Title

Marine environmental DNA (eDNA) for biodiversity assessments: a one-to-one comparison between eDNA and baited remote underwater video (BRUV) surveys.

Journal: Molecular Ecology Resources

Gert-Jan Jeunen[1*], Lara Urban[1*], Robert Lewis[2], Michael Knapp[1], Miles Lamare[2], William Rayment[2], Steve Dawson[2], and Neil J. Gemmell[1]

[*]Co-first authors

1. Department of Anatomy, University of Otago, PO Box 56, Dunedin 9054, New Zealand
2. Department of Marine Science, University of Otago, PO Box 56, Dunedin 9054, New Zealand

Corresponding Author:	Gert-Jan Jeunen	Lara Urban
	Department of Anatomy	Department of Anatomy
	University of Otago	University of Otago
	PO Box 56	PO Box 56
	Dunedin 9054, NZ	Dunedin 9054, NZ
	gert-jan.jeunen@otago.ac.nz	lara.h.urban@gmail.com

Abstract

Aquatic environmental DNA (eDNA) surveys have emerged as an alternative method for monitoring complex and vast marine ecosystems. One-to-one comparisons between existing survey techniques and eDNA approaches are essential to determine biases associated with this novel methodology. To date, such direct comparative studies have been scarce in the context of marine eDNA surveys. In this study, we conducted simultaneous baited remote underwater video (BRUV) and eDNA surveys to describe the fish community in Paterson Inlet, Stewart Island/Rakiura, New Zealand. BRUV detected three distinct families of bony fish (Actinopterygii) and four families of cartilaginous fish (Chondrichthyes). Three different eDNA assays, detected 32 (MiFish-U), 42 (MiFish-E), and 23 (16S-Fish) families, spanning the classes of Actinopterygii, Chondrichthyes, Hyperoartia, Mammalia, and Aves. Our direct comparison identified the need for (*i*) increased sampling, (*ii*) spatial pooling, and (*iii*) multiple targeted eDNA assays, to achieve similar detection rates of a given species in eDNA and BRUV monitoring. Diversity, ordination, and indicator species analyses identified distinct eDNA signals between different habitats in our relatively small sampling area, showcasing the high spatial resolution of eDNA approaches in marine habitats. Our results provide valuable insights into the potential biases associated with eDNA monitoring, as well as highlight the power of eDNA for detecting a broad range of taxa beyond traditional observational approaches, including terrestrial, invasive and migratory organisms.

Keywords: eDNA, BRUV, biodiversity monitoring, marine environment, fish diversity

Introduction

Effective environmental stewardship requires accurate measurements of the diversity, abundance and distribution of biological communities (Karnauskas, 2012; Matthews & Whittaker, 2015). These measurements, however, are difficult to obtain for many

communities, with marine systems posing additional challenges due to their inaccessibility (Colton & Swearer, 2010), the highly variable distribution of organisms (Acosta, 1997; Bacheler et al., 2017), and the need for visual species identification through morphological characterisation when traditional monitoring approaches are employed (e.g., underwater visual census (UVC), baited remote underwater video (BRUV), catch-per-unit-effort (CPUE); Thorngren et al., 2017; Yang et al., 2017). These challenges lead to detection biases across taxa, potentially resulting in inaccurate ecosystem health assessments (Monk, 2014; Pais & Cabral, 2018).

Many such biases have been highlighted for traditional monitoring approaches (Boulinier et al., 1998; Colton & Swearer, 2010; MacNeil et al., 2008; Monk, 2014). For example, BRUV monitoring, one of the best established non-destructive approaches to monitor bony (Actinopterygii) and cartilaginous (Chondrichthyes) fish in coastal and marine environments (Andradi-Brown et al., 2016; Stobart et al., 2015), uses a bait box in front of a camera to increase the detection probability of the residing fish community (Murphy & Jenkins, 2010). Unlike diver surveys, BRUV is not restricted to time and depth limitations (Stobart et al., 2015) and provides a standardized manner for assessing species diversity free of diver bias (Bacheler et al., 2017; Mallet & Pelletier, 2014; White et al., 2013). However, apex predators are usually overrepresented due to the use of bait (Cappo et al., 2004; Murphy & Jenkins, 2010; Willis & Babcock, 2000), while herbivores and small cryptic species are often missed (Andradi-Brown et al., 2016).

To avoid or quantify such biases, there is a need for novel non-destructive and inexpensive monitoring methods that generate accurate and high-resolution data (Dickens et al., 2011; Murphy & Jenkins, 2010; Stat et al., 2019). For example, environmental DNA (eDNA)

77 surveys (Ficetola et al., 2008; Garlapati et al., 2019) measure biodiversity through DNA
78 obtained from environmental samples (e.g., water, soil, sediment) and may therefore
79 circumvent the need for visual observations and taxonomic expertise (DiBattista et al., 2020;
80 Jeunen et al., 2018). To date, various eDNA studies (Garlapati et al., 2019) have assessed the
81 (*i*) accuracy of species detection (Jeunen et al., 2019a; Murakami et al., 2019; O'Donnell et
82 al., 2017; Port et al., 2016), (*ii*) ability to obtain species' abundance information (Sassoubre et
83 al., 2016a; Takahara et al., 2012; Uthicke et al., 2018), and (*iii*) possibility of retrieving
84 population-genetic data (Adams et al., 2019; Sigsgaard et al., 2020).
85
86 For eDNA monitoring to be a practical tool in environmental management, direct
87 comparisons between different eDNA and traditional survey techniques are crucial to gain an
88 understanding of the accuracy and observational biases specific to each methodology
89 (Schmid et al., 2016). Several studies have compared species detection between eDNA and
90 traditional monitoring methods such as BRUV, UVC, tow net, diver surveys, fyke-nets and
91 bottom trawling (Afzali et al., 2020; Bakker et al., 2017; Kelly et al., 2017; Port et al., 2016;
92 Stat et al., 2019; Thomsen et al., 2012, 2016; Valdivia-Carrillo et al., 2019). While Kelly et
93 al. (2017) and Thomsen et al. (2012) found that eDNA outperformed traditional monitoring
94 approaches, the majority of studies found only a partial overlap in the species detected. The
95 current recommendation is, therefore, a combined monitoring strategy to provide a more
96 holistic picture of marine communities (Afzali et al., 2020; Stat et al., 2019; Valdivia-Carrillo
97 et al., 2019). However, these comparative studies have been based on either (*i*) a single
98 metabarcoding assay (Afzali et al., 2020; Stat et al., 2019; Thomsen et al., 2012; Valdivia-
99 Carrillo et al., 2019), (*ii*) limited eDNA sampling without assessing the effect of sampling
100 effort or spatial pooling (Thomsen et al., 2012, 2016), or (*iii*) general distributional
101 observations as opposed to one-to-one comparisons between traditional and eDNA surveys

(Afzali et al., 2020; Bakker et al., 2017; Kelly et al., 2017; Port et al., 2016; Stat et al., 2019; Thomsen et al., 2016; Valdivia-Carrillo et al., 2019).

Here, we conduct a direct comparative study between eDNA and BRUV monitoring to describe the fish diversity at Paterson Inlet, Stewart Island/Rakiura, New Zealand. The application of three metabarcoding assays (MiFish-U, MiFish-E, 16S-Fish) allows us to determine biases and limitations associated with eDNA surveys with respect to sampling effort, spatial pooling, and assay choice.

Material and Methods

Study area

Our study area was situated in Paterson Inlet, the largest natural harbour (located at 46°55'S 168°03'E; total area: 65 km^2; length: 15 km; maximum depth: 45 m; Willan, 1981) of Stewart Island/Rakiura, New Zealand (FIGURE 1). Paterson Inlet is situated immediately South of Stewart Island's largest community, Oban (population size: ~ 400). Within the inlet, several marine protected areas have been established, as well as salmon, oyster and mussel farms (FIGURE 1). The entrance of Paterson Inlet faces northward and is constricted by Ulva Island, resulting in a largely sheltered and soft-bottom habitat disrupted with rock formations surrounded by kelp forest (Smith et al., 2005). Paterson Inlet is relatively well-flushed with an estimated mean residence time of water of five days (Pridmore & Rutherford, 1990). Current speeds in the inlet are reported as 15 to 20 cm s^{-1} (O'Callaghan, 1999). The inlet experiences semi-diurnal tidal mixing, with additional water exchange with the adjacent coastal waters occurring through periodic events of wind-driven and baroclinic forcing (O'Callaghan, 1999). Thermal stratification of the water can occur during the warmer summer months, while the water column is well-mixed over winter (O'Callaghan, 1999).

Four sampling sites were simultaneously surveyed by BRUV and aquatic eDNA monitoring: Papatiki Bay (PAB) is located closest to Foveaux Strait on the West side of Bradshaw Peninsula at the entrance of Paterson Inlet. Trumpeter Point (TP) is located roughly on the halfway mark on the Southern coastline of Paterson Inlet. Prices Inlet (PI) is located opposite of TP on the Northern coastline. Sawdust Bay (SB and SDB) is located furthest inland at the entrance to North Arm, and was surveyed twice within three days to obtain temporal replicates (FIGURE 1).

BRUV study

Surveys took place at the four study locations (see *Study area*) between October 23rd and 28th, 2017. Video images were obtained using a downward-facing BRUV system equipped with two GoPro Hero 4 Silver cameras mounted in waterproof housings in a stainless-steel frame (Lewis et al., 2020). The cameras were positioned 1.2 m above a 1 m long pole marked with 10 cm graduations. A bait box was attached in the middle of the pole. Two Qudos 300 lumen LED lights were used to illuminate the field of view. Pilchard (*Sardinops neopilchardus*), barracouta (*Thyrsites atun*), and salmon (*Oncorhynchus tshawytscha*) were used as chum for the BRUV deployment. The bait box was deployed at a depth of 5-8 m within a radius of 2 m of the seabed. Chumming continued steadily throughout two consecutive one-hour BRUV deployments at each location. Video footage was examined using Avidemux 2.7.1, which allows for frame-by-frame viewing. Individuals were taxonomically identified via their morphological characteristics.

eDNA sampling and extraction

151 Prior to field and laboratory work, we sterilized all equipment and bench spaces by a 10
152 minute exposure to 10% bleach solution (Prince & Andrus, 1992). We rinsed all sampling
153 bottles (2L, HDPE Natural, EPI Plastics) twice with ultrapure water (UltraPure™ Distilled
154 Water, Invitrogen), submerged them in 10% bleach for 10 minutes, and rinsed twice again
155 with ultrapure water. All laboratory work prior to amplification was performed in a dedicated
156 environmental DNA PCR-free clean laboratory.
157
158 We monitored contamination by introducing negative controls at each step: sampling controls
159 consisted of two sampling bottles filled with 500 mL ultrapure water, DNA capture controls
160 were added by filtering 500 mL ultrapure water, and DNA extraction controls consisted of
161 500 µL ultrapure water. Negative controls were processed alongside the samples. None of the
162 species used for chumming were detected in our negative control samples.
163
164 Environmental DNA samples were taken directly prior to BRUV deployment (see *BRUV*
165 *study*). Surface water samples of 2 L were collected at the four locations, with three different
166 sites per location representing different habitats (*e.g.*, sandy bank or rocky shore) and five
167 samples per site as sample replicates (FIGURE 1; SUPPLEMENT 1). Sawdust Bay (SB, SDB)
168 was sampled twice, resulting in the overall collection of 75 samples. Samples were
169 transported to the Marine Sciences field station in Oban directly after BRUV deployment and
170 filtered the same day.
171
172 Sample processing followed the recommendations of Jeunen et al. (2018): Briefly, water
173 samples were filtered over a 1.2 µm cellulose-nitrate filter (CN, Whatman™) using a vacuum
174 filtration pump (Laboport®, KNF Neuberger, Inc.) and an in-house made filtration manifold.
175 Filters were rolled up, cut into two, placed in two 2 mL LoBind Eppendorf tubes, and stored

176 at -20°C. Filters were transported at -20°C to the dedicated PCR-free laboratory at the
177 University of Otago, Dunedin for subsequent processing. DNA was extracted from the filters
178 following the standard protocol of the Qiagen DNeasy Blood & Tissue Kit (Qiagen GmbH,
179 Hilden, Germany), with modifications described in Jeunen et al. (2018). DNA extracts were
180 stored at -20°C until further processing.
181
182 *Library preparation and sequencing*
183 Library preparation followed the protocol described in Berry et al. (2017) and Jeunen et al.
184 (2019b). We used three metabarcoding assays to describe the fish community at each
185 sampling site (SUPPLEMENT 2), one assay targeting the 16S rRNA gene region (16S-Fish;
186 Berry et al., 2017) and two assays targeting the 12S rRNA hypervariable gene region
187 (MiFish-U/E; Miya et al., 2015).
188
189 Prior to library preparation, input DNA for each sample was optimized using a dilution series
190 (neat, 1/10, 1/20) to identify inhibitors, and low-template samples (Murray et al., 2015).
191 Amplification was carried out in 25 µL reactions, prepared with 1x SensiFAST™ SYBR® Lo-
192 ROX Kit (Bioline, Meridian Bioscience), 0.4 µmol/L of each primer (Integrated DNA
193 Technologies, Australia) and 2 µL of DNA. qPCR conditions included an initial denaturing
194 step at 95 °C for 10 minutes; followed by 50 cycles of 20 s at 98 °C, 15 s at 60 °C, and 15 s at
195 72 °C for the MiFish-E/U assays, and 50 cycles of 30 s at 95 °C, 30 s at 54 °C, and 45 s at 72
196 °C for the 16S-Fish assay; and a final extension of 10 minutes at 72°C.
197
198 A one-step amplification protocol was used for library building using fusion primers
199 containing a modified Illumina sequencing adapter, a barcode tag (6-8 bp in length), and the
200 template-specific primer. Each sample was amplified in duplicate and assigned a unique

barcode combination to allow pooling of samples post-qPCR. qPCR conditions followed the amplification protocol described above. qPCR duplicates of each sample were pooled to reduce stochastic effects due to PCR amplification. Samples were then pooled to approximately equal molarity based on Ct-value and end-point qPCR fluorescence. Samples were normalized on a Bioanalyzer 2100 (Agilent, USA) to produce a single library.

Due to differences in cycle number between samples and negative controls, the negative controls were spiked into the library to allow for optimal concentration of the library. The library was then size-selected and purified using AMPure XP Beads (BioLabs Inc., USA) prior to final library quantitation on a Bioanalyzer 2100 and Qubit. Sequencing was performed on an Illumina MiSeq® (300 cycle, single end) at the Otago Genomics Sequencing Facility, following the manufacturer's protocols and with PhiX to minimize issues associated with low-complexity libraries.

Data preprocessing

Raw fastq files were quality-checked using FastQC (v. 0.11.9) (Bioinformatics, 2011). Sequences were demultiplexed and assigned to samples using the ngsfilter function in OBITools (v. 1.2.11-1; Boyer et al., 2016). Sequences longer than 250 bp were discarded using the obigrep function and split into fastq files per sample using the obisplit module. Fastq files were imported into USEARCH (v. 11.0.667; Edgar, 2010) and quality-filtered based on total expected errors (MaxEE = 1), a minimum length of 150 bp, and presence of ambiguous bases using the fastq_filter function. Sequences passing quality filtering were pooled into a single file, quality checked again by FastQC, and dereplicated into unique sequences using the fastx_uniques module. Sequences occurring <10 times were discarded. Clustering of sequences into ZOTUs (Zero-radius Operational Taxonomic Units) was done

using USEARCH with the modules unoise3 and otutab. OBITools ecoPCR (v. 1.0.1) was used to perform *in silico* PCR on the EMBL database (downloaded on 17-08-2019) to generate custom reference databases per metabarcoding assay. The OBITools ecotag module was used to assign taxonomy to each ZOTU. Read assignments were subsequently pooled on the taxonomic family level and reads that did not achieve a taxonomic resolution at the family level were discarded.

The read counts were binarized to presence-absence data per sample: To avoid issues relating to cross-contamination and tag jumping, we assigned presence of a family only in the case of more than one read per sample. Additionally, to account for variable read counts across samples, we implemented an alternative approach and assigned presence if more than 0.01% (or 0.0001%) of sample-specific reads mapped to a specific family. These approaches did not have a substantial effect on our analyses (data not shown).

The 75 samples were then pooled across sample replicates (five samples for each of the three habitats in a sampling area), resulting in 15 samples, or additionally pooled across the different habitats within a sampling area, resulting in five samples. To do so, we either applied sum-pooling (i.e., presence according to at least one sample leads to presence at the next highest pooling level), or max-pooling (i.e., majority vote across samples decides about presence or absence on the next highest pooling level).

Statistical analyses

We analysed the data in the programming languages Python (*v.* 3.45) and R (*v.* 3.5.3). For BRUV-eDNA comparisons, we assessed the proportion, ρ, of BRUV-positive samples that were eDNA-positive across seven observed families for each metabarcoding assay

individually and for the pooled assays. Additionally, we pooled the samples per habitat and per location to explore the effect of sample and spatial pooling respectively. Pooling of samples followed either sum- or max-pooling (see *Data preprocessing*).

We performed and visualised Multiple Correspondence Analysis (MCA) using the Python *mca* package (Greenacre & Blasius, 2006) and customized scripts from Urban et al. (2020). We used the R libraries *betapart* to calculate beta richness indices (Baselga & Orme, 2012) and *indicspecies* to perform indicator taxa analysis (Cáceres & Legendre, 2009). Specifically, we calculated the indicator value indices and Phi coefficients of association between within-bay (SB, SDB), marine (PAB) and intermediate (PI, TP) samples, correcting for the unequal sample numbers between habitats. For the indicator value analysis, we considered taxa at a nominal P-value threshold of 0.05, and high positive predictive value, ***P,*** and sensitivity, ***S,*** (>0.8; n=9,999 permutations). We used correlation analysis to confirm the ecological preference of the respective taxa. We then extended the indicator value analysis to include combinations of taxa (up to five) and assessed ***P*** and ***S*** and their confidence intervals ($n_{bootstrapping}$ = 9,999).

Results

BRUV observations

BRUV detected seven distinct fish families across all five sampling sites, i.e., Scyliorhinidae (catsharks), Hexanchidae (cow sharks), Triakidae (houndsharks), Rajidae (skates), Congiopodidae (pigfishes), Labridae (wrasses), and Pinguipedidae (sandperches). The highest number of families was observed at Sawdust Bay (SB/SDB, n=5). Labridae was the most commonly observed family at every sampling site. Congiopodidae and Hexanchidae, on

the other hand, were only detected at Sawdust Bay, with Congiopodidae represented by a single individual.

eDNA results

After quality filtering, we obtained 7,691,672 high-quality DNA reads across the three metabarcoding assays. Samples that were not assigned a single read for at least one of the three assays (i.e., PAB_1_5, PAB_2_2, PAB_2_3, PAB_2_4, and PAB_3_5) were removed from subsequent analyses. A total of 4,581,483 reads (59.56%) were assigned to at least taxonomic family level by OBITools (MiFish-E: n=1,633,649; MiFish-U: n=1,038,014; 16S-Fish: n=1,909,820; MATERIAL & METHODS). Per sampling location, we obtained an average number of reads (± S.D.) of 23,337.8 (± 9,679.4), 14,828.8 (± 10,216.9), and 27,283.1 (± 25,226.7), for the MiFish-E, MiFish-U, and 16S-Fish assay, respectively (SUPPLEMENT 4).

In silico PCR results per metabarcoding assay showed that all assays combined were theoretically able to detect 1,464 unique vertebrate families (MiFish-E: n=940; MiFish-U: n=874; 16S-Fish: n=1,166; SUPPLEMENT 3; MATERIAL & METHODS).

Across our samples, MiFish-E detected a total of 42 families, covering 34 orders within the classes Actinopterygii (ray-finned fishes), Aves (birds), Chondrichthyes (cartilaginous fishes), and Mammalia (mammals). Labridae, Hominidae (hominids), and Retropinnidae (smelts and graylings) were detected at all sampling locations, while Callorhinchidae (elephantfish), Leporidae (rabbits and hares), Trachipteridae (ribbonfish), Bovidae (bovids), and Phocidae (true seals) were only detected at a single sampling location. MiFish-E detected most families at SB (10.1 ± 3.1), with the lowest number of families being observed at TP (6.5 ± 2.1).

MiFish-U detected a total of 32 families, covering 25 orders within the classes Actinopterygii, Aves, Chondrichthyes, Hyperoartia (lampreys), and Mammalia. Labridae and Retropinnidae were detected at all sampling locations, while Bovichtidae (thornfishes), Sebastidae (scorpionfishes), and Syngnathidae (seahorses, pipefishes, and seadragons) were only detected at a single sampling location. MiFish-U detected most families at PAB (8.4 ± 1.8), with the lowest number of families being observed at TP (5.0 ± 2.7).

16S-Fish detected a total of 23 families, covering 16 orders within the classes Actinopterygii and Chondrichthyes. Labridae and Pinguipedidae were detected at all sampling locations, while Sebastidae, Trachichtyidae (roughies), Triakidae and Cheilodactylidae (morwongs) were only detected at a single sampling location. 16S-Fish detected most families at PI (7.6 ± 1.1), with the lowest number of families being observed at SDB (4.9 ± 1.4) (SUPPLEMENT 4).

Comparison of eDNA and BRUV

Next, we performed one-to-one comparisons between eDNA and BRUV results to compare both monitoring approaches and to determine biases associated with eDNA metabarcoding (MATERIAL & METHODS). Each of the seven families observed by BRUV was detected by at least one of the eDNA assays: *In silico* PCR analysis predicted the detection of all seven observed families by the 16S-Fish assay, while two families (Congiopodidae, Pinguipedidae) were predicted to not be detectable by the MiFish-E/U assays due to a lack of a reference sequence for these families. *In situ*, 16S-Fish did not detect the families Scyliorhinidae and Hexanchidae. This false-negative result might be explained by a 3 bp/1 bp mismatch at the 3'-end of the forward primer for Scyliorhinidae and Hexanchidae, respectively (SUPPLEMENT 3). The MiFish-U assay *in situ* failed to detect Scyliorhinidae, Triakidae, and Rajidae. All

three families showed the same 3 bp and 2 bp mismatch on the forward and reverse primer, respectively. The lack of sensitivity of the MiFish-U assay to detect Chondrichthyes has already been shown by Miya et al. (2015). Despite the optimisation of the MiFish-E assay for the class Chondrichthyes, this assay *in situ* failed to detect the family Rajidae, which is characterized by a single base pair mismatch in both, the forward and reverse primer binding sites (SUPPLEMENT 3).

Comparison of ρ values, the proportion of BRUV-positive samples that were eDNA-positive across the seven observed families (MATERIAL & METHODS), showed that only the pooling of data across (*i*) all metabarcoding assays, (*ii*) spatial habitats, and (*iii*) sample replicates led to an accurate reflection of the BRUV observations (FIGURE 2). Furthermore, only sum-pooling as opposed to max-pooling led to an increase in ρ (FIGURE 2), with max-pooling roughly keeping the detection performance on the level of the next-lowest pooling approach (SUPPLEMENT 5).

When the results from all three metabarcoding assays were combined and pooled across all 15 samples collected at the sampling site (sample and spatial pooling), eDNA monitoring accurately reflected the observed fish diversity across locations based on BRUV detection (FIGURE 2c, f). This fully pooled eDNA approach further detected the seven visually observed families at more locations than BRUV. In particular, Rajidae were detected at three more locations compared to BRUV (SB, SDB, PAB). The largest difference was at PAB, where eDNA detected four more families than BRUV (Congiopodidae, Pinguipedidae, Hexanchidae, Rajidae). These families were observed in BRUV samples elsewhere. *Vice versa*, the only family occurrences that were not detected by eDNA monitoring despite the documented presence by BRUV were Triakidae at PAB and Congiopodidae at SDB.

350

351 *eDNA monitoring - Taxonomic diversity*

352 In addition to the seven families observed by BRUV, eDNA monitoring detected 49
353 additional families, ranging from marine and freshwater fish to seabirds and mammals. We
354 detected a total of 56 families across the three metabarcoding assays and locations (**γ**
355 richness), with **α** richness ranging from 29 at TP to 36 families at PI (median across
356 locations: 33). With respect to **ß** richness, we observed high diversity between locations, with
357 a Jaccard Index (JI) of 0.958 and a Simpson Index (SI) of 0.881 (FIGURE 3A; Baselga and
358 Orme, 2012; MATERIAL & METHODS); SI represents the proportion of **ß** richness due to
359 taxonomic composition, as opposed to nestedness effects that are additionally being captured
360 by JI (MATERIAL & METHODS). We observed that combining the three individual eDNA
361 assays consistently improved diversity estimates (TABLE 1).

362

363 *eDNA – Ecosystem divergence*

364 MCA of presence-absence data across all samples separates the SB/SDB location from the
365 remaining ones along the first axis (25.87% explained variance, FIGURE 3C), reflecting the
366 ecological separation between SB/SDB as an estuarine environment, PAB as a near-marine
367 habitat in proximity to the open ocean (Foveaux Strait), and TP and PI as intermediate
368 locations (FIGURE 3C). In the MCA, samples of the same location cluster together, but their
369 relatively wide ranges (approximated by elliptic shapes in FIGURE 3C) underline the necessity
370 of sample replication for accurately distinguishing locations in terms of their taxonomic
371 composition. The five samples per site (represented by point shapes in FIGURE 3C) form
372 further subclusters, pointing towards slight variability across spatially proximate sites. The
373 ranges of SB and SDB samples are highly overlapping, reflecting successful temporal

374 replication due to spatial and sample pooling. MCAs applied to the MiFish-E/U assays do not
375 distinguish the distinct environments as well as the pooled assay (SUPPLEMENT 6).
376
377 Analysis of the relative contribution to the first MCA axis revealed a strong impact of the
378 overrepresentation of freshwater taxa at SB/SDB (FIGURE 3D, red bars): For example,
379 Anguillidae is a family of freshwater eels, and Anatidae (ducks) were observed to aggregate
380 near SB/SDB (personal observation). On the other hand, the MCA axis identified the
381 presence of Salmonidae, Otariidae, and Centrolophidae as strong contributors to the
382 separation of the more marine environment. The Salmonidae DNA signal has most probably
383 originated from the nearby salmon farm (FIGURE 1). Centrolophidae are typically being
384 fished in Stewart Island waters (Ayling, 1987), and New Zealand fur seals (Otariidae)
385 maintain an established colony close to PAB.
386
387 *eDNA - Indicator species analysis*
388 Stringent analysis of indicator species across all samples showed strong predictability of
389 habitat based on taxonomic communities described by our pooled eDNA assay (FIGURE 3B),
390 especially for the marine and within-bay habitats. We detected significant roles of
391 Salmonidae and Otariidae for the marine, and of Anguillidae for the within-bay habitats ($\boldsymbol{P}$ >
392 0.8; $\boldsymbol{S}$ > 0.8; SUPPLEMENT 7), confirming their strong contribution to spatial segregation
393 identified by MCA. Indicator value analysis based on combinations of taxa further revealed
394 that Otariidae and Salmonidae alone were able to completely resolve marine habitat
395 affiliation with maximum positive predictive value and sensitivity ($\boldsymbol{P}$ = 1; $\boldsymbol{S}$ = 1). A
396 combination of Salmonidae and Cheilodactylidae revealed the same strong association, with
397 Cheilodactylidae being a typical family of the Southern Hemisphere's coastal marine
398 environments. The presence of Anatidae, Eleotridae, Galaxiidae and Retropinnidae achieved

399 maximum predictability of within-bay habitat (***P*** = 0.93±0.08; ***S*** = 0.90±0.11), partially
400 reflecting spatial composition detected by MCA. Eleotridae and Retropinnidae are known to
401 inhabit freshwater habitats, with adult Eleotridae mainly living in freshwater streams and
402 brackish water, and with Retropinnidae inhabiting shallow-depth estuaries and rivers.
403
404 *eDNA - Taxa of interest*
405 Besides assessing within- and between-habitat diversities, eDNA as opposed to BRUV
406 monitoring discovered taxa of interest such as elusive, endangered or invasive species, as
407 well as terrestrial and freshwater taxa. For example, our eDNA approach supported the
408 presence of Phalangeridae at all locations except for PAB by a large number of reads
409 (n>10,000). The only representative of this family that is known to occur in New Zealand is
410 *Trichosurus vulpecula*, the invasive common brushtail possum. Another invasive, Muridae
411 (rodents) were detected by MiFish-E at the sampling sites PI, SB, and SDB. We further
412 detected evidence of Cervidae (deer) and Suidae (boars) which have been introduced to
413 Stewart Island/Rakiura for hunting purposes, and of a variety of bird families which use the
414 inlet as a foraging area, such as Laridae (gulls), Anatidae (ducks), Haematopodidae
415 (oystercatchers) and Procellariidae (petrels), or as nesting grounds, such as Spheniscidae
416 (penguins) (SUPPLEMENT 4).
417
418 **Discussion**
419 *Direct comparison between BRUV and eDNA surveys*
420 Our one-to-one comparison indicated the need for increased sampling effort and the use of
421 multiple metabarcoding assays in eDNA surveys to allow for the exact recovery of taxonomic
422 compositions as detected by BRUV. Increased sampling effort will capture the observed
423 variability in eDNA signals between samples collected from a single sampling site. This

variability most likely originates from the high complexity of the DNA signal and low DNA concentration of environmental samples (Grey et al., 2018). The parallel application of various primer sets, on the other hand, has previously been shown to increase the number of detectable species by reducing the impact of preferential amplification and incomplete reference databases (Alexander et al., 2020; Jeunen et al., 2019a; Stat et al., 2017). "Blind spots" in individual eDNA assays due to, for example, variable PCR efficacy, primer binding/ degeneracy, assay sensitivity or amplicon length, have been shown to lead to false negative results (Hajibabaei et al., 2019; Nester et al., 2020). Our results indicate that these effects on assay choice are mitigated when applying multiple assays to cover a certain range and variability of taxa. While previous studies have compared various eDNA assays (Hajibabaei et al., 2019), here we have corroborated our findings via direct observational comparisons.

When pooling the data generated by the three metabarcoding assays from all fifteen samples collected per site, our eDNA survey proved to be more sensitive than BRUV for the seven visually observed families. Increased sensitivity was, in particular, observed for the Rajidae family, which we detected at three more locations with the help of eDNA as compared to BRUV monitoring. One explanation might be their lack of attraction to the BRUV bait, resulting in the dependency of a random occurrence of an individual under the camera for BRUV detection. With respect to locations, the taxonomic diversity identified at PAB profited most from eDNA analysis. The detection of the seven visually observed families at more sampling sites when using eDNA than was observed with BRUV might be explained by a low level of DNA dispersal throughout Patterson Inlet (Kelly et al., 2018). Alternatively, the eDNA signal detected might originate from a dead individual or from faeces from a predator/scavenger (Berry et al., 2017; Young et al., 2020). Lastly, the downward facing

448 BRUV system may have had a reduced monitoring field, which has resulted in a high rate of
449 false negatives (Mallet & Pelletier, 2014).
450
451 Despite the ability to detect all seven families observed by BRUV with our eDNA survey,
452 two family occurrences could not be confirmed by eDNA monitoring at two specific
453 instances, i.e., Triakidae at PAB and Congiopodidae at SDB. Both instances might be
454 explained by our sampling design with collection of eDNA samples prior to BRUV
455 deployment. While this design avoided the detection of chum that was used for attracting fish
456 within the BRUV survey, our eDNA signal might have captured a slightly different temporal
457 snapshot of the fish community. It is additionally probable that the bait impacted the fish
458 composition in the surrounding area of our sampling sites within the two hours of BRUV
459 surveying after eDNA sample collection. For example, Triakidae at PAB were only observed
460 after a substantial time lag (at 00:58:04 since the start of BRUV recording). We hypothesize
461 that this shark family was attracted by the BRUV bait and therefore remained undetected by
462 preceding eDNA sampling. As Hexanchidae were also present at PAB, avoidance behaviour
463 of Triakidae might explain the late occurrence of this species. With respect to
464 Congiopodidae, only a single fish was observed at SDB by BRUV, potentially indicating a
465 detection limit for eDNA surveys. We however showed that both Triakidae and
466 Congiopodidae can generally be discovered by our pooled eDNA approach.
467
468 Our results show the necessity of a robust experimental design for eDNA metabarcoding
469 surveys to limit the risk of false-negative detections. In contrast to several previously
470 conducted comparative studies (Afzali et al., 2020; Bakker et al., 2017; Port et al., 2016; Stat
471 et al., 2019; Thomsen et al., 2016; Valdivia-Carrillo et al., 2019), all families observed by our
472 traditional monitoring approach were detected by eDNA. However, the diversity of our target

taxonomic group was low in our sampling area, especially compared to a highly diverse system such as a tropical reef (Stat et al., 2019). Nevertheless, an increasing number of publications are showing that with increased sampling effort (Grey et al., 2018), inclusion of different substrates (Koziol et al., 2019), and use of multiple targeted metabarcoding assays (Jeunen et al., 2019a), eDNA surveys have the potential to surpass the sensitivity and taxonomic diversity limits of traditional monitoring techniques. The sampling effort and assay choice will, however, be dependent on the area of interest and research question. Hence, we recommend conducting an initial pilot experiment including an extensive number of samples collected at a limited number of sampling locations in the area of interest to determine the necessary number of samples per sampling location. Furthermore, *in silico* PCR analyses will provide the necessary information for an informed assay choice by reporting on (*i*) potential problems with amplification efficiency due to mismatches in the primer-binding sites, and (*ii*) the completeness of the reference database.

Increased taxonomic diversity

Besides the seven families detected by BRUV, our eDNA survey detected an additional 49 families, ranging from marine and freshwater fish to seabirds and mammals. Marine fish families only detected by eDNA included herbivores, elusive and cryptic taxa, such as Bothidae (flounders), Tripterygiidae (triplefins), Rhombosoleidae (flatfish), and Bovichtidae (thornfishes). BRUV monitoring is known to overrepresent apex predators and overlook other, elusive, shy or cryptic, taxa (Acosta, 1997; Bacheler et al., 2017; Colton & Swearer, 2010). Additionally, all three assays in our eDNA survey detected representative species of the Syngnathidae family (seahorses), while previously claimed to be undetectable with existing eDNA assays (Nester et al., 2020). Our eDNA survey also detected families that are unable or unlikely to be detected by BRUV monitoring. For example, Phocidae (earless seals)

498 are known to migrate through the area; Anatidae (ducks), Haematopodidae (oystercatchers),
499 Procellaridae (petrels), and Laridae (gulls) use the shallow mudflats as a foraging ground; and
500 Sphenicidae (penguins) have nesting grounds on the banks of Patterson Inlet. Finally, DNA
501 signals were picked up from organisms in the surrounding areas. Galaxiidae (galaxids) and
502 Eleotridae (sleeper gobies) are two families of freshwater fish residing in the streams and
503 rivers around Patterson Inlet, while the DNA signal from Trachichthyidae (roughies) and
504 Callorhinchidae (elephant sharks) probably originate from the deeper waters of Foveaux
505 Strait. DNA signals from terrestrial mammals were also detected, such as Cervidae (deer),
506 Suidae (boars), Canidae (canids), and Muridae (rodents), indicating the power of eDNA
507 metabarcoding surveys for detecting an increased proportion of biodiversity in a non-invasive
508 and inexpensive manner compared to BRUV.
509
510 Although an increased number of families were detected by eDNA, taxonomic identification
511 and assignment is limited by the reference database and hindered by the taxonomic resolution
512 of short DNA fragments (Stat et al., 2017). To reduce the risk of misidentification and assure
513 correct prediction of presence of each taxon, we filtered the sequencing data stringently and
514 analysed the taxonomic assignments on the family level. However, external expertise in New
515 Zealand marine taxonomy still revealed the presence of an unlikely family in our eDNA
516 dataset (see *Acknowledgement*). Sciaenidae (drums), identified by our 16S-Fish assay,
517 typically occur in warm-temperate and tropical waters, and have not been detected around
518 Stewart Island. Further investigation of the reads classified as Sciaenidae by applying
519 BLAST (Altschul et al., 1990) to the NCBI database revealed that the Latridae and
520 Cheilodactylidae families, which are both native to Southern New Zealand waters, resulted in
521 generally better alignment scores. However, as no full-length sequence of the targeted 16S
522 region of these two families was available in the database, these sequences were discarded in

523 our initial taxonomic assignment where we used a threshold of 100 % coverage of the
524 sequence. Hence, our sequences were assigned to the Sciaenidae family (SUPPLEMENT 7).
525 This discovery provides insight into the potential risk of taxonomic misclassifications in
526 eDNA surveys and highlights the dependency of any eDNA survey on complete and unbiased
527 databases.
528
529 While our results show that eDNA metabarcoding surveys are able to detect a greater portion
530 of the biodiversity compared to BRUV, eDNA is currently unable to provide essential
531 information needed for successful fisheries management and conservation. Only weak
532 correlations between eDNA signal strength, qPCR efficiency for species-specific assays, or
533 number of sequencing reads with abundance or biomass have been detected thus far
534 (Andruszkiewicz et al., 2017; Sassoubre et al., 2016b; Stoeckle et al., 2017). It is technically
535 possible to retrieve sex ratios from environmental samples when employing sex-specific
536 markers, but eDNA studies have not yet been leveraged to investigate this issue (Brunelli &
537 Thorgaard, 2004; Dan et al., 2013). Vital status as well as weight, age and health estimates,
538 on the other hand, cannot currently be retrieved from environmental DNA surveys, but such
539 parameters can be monitored by traditional methods (Lewis et al., 2020; Mallet & Pelletier,
540 2014). Recently, eRNA studies have been employed to differentiate between signals
541 originating from live or dead organisms (Laroche et al., 2017; Pochon et al., 2017; Wood et
542 al., 2020) and new tools may eventuate for determining the age of species via population
543 level analyses (Adams et al., 2019) of e.g. epigenetic signals in eDNA data (Sigsgaard et al.,
544 2020). However, for the foreseeable future, we recommend the ongoing use of a combination
545 of survey techniques to monitor the coastal environment, with eDNA surveys providing an
546 overview of the diversity of the area and traditional techniques delivering in-depth
547 information about taxa and populations of interest (Stat et al., 2019).

548

549 *Spatial resolution of eDNA in an estuarine environment*

550 The diversity we recorded in our eDNA survey showed a fine-scale spatial gradient between
551 oceanic influx (PAB sampling site) and freshwater input (SB/SDB sampling site) (FIGURE
552 3C, D) despite high levels of water exchange from current speeds and wind-driven as well as
553 baroclinic forcing (O'Callaghan, 1999). The eDNA signals from the four different locations
554 were significantly different with respect to taxon richness and composition. Similar eDNA
555 signals were retrieved, on the other hand, for the temporal Sawdust Bay (SB and SDB)
556 replication, indicating a temporally stable eDNA signal of the residing community. Assessing
557 the temporal resolution of eDNA was, however, outside the scope of this experiment. While
558 seasonal patterns have been observed through eDNA surveys (Berry et al., 2019), additional
559 work is needed to determine the effect of migratory organisms on the temporal resolution of
560 marine eDNA.

561

562 Our MCA and indicator species analyses revealed the spatial structure of the eDNA signal
563 was driven by taxa with a strong habitat-preference, which are known to occur in Patterson
564 Inlet. Our results further show that the pooling of eDNA assays improves the resolution of
565 inter-locational changes in taxonomic composition. We propose that our pooled eDNA
566 approach can be leveraged to define spatial taxonomic gradients and could therefore assist in
567 the design of future marine protected areas.

568

569 High spatial resolutions have been recorded in previous research (Jeunen et al., 2019b, 2019a;
570 Port et al., 2016; West et al., 2020). However, the reported spatial scale ranges from meters to
571 kilometres and might be impacted by oceanographic processes (Jeunen et al., 2019b).
572 Additionally, the detection strategy in eDNA surveys (species-specific amplification, targeted

metabarcoding, universal metabarcoding) might also have an impact on spatial resolution due to the increased complexity of the signal and inability to detect low-abundant over high-abundant signals ("dilution effect"). Unlike the well-defined sampling area of a BRUV survey, varying spatial resolutions that are specific to a sampling area for eDNA surveys hinder the ability to define the sampled area, potentially complicating the experimental sampling design.

Taxa of interest

Besides marine taxa, our eDNA survey detected terrestrial species, such as the common brushtail possum (*T. vulpecula*), rodents, deer, and boars. These eDNA signals most likely originate from freshwater sources such as river or rainfall being discharged into the estuarine system. Previous research determined rivers to be a better sampling medium compared to sediment/soil for semi-aquatic and terrestrial mammals (Sales et al., 2020; Yang et al., 2020). Our results extend this finding to nearby estuarine and coastal environments, opening up the possibility of surveillance of invasive terrestrial species on remote offshore islands by collecting water samples along the coastline. For example, two of the detected terrestrial taxa in our eDNA survey, the common brushtail possum and rodents, are currently actively being removed from New Zealand islands, including Stewart Island, through trapping (Jackson et al., 2016). This costly eradication incentive might be aided by routine monitoring via eDNA surveys. However, additional research is required to determine the sensitivity of eDNA surveys conducted in coastal environments in detecting invasive terrestrial species (Russell & Holmes, 2015).

Conclusion

Effective monitoring methods delivering accurate measurements on the residing community are essential for successful management and conservation efforts. In this study, we provide evidence for the power of eDNA metabarcoding surveys to describe the fish community in an estuarine environment. However, our data shows the importance of a robust experimental design and the need for increased sampling effort (Grey et al., 2018), as well as for applying multiple assays to reduce the risk of false-negative species detections (Jeunen et al., 2019a). Furthermore, the high spatial resolution of eDNA allows for the differentiation between communities on small spatial scales. Environmental DNA surveys could therefore be used to provide information on ecological gradients and the impact of marine protected areas in a non-invasive manner. As eDNA surveys are currently unable to retrieve important metrics on the residing community (e.g., sex ratios, size, weight, abundance), we recommend the combined use of eDNA surveys and traditional monitoring techniques to allow for the detection of a broader range of the taxonomic diversity in the area of interest and the detailed information on taxa of interest.

Data availability

Dereplicated FASTQ files from all eDNA assays will be made available via the Sequence Read Archive (SRA) upon acceptance of this manuscript. Bioinformatic and statistical code will be made available on GitHub upon acceptance of this manuscript.

Acknowledgement

We thank Clive Roberts from the Te Papa Tongarewa Museum for valuable feedback on the occurrence of taxa in New Zealand waters; Kate McDonald and University of Otago's Portobello Marine Lab technicians for their help during sampling; and Joanne Gillum, as well as Sara Ferreira for their assistance in the laboratory. The sequencing data were generated by

Otago Genomics. Data preprocessing and taxonomic assignment was generated with the help of the BOROS server at the Department of Anatomy, University of Otago.

Funding

GJJ was supported by grants from the University of Otago. LU was funded by a Feodor Lynen Research Fellowship provided by the Alexander von Humboldt Foundation, Germany, and grants to NJG from the University of Otago. Laboratory resources, boat time, and field support were funded through the University of Otago.

Author contributions

GJJ, MK, ML, RL, WR, and NJG conceived the study. GJJ and RL realised the field work. GJJ completed the lab work. GJJ, LU, RL, WR, and NJG contributed to the processing and interpretation of the data. LU performed the statistical analyses. All authors contributed to the writing of the manuscript.

638 **Tables**

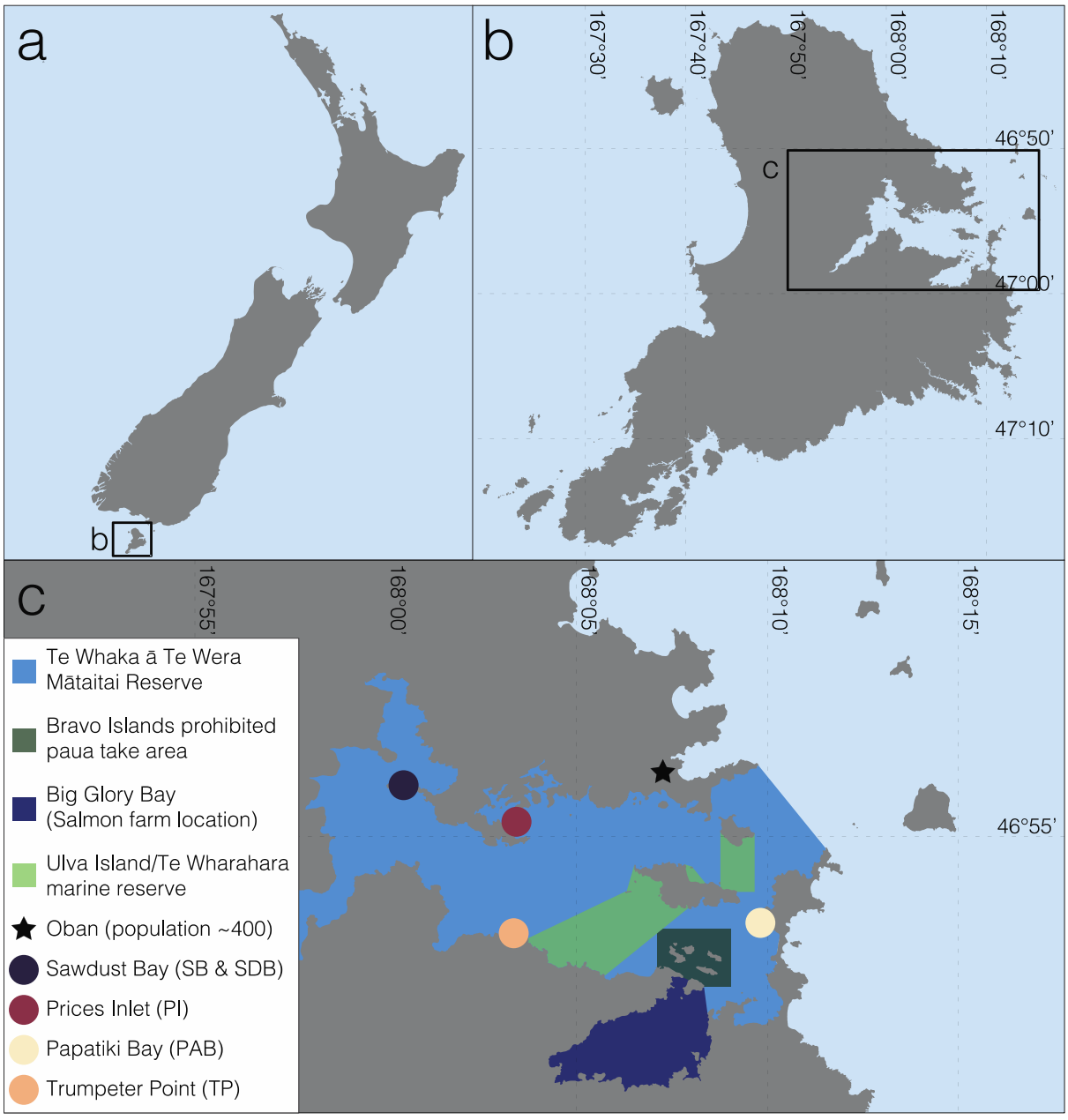
639 **Table 1.** Taxonomic richness detected across eDNA analyses (16S-Fish, MiFish-U, MiFish-
640 E, and pooled assays ("All")). **γ** richness across all locations, median **α** richness and **ß**
641 richness as represented by Jaccard Index (JI) and Simpson Index (SI) are shown.

Assay	**γ**	**α** (median)	**ß** (JI)	**ß** (SI)
All	56	33	0.958	0.881
16S-Fish	23	13	0.924	0.813
MiFish-U	32	17	0.937	0.840
MiFish-E	42	25	0.950	0.855

642

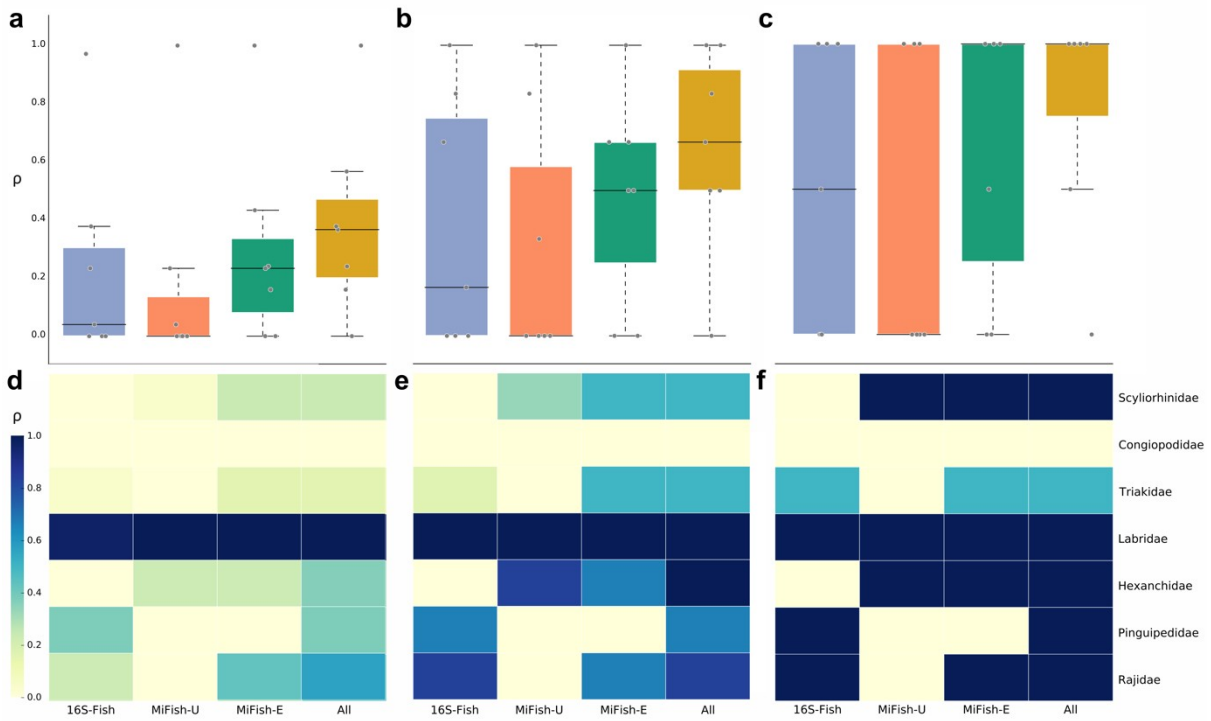
643

644 **Figures**



645

646 **Figure 1.** Sampling locations. The study area is located in the South of New Zealand/Aotearoa (**a**), in
647 the largest natural harbour of Stewart Island/Rakiura (**b**), Paterson Inlet (**c**). The within-bay location,
648 Sawdust Bay, was sampled twice (samples SB & SDB). The Papatiki Bay (PAB) sampling location is
649 located close to the open sea, and Prices Inlet (PI) and Trumpeter Point (TP) are intermediate
650 locations. The only settlement on the island, Oban, is indicated by a star. Various established marine
651 reserves, prohibited zones and the location of a salmon farm are indicated by colour (adopted from
652 https://www.fisheries.govt.nz/dmsdocument/931/).



653

654 **Figure 2.** One-to-one comparisons between eDNA analyses (16S-Fish, MiFish-U, Mifish-E, and
655 pooled ("All") assays) and BRUV results. (**a-c)** Proportion, **ρ**, of BRUV-positive samples that were
656 eDNA-positive across the seven observed families (y-axis), per eDNA assay (x-axis). Boxes indicate
657 1st and 3rd quartile of the data distribution, and whiskers indicate Q1-1.5*IQR (interquartile range) and
658 Q3+1.5*IQR, respectively. (**d-f**) Detection rate of individual taxa (y-axis) per eDNA assay (x-axis)
659 across BRUV-positive locations. From left to right: Different levels of sample and spatial pooling of
660 the individual samples: (**a, d**) Sample-level. (**b, e**) Site-level after sample sum-pooling. (**c, f**)
661 Location-level after additional spatial sum-pooling.

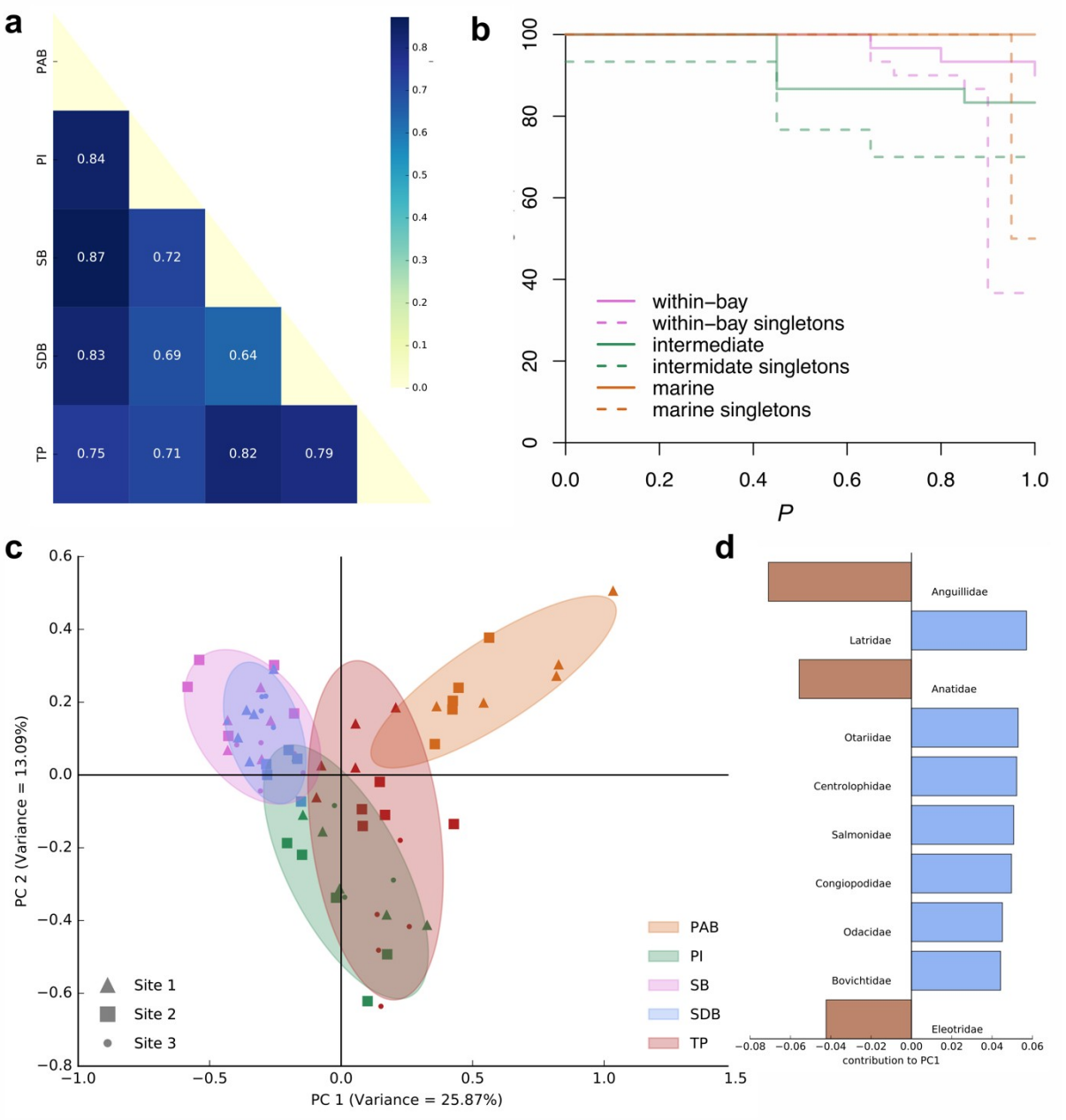


Figure 3. Pooled eDNA monitoring results. (**a**) ß richness between locations as represented by pairwise Simpson Index (SI). (**b**) Strong habitat predictability by indicator species analysis for within-bay (SB, SDB), intermediate (PI, TP) and marine (PAB) locations. The coverage ([%]; y-axis) represents the proportion of sites of the target site group at which one or another indicator is found, and ***P*** represents the positive predictive value of the indicator species analysis. Per location, we performed the analysis based on a single ("singleton"; dashed line) taxon or on combinations of taxa (up to five; continuous line). (**c**) Multiple Correspondence Analysis (MCA) of taxonomic composition of all samples. Samples within a habitat are distinguished by shape, sampling sites by colour. The ellipses show the approximate span of sample composition per sample location. SB and SDB ranges as temporal replicates are highly overlapping. (**d**) MCA contribution plot showing the contribution of each taxon to the variance capture by the first principal component (PC1).

675 **References**

676 Acosta, A. (1997). Use of multi-mesh gillnets and trammel nets to estimate fish species
677 composition in coral reef and mangroves in the southwest coast of Puerto Rico.
678 *Caribbean Journal of Science*, *33*, 45–57.
679 Adams, C. I. M., Knapp, M., Gemmell, N. J., Jeunen, G.-J., Bunce, M., Lamare, M. D., &
680 Taylor, H. R. (2019). Beyond biodiversity: can environmental DNA (eDNA) cut it as a
681 population genetics tool? *Genes*, *10*(3), 192.
682 Afzali, S. F., Bourdages, H., Laporte, M., Mérot, C., Normandeau, E., Audet, C., &
683 Bernatchez, L. (2020). Comparing environmental metabarcoding and trawling survey of
684 demersal fish communities in the Gulf of St. Lawrence, Canada. *Environmental DNA*, *n/*
685 *a*(n/a). https://doi.org/10.1002/edn3.111
686 Alexander, J. B., Bunce, M., White, N., Wilkinson, S. P., Adam, A. A. S., Berry, T., Stat, M.,
687 Thomas, L., Newman, S. J., Dugal, L., & Richards, Z. T. (2020). Development of a
688 multi-assay approach for monitoring coral diversity using eDNA metabarcoding. *Coral*
689 *Reefs*, *39*(1), 159–171. https://doi.org/10.1007/s00338-019-01875-9
690 Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local
691 alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410.
692 https://doi.org/10.1016/S0022-2836(05)80360-2
693 Andradi-Brown, D. A., Macaya-Solis, C., Exton, D. A., Gress, E., Wright, G., & Rogers, A.
694 D. (2016). Assessing Caribbean Shallow and Mesophotic Reef Fish Communities Using
695 Baited-Remote Underwater Video (BRUV) and Diver-Operated Video (DOV) Survey
696 Techniques. *PLOS ONE*, *11*(12), e0168235.
697 https://doi.org/10.1371/journal.pone.0168235
698 Andruszkiewicz, E. A., Sassoubre, L. M., & Boehm, A. B. (2017). Persistence of marine fish
699 environmental DNA and the influence of sunlight. *PLoS ONE*, *12*(9), 1–18.
700 https://doi.org/10.1371/journal.pone.0185043
701 Ayling, T. (1987). *Collins guide to the sea fishes of New Zealand / by Tony Ayling ; with 48*
702 *colour plates by Geoffrey J. Cox and 475 line drawings by the author* (G. J. Cox 1952-
703 (ed.)). Collins.
704 Bacheler, N., Geraldi, N., Burton, M., Muñoz, R., & Kellison, G. (2017). Comparing relative
705 abundance, lengths, and habitat of temperate reef fishes using simultaneous underwater
706 visual census, video, and trap sampling. *Marine Ecology Progress Series*, *574*.
707 https://doi.org/10.3354/meps12172
708 Bakker, J., Wangensteen, O. S., Chapman, D. D., Boussarie, G., Buddo, D., Guttridge, T. L.,
709 Hertler, H., Mouillot, D., Vigliola, L., & Mariani, S. (2017). Environmental DNA
710 reveals tropical shark diversity in contrasting levels of anthropogenic impact. *Scientific*
711 *Reports*, *7*(1), 16886. https://doi.org/10.1038/s41598-017-17150-2
712 Baselga, A., & Orme, C. D. L. (2012). betapart: an R package for the study of beta diversity.
713 *Methods in Ecology and Evolution*, *3*(5), 808–812. https://doi.org/10.1111/j.2041-
714 210X.2012.00224.x
715 Berry, T. E., Osterrieder, S. K., Murray, D. C., Coghlan, M. L., Richardson, A. J., Grealy, A.
716 K., Stat, M., Bejder, L., & Bunce, M. (2017). DNA metabarcoding for diet analysis and
717 biodiversity: A case study using the endangered Australian sea lion (Neophoca cinerea).
718 *Ecology and Evolution*, *7*(14), 5435–5453. https://doi.org/10.1002/ece3.3123
719 Berry, T. E., Saunders, B. J., Coghlan, M. L., Stat, M., Jarman, S., Richardson, A. J., Davies,
720 C. H., Berry, O., Harvey, E. S., & Bunce, M. (2019). Marine environmental DNA
721 biomonitoring reveals seasonal patterns in biodiversity and identifies ecosystem
722 responses to anomalous climatic events. *PLoS Genetics*, *15*(2), e1007943–e1007943.
723 https://doi.org/10.1371/journal.pgen.1007943

724 Bioinformatics, B. (2011). FastQC: a quality control tool for high throughput sequence data.
725 *Cambridge, UK: Babraham Institute*.
726 Boulinier, T., Nichols, J. D., Sauer, J. R., Hines, J. E., & Pollock, K. H. (1998).
727 ESTIMATING SPECIES RICHNESS: THE IMPORTANCE OF HETEROGENEITY
728 IN SPECIES DETECTABILITY. *Ecology*, *79*(3), 1018–1028.
729 https://doi.org/10.1890/0012-9658(1998)079[1018:ESRTIO]2.0.CO;2
730 Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). obitools: a
731 unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*,
732 *16*(1), 176–182. https://doi.org/doi:10.1111/1755-0998.12428
733 Brunelli, J. P., & Thorgaard, G. H. (2004). A New Y-Chromosome-Specific Marker for
734 Pacific Salmon. *Transactions of the American Fisheries Society*, *133*(5), 1247–1253.
735 https://doi.org/10.1577/T03-049.1
736 Cáceres, M. De, & Legendre, P. (2009). Associations between species and groups of sites:
737 indices and statistical inference. *Ecology*, *90*(12), 3566–3574.
738 Cappo, M., Speare, P., & De'ath, G. (2004). Comparison of baited remote underwater video
739 stations (BRUVS) and prawn (shrimp) trawls for assessments of fish biodiversity in
740 inter-reefal areas of the Great Barrier Reef Marine Park. *Journal of Experimental*
741 *Marine Biology and Ecology*, *302*(2), 123–152.
742 https://doi.org/https://doi.org/10.1016/j.jembe.2003.10.006
743 Colton, M., & Swearer, S. (2010). A comparison of two survey methods: Differences
744 between underwater visual census and baited remote underwater video. *Marine Ecology-*
745 *Progress Series - MAR ECOL-PROGR SER*, *400*, 19–36.
746 https://doi.org/10.3354/meps08377
747 Dan, C., Mei, J., Wang, D., & Gui, J.-F. (2013). Genetic differentiation and efficient sex-
748 specific marker development of a pair of Y- and X-linked markers in yellow catfish.
749 *International Journal of Biological Sciences*, *9*(10), 1043–1049. https://doi.org/10.7150/
750 ijbs.7203
751 DiBattista, J. D., Reimer, J. D., Stat, M., Masucci, G. D., Biondi, P., De Brauwer, M.,
752 Wilkinson, S. P., Chariton, A. A., & Bunce, M. (2020). Environmental DNA can act as a
753 biodiversity barometer of anthropogenic pressures in coastal ecosystems. *Scientific*
754 *Reports*, *10*(1), 8365. https://doi.org/10.1038/s41598-020-64858-9
755 Dickens, L. C., Goatley, C. H. R., Tanner, J. K., & Bellwood, D. R. (2011). Quantifying
756 relative diver effects in underwater visual censuses. *PloS One*, *6*(4), e18965.
757 Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST.
758 *Bioinformatics (Oxford, England)*, *26*(19), 2460–2461.
759 https://doi.org/10.1093/bioinformatics/btq461
760 Ficetola, G. F., Miaud, C., Pompanon, F., & Taberlet, P. (2008). Species detection using
761 environmental DNA from water samples. *Biology Letters*, *4*(4), 423 LP – 425.
762 http://rsbl.royalsocietypublishing.org/content/4/4/423.abstract
763 Garlapati, D., Charankumar, B., Ramu, K., Madeswaran, P., & Ramana Murthy, M. V.
764 (2019). A review on the applications and recent advances in environmental DNA
765 (eDNA) metagenomics. *Reviews in Environmental Science and Bio/Technology*, *18*(3),
766 389–411. https://doi.org/10.1007/s11157-019-09501-4
767 Greenacre, M., & Blasius, J. (2006). *Multiple correspondence analysis and related methods*.
768 CRC press.
769 Grey, E. K., Bernatchez, L., Cassey, P., Deiner, K., Deveney, M., Howland, K. L.,
770 Lacoursière-Roussel, A., Leong, S. C. Y., Li, Y., Olds, B., Pfrender, M. E., Prowse, T.
771 A. A., Renshaw, M. A., & Lodge, D. M. (2018). Effects of sampling effort on
772 biodiversity patterns estimated from environmental DNA metabarcoding surveys.
773 *Scientific Reports*, *8*(1), 8843. https://doi.org/10.1038/s41598-018-27048-2

774 Hajibabaei, M., Porter, T. M., Wright, M., & Rudar, J. (2019). COI metabarcoding primer
775 choice affects richness and recovery of indicator taxa in freshwater systems. *PLOS ONE*,
776 *14*(9), e0220953. https://doi.org/10.1371/journal.pone.0220953
777 Jackson, M., Hartley, S., & Linklater, W. (2016). Better food-based baits and lures for
778 invasive rats Rattus spp. and the brushtail possum Trichosurus vulpecula: a bioassay on
779 wild, free-ranging animals. *Journal of Pest Science*, *89*(2), 479–488.
780 https://doi.org/10.1007/s10340-015-0693-8
781 Jeunen, G. J., Knapp, M., Spencer, H. G., Lamare, M. D., Taylor, H. R., Stat, M., Bunce, M.,
782 & Gemmell, N. J. (2019a). Environmental DNA (eDNA) metabarcoding reveals strong
783 discrimination among diverse marine habitats connected by water movement. *Molecular*
784 *Ecology Resources*, *19*(2), 426–438. https://doi.org/10.1111/1755-0998.12982
785 Jeunen, G. J., Knapp, M., Spencer, H. G., Taylor, H. R., Lamare, M. D., Stat, M., Bunce, M.,
786 & Gemmell, N. J. (2018). Species-level biodiversity assessment using marine
787 environmental DNA metabarcoding requires protocol optimization and standardization.
788 *Ecology and Evolution*, *9*(3), 1323–1335. https://doi.org/doi:10.1002/ece3.4843
789 Jeunen, G. J., Lamare, M. D., Knapp, M., Spencer, H. G., Taylor, H. R., Stat, M., Bunce, M.,
790 & Gemmell, N. J. (2019b). Water stratification in the marine biome restricts vertical
791 environmental DNA (eDNA) signal dispersal. *Environmental DNA*.
792 https://onlinelibrary.wiley.com/doi/abs/10.1002/edn3.49
793 Karnauskas, M. (2012). Comparisons between abundance estimates from underwater visual
794 census and catch-per-unit-effort in a patch reef system . *Marine Ecology Progress*
795 *Series*, *468*, 217–230. https://www.int-res.com/abstracts/meps/v468/p217-230/
796 Kelly, R. P., Closek, C. J., O'Donnell, J. L., Kralj, J. E., Shelton, A. O., & Samhouri, J. F.
797 (2017). Genetic and Manual Survey Methods Yield Different and Complementary
798 Views of an Ecosystem. *Frontiers in Marine Science*, *3*, 283.
799 https://doi.org/10.3389/fmars.2016.00283
800 Kelly, R. P., Gallego, R., & Jacobs-Palmer, E. (2018). The effect of tides on nearshore
801 environmental DNA. *PeerJ*, *6*, e4521. https://doi.org/10.7717/peerj.4521
802 Koziol, A., Stat, M., Simpson, T., Jarman, S., DiBattista, J. D., Harvey, E. S., Marnane, M.,
803 McDonald, J., & Bunce, M. (2019). Environmental DNA metabarcoding studies are
804 critically affected by substrate selection. *Molecular Ecology Resources*, *19*(2), 366–376.
805 https://doi.org/doi:10.1111/1755-0998.12971
806 Laroche, O., Wood, S. A., Tremblay, L. A., Lear, G., Ellis, J. I., & Pochon, X. (2017).
807 Metabarcoding monitoring analysis: the pros and cons of using co-extracted
808 environmental DNA and RNA data to assess offshore oil production impacts on benthic
809 communities. *PeerJ*, *5*, e3347. https://doi.org/10.7717/peerj.3347
810 Lewis, R., Dawson, S., & Rayment, W. (2020). Estimating population parameters of
811 broadnose sevengill sharks (Notorynchus cepedianus) using photo identification capture-
812 recapture. *Journal of Fish Biology*, *n/a*(n/a). https://doi.org/10.1111/jfb.14453
813 MacNeil, M. A., Graham, N. A. J., Conroy, M. J., Fonnesbeck, C. J., Polunin, N. V. C.,
814 Rushton, S. P., Chabanet, P., & McClanahan, T. R. (2008). Detection heterogeneity in
815 underwater visual-census data. *Journal of Fish Biology*, *73*(7), 1748–1763.
816 https://doi.org/10.1111/j.1095-8649.2008.02067.x
817 Mallet, D., & Pelletier, D. (2014). Underwater video techniques for observing coastal marine
818 biodiversity: A review of sixty years of publications (1952–2012). *Fisheries Research*,
819 *154*, 44–62. https://doi.org/https://doi.org/10.1016/j.fishres.2014.01.019
820 Matthews, T. J., & Whittaker, R. J. (2015). On the species abundance distribution in applied
821 ecology and biodiversity management. *Journal of Applied Ecology*, *52*(2), 443–454.
822 https://doi.org/10.1111/1365-2664.12380
823 Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T.,

Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., & Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *Royal Society Open Science*, *2*(7), 150088. https://doi.org/10.1098/rsos.150088

Monk, J. (2014). How long should we ignore imperfect detection of species in the marine environment when modelling their distribution? *Fish and Fisheries*, *15*(2), 352–358. https://doi.org/10.1111/faf.12039

Murakami, H., Yoon, S., Kasai, A., Minamoto, T., Yamamoto, S., Sakata, M. K., Horiuchi, T., Sawada, H., Kondoh, M., Yamashita, Y., & Masuda, R. (2019). Dispersion and degradation of environmental DNA from caged fish in a marine environment. *Fisheries Science*, *85*(2), 327–337. https://doi.org/10.1007/s12562-018-1282-6

Murphy, H., & Jenkins, G. (2010). Observational methods used in marine spatial monitoring of fishes and associated habitats: A review. *Marine and Freshwater Research - MAR FRESHWATER RES*, *61*. https://doi.org/10.1071/MF09068

Murray, D. C., Coghlan, M. L., & Bunce, M. (2015). From Benchtop to Desktop: Important Considerations when Designing Amplicon Sequencing Workflows. *PLOS ONE*, *10*(4), 1–21. https://doi.org/10.1371/journal.pone.0124671

Nester, G. M., De Brauwer, M., Koziol, A., West, K. M., DiBattista, J. D., White, N. E., Power, M., Heydenrych, M. J., Harvey, E., & Bunce, M. (2020). Development and evaluation of fish eDNA metabarcoding assays facilitate the detection of cryptic seahorse taxa (family: Syngnathidae). *Environmental DNA*, *n/a*(n/a). https://doi.org/10.1002/edn3.93

O'Callaghan, M. D. (1999). *Exchange of nutrients from Big Glory Bay, Stewart Island*. University of Otago.

O'Donnell, J. L., Kelly, R. P., Shelton, A. O., Samhouri, J. F., Lowell, N. C., & Williams, G. D. (2017). Spatial distribution of environmental DNA in a nearshore marine habitat. *PeerJ*, *5*, e3044. https://doi.org/10.7717/peerj.3044

Pais, M. P., & Cabral, H. N. (2018). Effect of underwater visual survey methodology on bias and precision of fish counts: a simulation approach. *PeerJ*, *6*, e5378–e5378. https://doi.org/10.7717/peerj.5378

Pochon, X., Zaiko, A., Fletcher, L. M., Laroche, O., & Wood, S. A. (2017). Wanted dead or alive? Using metabarcoding of environmental DNA and RNA to distinguish living assemblages for biosecurity applications. *PLOS ONE*, *12*(11), e0187636. https://doi.org/10.1371/journal.pone.0187636

Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J., Yamahara, K. M., & Kelly, R. P. (2016). Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Molecular Ecology*, *25*(2), 527–541. https://doi.org/10.1111/mec.13481

Pridmore, R., & Rutherford, J. C. (1990). *Factors affecting phytoplankton abundance in Big Glory Bay, Stewart Island: a modelling study*. Water Quality Centre.

Prince, A. M., & Andrus, L. (1992). PCR: how to kill unwanted DNA. *BioTechniques*, *12*(3), 358–360.

Russell, J. C., & Holmes, N. D. (2015). Tropical island conservation: Rat eradication for species recovery. *Biological Conservation*, *185*, 1–7. https://doi.org/https://doi.org/10.1016/j.biocon.2015.01.009

Sales, N. G., McKenzie, M. B., Drake, J., Harper, L. R., Browett, S. S., Coscia, I., Wangensteen, O. S., Baillie, C., Bryce, E., Dawson, D. A., Ochu, E., Hänfling, B., Lawson Handley, L., Mariani, S., Lambin, X., Sutherland, C., & McDevitt, A. D. (2020). Fishing for mammals: Landscape-level monitoring of terrestrial and semi-aquatic communities using eDNA from riverine systems. *Journal of Applied Ecology*,

874 *57*(4), 707–716. https://doi.org/10.1111/1365-2664.13592
875 Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016a).
876 Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three
877 Marine Fish. *Environmental Science and Technology*, *50*(19), 10456–10464.
878 https://doi.org/10.1021/acs.est.6b03114
879 Sassoubre, L. M., Yamahara, K. M., Gardner, L. D., Block, B. A., & Boehm, A. B. (2016b).
880 Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three
881 Marine Fish. *Environmental Science & Technology*, *50*(19), 10456–10464.
882 https://doi.org/10.1021/acs.est.6b03114
883 Schmid, K., Reis-Filho, J., Harvey, E., & Giarrizzo, T. (2016). Baited remote underwater
884 video as a promising nondestructive tool to assess fish assemblages in clearwater
885 Amazonian rivers: testing the effect of bait and habitat type. *Hydrobiologia*, *784*. https://
886 doi.org/10.1007/s10750-016-2860-1
887 Sigsgaard, E. E., Jensen, M. R., Winkelmann, I. E., Møller, P. R., Hansen, M. M., &
888 Thomsen, P. F. (2020). Population-level inferences from environmental DNA—Current
889 status and future perspectives. *Evolutionary Applications*, *13*(2), 245–262.
890 https://doi.org/10.1111/eva.12882
891 Smith, A. M., McGourty, C. R., Kregting, L., & Elliot, A. (2005). Subtidal Galeolaria hystrix
892 (Polychaeta: Serpulidae) reefs in Paterson Inlet, Stewart Island, New Zealand. *New*
893 *Zealand Journal of Marine and Freshwater Research*, *39*(6), 1297–1304. https://doi.org/
894 10.1080/00288330.2005.9517394
895 Stat, M., Huggett, M. J., Bernasconi, R., DiBattista, J. D., Berry, T. E., Newman, S. J.,
896 Harvey, E. S., & Bunce, M. (2017). Ecosystem biomonitoring with eDNA:
897 metabarcoding across the tree of life in a tropical marine environment. *Scientific*
898 *Reports*, *7*(1), 12240. https://doi.org/10.1038/s41598-017-12501-5
899 Stat, M., John, J., DiBattista, J. D., Newman, S. J., Bunce, M., & Harvey, E. S. (2019).
900 Combined use of eDNA metabarcoding and video surveillance for the assessment of fish
901 biodiversity. *Conservation Biology*, *33*(1), 196–205.
902 https://doi.org/doi:10.1111/cobi.13183
903 Stobart, B., Díaz, D., Álvarez, F., Alonso, C., Mallol, S., & Goñi, R. (2015). Performance of
904 Baited Underwater Video: Does It Underestimate Abundance at High Population
905 Densities? *PLOS ONE*, *10*(5), e0127559. https://doi.org/10.1371/journal.pone.0127559
906 Stoeckle, M. Y., Soboleva, L., & Charlop-Powers, Z. (2017). Aquatic environmental DNA
907 detects seasonal fish abundance and habitat preference in an urban estuary. *PLoS ONE*,
908 *12*(4), 1–15. https://doi.org/10.1371/journal.pone.0175186
909 Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., & Kawabata, Z. (2012). Estimation of
910 Fish Biomass Using Environmental DNA. *PLOS ONE*, *7*(4), 1–8.
911 https://doi.org/10.1371/journal.pone.0035868
912 Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M., & Willerslev, E.
913 (2012). Detection of a Diverse Marine Fish Fauna Using Environmental DNA from
914 Seawater Samples. *PLOS ONE*, *7*(8), e41732.
915 https://doi.org/10.1371/journal.pone.0041732
916 Thomsen, P. F., Møller, P. R., Sigsgaard, E. E., Knudsen, S. W., Jørgensen, O. A., &
917 Willerslev, E. (2016). Environmental DNA from Seawater Samples Correlate with
918 Trawl Catches of Subarctic, Deepwater Fishes. *PLOS ONE*, *11*(11), e0165252.
919 https://doi.org/10.1371/journal.pone.0165252
920 Thorngren, L., Dunér Holthuis, T., Lindegarth, S., & Lindegarth, M. (2017). Developing
921 methods for assessing abundance and distribution of European oysters (Ostrea edulis)
922 using towed video. *PLoS ONE*, *12*(11), e0187870.
923 https://doi.org/10.1371/journal.pone.0187870

924 Urban, L., Holzer, A., Baronas, J. J., Hall, M., Braeuninger-Weimer, P., Scherm, M. J., Kunz,
925 D. J., Perera, S. N., Martin-Herranz, D. E., Tipper, E. T., Salter, S. J., & Stammnitz, M.
926 R. (2020). Freshwater monitoring by nanopore sequencing. *BioRxiv*, 2020.02.06.936302.
927 https://doi.org/10.1101/2020.02.06.936302
928 Uthicke, S., Lamare, M., & Doyle, J. R. (2018). eDNA detection of corallivorous seastar
929 (Acanthaster cf. solaris) outbreaks on the Great Barrier Reef using digital droplet PCR.
930 *Coral Reefs*, *37*(4), 1229–1239. https://doi.org/10.1007/s00338-018-1734-6
931 Valdivia-Carrillo, T., Rocha-Olivares, A., Reyes-Bonilla, H., Domínguez-Contreras, J. F., &
932 Munguia-Vega, A. (2019). Beyond traditional biodiversity fish monitoring:
933 environmental DNA metabarcoding and simultaneous underwater visual census detect
934 different sets of a complex fish community at a marine biodiversity hotspot. *BioRxiv*,
935 806729. https://doi.org/10.1101/806729
936 West, K. M., Stat, M., Harvey, E. S., Skepper, C. L., DiBattista, J. D., Richards, Z. T.,
937 Travers, M. J., Newman, S. J., & Bunce, M. (2020). eDNA metabarcoding survey
938 reveals fine-scale coral reef community variation across a remote, tropical island
939 ecosystem. *Molecular Ecology*, *29*(6), 1069–1086. https://doi.org/10.1111/mec.15382
940 White, J., Simpfendorfer, C., Tobin, A., & Heupel, M. (2013). Application of baited remote
941 underwater video surveys to quantify spatial distribution of elasmobranchs at an
942 ecosystem scale. *Journal of Experimental Marine Biology and Ecology*, *448*, 281–288.
943 https://doi.org/10.1016/j.jembe.2013.08.004
944 Willan, R. C. (1981). Soft-bottom assemblages of Paterson Inlet, Stewart Island. *New*
945 *Zealand Journal of Zoology*, *8*(2), 229–248.
946 https://doi.org/10.1080/03014223.1981.10427964
947 Willis, T., & Babcock, R. (2000). A baited underwater video system for the determination of
948 relative density of carnivorous reef fish. *Marine and Freshwater Research*, *51*, 755–763.
949 Wood, S. A., Biessy, L., Latchford, J. L., Zaiko, A., von Ammon, U., Audrezet, F., Cristescu,
950 M. E., & Pochon, X. (2020). Release and degradation of environmental DNA and RNA
951 in a marine system. *Science of The Total Environment*, *704*, 135314.
952 https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.135314
953 Yang, H., Du, H., Qi, H., Yu, L., Hou, X., Zhang, H., Li, J., Wu, J., Wang, C., Zhou, Q., &
954 Wei, Q. (2020). Simultaneously monitoring aquatic and terrestrial biodiversity using
955 riverine water eDNA: seasonal variation of monitoring effectiveness. *BioRxiv*,
956 2020.06.20.162388. https://doi.org/10.1101/2020.06.20.162388
957 Yang, J., Zhang, X., Xie, Y., Song, C., Zhang, Y., Yu, H., & Burton, G. A. (2017).
958 Zooplankton Community Profiling in a Eutrophic Freshwater Ecosystem-Lake Tai Basin
959 by DNA Metabarcoding. *Scientific Reports*, *7*, 1773. https://doi.org/10.1038/s41598-
960 017-01808-y
961 Young, M. J., Dutoit, L., Robertson, F., van Heezik, Y., Seddon, P. J., & Robertson, B. C.
962 (2020). Species in the faeces: DNA metabarcoding as a method to determine the diet of
963 the endangered yellow-eyed penguin. *Wildlife Research*, *47*(6), 509–522. https://doi.org/
964 10.1071/WR19246
965