*	*Odax pullus*	greenbone	1			TRUE

Class (cont.)	Order (cont.)	Family (cont.)	Genus (cont.)	Species (cont.)	Common name (cont.)	BRUV (cont.)	eDNA (cont.)	OBIS (cont.)	Literature (cont.)
	Lutjaniformes	Lutjanidae	*Lutjanus*	*Lutjanus sanguineus*	humphead snapper		1		
	Mugiliformes	Mugilidae	*Aldrichetta*	*Aldrichetta forsteri*	yellow-eye mullet		2		TRUE
	Myctophiformes	Myctophidae	*Gymnoscopelus**	*Gymnoscopelus nicholsi**			1		
			Hygophum	*Hygophum hygomii*			1		
	Ophidiiformes	Bythitidae	*Fiordichthys*	*Fiordichthys slartibartfasti*					TRUE
	Osmeriformes	Retropinnidae	*Retropinna*	*Retropinna retropinna*	cucumberfish				TRUE
	Ovalentaria	Plesiopidae	*Acanthoclinus*	*Acanthoclinus fuscus*					TRUE
				Acanthoclinus littoreus					TRUE
				Acanthoclinus marilynae					TRUE
				Acanthoclinus matti				TRUE	TRUE
				Acanthoclinus rua					TRUE
	Pempheriformes	Banjosidae	*Banjos**	*Banjos banjos**			6		
		Percophidae	*Hemerocoetes*	*Hemerocoetes monopterygius*				TRUE	
		Polyprionidae	*Polyprion*	*Polyprion oxygeneios*					TRUE
	Perciformes	Bovichtidae	*Bovichtus**	*Bovichtus diacanthus**			1		
				*Bovichtus variegatus**	thornfish				TRUE
		Callanthiidae	*Callanthias*	*Callanthias allporti*					TRUE
				Callanthias japonicus			1		
		Nototheniidae	*Notothenia*	*Notothenia angustata*	Maori chief			TRUE	
		Percidae	*Sander**	*Sander lucioperca**	pikeperch		1		
		Scorpaenidae	*Scorpaena*	*Scorpaena cardinalis*	red rock cod	1			
				Scorpaena papillosa				TRUE	TRUE
				Scorpaena pepo	pumpkin scorpionfish		1		
		Sebastidae	*Helicolenus*	*Helicolenus hilgendorfi*			1		
				Helicolenus percoides		6			TRUE
		Serranidae	*Caesioperca*	*Caesioperca lepidoptera*		8		TRUE	TRUE
			*Caprodon**	*Caprodon schlegelii**	sunrise perch		27		
			Hypoplectrodes	*Hypoplectrodes huntii*		2			TRUE
			Lepidoperca	*Lepidoperca tasmanica*				TRUE	TRUE
		Triglidae	*Chelidonichthys*	*Chelidonichthys kumu*	bluefin gurnard	1			

Class (cont.)	Order (cont.)	Family (cont.)	Genus (cont.)	Species (cont.)	Common name (cont.)	BRUV (cont.)	eDNA (cont.)	OBIS (cont.)	Literature (cont.)
				Chelidonichthys spinosus	red gurnard		1		
	Pleuronectiformes	Rhombosoleidae	*Peltorhamphus*	*Peltorhamphus latus*	speckled sole			TRUE	
			Rhombosolea	*Rhombosolea plebeia*	New Zealand flounder				TRUE
	Salmoniformes	Salmonidae	*Oncorhynchus*	*Oncorhynchus mykiss*	rainbow trout		1		
	Scombriformes	Gempylidae	*Thyrsites*	*Thyrsites atun*	snoek	1			TRUE
		Scombridae	*Katsuwonus*	*Katsuwonus pelamis*	skipjack tuna		1		
			Scomber	*Scomber japonicus*	chub mackerel		1		
	Stomiiformes	Sternoptychidae	*Maurolicus*	*Maurolicus muelleri*	pearlsides		9		
	Tetraodontiformes	Monacanthidae	*Meuschenia*	*Meuschenia scaber*	velvet leatherjacket	6		TRUE	TRUE
			*Scobinichthys**	*Scobinichthys granulatus**	rough leatherjacket		11		
	Trachichthyiformes	Monocentridae	*Monocentris*	*Monocentris japonicus*			2		
		Trachichthyidae	*Paratrachichthys*	*Paratrachichthys trailli*	sandpaper fish				TRUE
	undefined	Opistognathidae	*Opistognathus**	*Opistognathus iyonis**			3		
				*Opistognathus liturus**	seto-amadai		2		
				*Opistognathus punctatus**	finespotted jawfish		1		
				*Opistognathus sp.**			1		
	Uranoscopiformes	Pinguipedidae	*Parapercis*	*Parapercis colias*	New Zealand blue cod	21		TRUE	TRUE
				Parapercis decemfasciata			10		
				Parapercis gilliesii	yellow weaver				TRUE
Chondrichthyes	Carcharhiniformes	Carcharhinidae	*Prionace*	*Prionace glauca*	blue shark			TRUE	
		Scyliorhinidae	*Cephaloscyllium*	*Cephaloscyllium isabellum*		4			TRUE
		Triakidae	*Galeorhinus*	*Galeorhinus galeus*	tope shark	2			
			Mustelus	*Mustelus lenticulatus*	spotted estuary smooth-hound	1			
				Mustelus manazo	starspotted smooth-hound		1		
	Hexanchiformes	Hexanchidae	*Notorynchus*	*Notorynchus cepedianus*	broadnose sevengill shark	2	1		
	Lamniformes	Alopiidae	*Carcharodon*	*Carcharodon carcharias*	great white shark			TRUE	
			Isurus	*Isurus oxyrinchus*	shortfin mako shark			TRUE	
	Squaliformes	Squalidae	*Squalus*	*Squalus acanthias*	spiny dogfish	2		TRUE	TRUE
				Squalus suckleyi	Puget Sound dogfish		3		