Supplementary Table 1. List of resources for eDNA metabarcoding data analysis applied to sustainable fisheries management. Note: this list does not pretend to be exhaustive. The tools listed were selected because 1) these appears cited in current marine or fisheries-related eDNA metabarcoding literature; 2) these are open access; 3) these are well-documented, or have been long-term used, or present unique algorithms useful for data analyses of eDNA metabarcoding.

	Tool	Tasks
	PIPELINES	
1	**OBITOOLS**. (Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). obitools: A unix-inspired software package for DNA metabarcoding. *Molecular ecology resources*, *16*(1), 176-182.) http://metabarcoding.org/obitools/doc.	**MODULAR PIPELINE** • Metabarcode design and quality assessment • File format conversions • Sequence annotations • Computations on sequences • Sequence sampling and filtering • Statistics over sequence file • Utilities
2	**MiFish (**Sato, Y., Miya, M., Fukunaga, T., Sado, T., & Iwasaki, W. (2018). MitoFish and MiFish pipeline: a mitochondrial genome database of fish with an analysis pipeline for environmental DNA metabarcoding. *Molecular biology and evolution*, *35*(6), 1553-1555.) http://mitofish.aori.u-tokyo.ac.jp	• Quality check of FASTQ file: **FastQC** • Tail trimming: **SolexaQA** • Paired-end read assembly: **FLASH** • Primer removal: **TagCleaner** • Read clustering: **Uclust/usearch** • Detection of chimeric sequences: **UCHIME** • Sequence similarity search: **BLAST+ version 2.2.29** • Multiple alignment: **MAFFT** • Phylogenetic analysis: **Molphy**

3	**Barque** (Mathon, L., Valentini, A., Guérin, P. E., Normandeau, E., Noel, C., Lionnet, C., ... & Manel, S. (2021). Benchmarking bioinformatic tools for fast and accurate eDNA metabarcoding species identification. *Molecular Ecology Resources*, *21*(7), 2565-2579.) https://github.com/enormandeau/barque	**MODULAR PIPELINE** • Filter and trim raw reads (trimmomatic) • Merge paired-end reads (flash) • Split merged reads by amplicon (Python script) • Look for chimeras (optional, vsearch --vsearch_global) • Merge unique reads (Python script) • Find species associated with each unique read (vsearch) • Summarize results (Python script) **Remarks:** Annotates reads or OTUS
4	**SLIM** *(*Dufresne, Y., Lejzerowicz, F., Apotheloz Perret-Gentil, L., Pawlowski, J., & Cordier, T. (2019). SLIM : a flexible web application for the reproducible processing of environmental DNA metabarcoding data. BMC Bioinformatics, 20(1), 88. https://doi.org/10.1186/s12859-019-2663-2) *https://github.com/yoann-dufresne/SLIM*	**MODULAR PIPELINE** • Demultiplexing the libraries DTD • Joining the paired-end reads (Pandaseq,vsearch mergepair, CASPER) • Chimera removal (vsearch uchime) • ASVs inference / OTUs clustering (DADA2; vsearch uclust; swarm) • Taxonomic assignement (vsearch usearch; IDTAXA) • Post-clustering (LULU)
5	**ANACAPA toolkit** (Curd, E. E., Gold, Z., Kandlikar, G. S., Gomer, J., Ogden, M., O'Connell, T., ... & Meyer, R. S. (2019). Anacapa Toolkit: An environmental DNA toolkit for processing multilocus metabarcode datasets. *Methods in Ecology and Evolution*, *10*(9), 1469-1475.)	**MODULAR PIPELINE** • Building reference libraries using **CRUX**

	(https://github.com/limey-bean/Anacapa/)	• Running quality control (QC) and assigning Amplicon Sequence Variants (ASV) using Dada2 (Sequence QC and ASV Parsing), • Assigning taxonomy using Bowtie 2 and a Bowtie 2 specific Bayesian Least Common Ancestor (BLCA) (Assignment) • Running exploratory data analysis and generating ecological diversity summary statistics for a set of samples using an R package ranacapa.
6	**FACEPAI** (Fast And Consistent Environmental DNA Processing AND Identification; Wahlberg, Emma. "FACEPAI: a script for fast and consistent environmental DNA processing and identification." *BMC ecology* 19.1 (2019): 1-6.)	• Conversion and Preparation of Reference Sequences (CaPReSe) • Filtering • Clustering • Assigning molecular Operational Taxonomic Units (MOTUs)
7	**eDNAFlow** (Mousavi-Derazmahalleh, M., Stott, A., Lines, R., Peverley, G., Nester, G., Simpson, T., ... & Christophersen, C. T. (2021). eDNAFlow, an automated, reproducible and scalable workflow for analysis of environmental DNA sequences exploiting Nextflow and Singularity. *Molecular Ecology Resources*, *21*(5), 1697-1704.) https://github.com/mahsa-mousavi/eDNAFlow	**MODULAR PIPELINE** • Quality filtering & demultiplexing • Threshold for forming ZOTUs • Setting blast parameters • LULU • Choice of USEARCH32 vs USEARCH64 • LCA (Lowest Common Ancestor) script for assigning taxonomy Remarks: the assigning taxonomy scripts can be used for any OTU, ZOTU, and or ASV files
8	**PIMBA** (Oliveira, R. R., Silva, R., Nunes, G. L., & Oliveira, G. (2021, November). Pimba: a pipeline for metabarcoding analysis. In *Brazilian Symposium on Bioinformatics* (pp. 106-116). Springer, Cham.)	• Preprocessing: demultiplexing and quality treatment of reads

	https://github.com/reinator/pimba	• Clustering: OTUs (with errors correction) • taxonomic assignment • alfa and beta diversity plots are built by Phyloseq including rarefaction curves and Principal Coordinates Analysis (PCoA) Remarks: only three command lines
9	**PEMA** (Zafeiropoulos, H., Viet, H. Q., Vasileiadou, K., Potirakis, A., Arvanitidis, C., Topalis, P., ... & Pafilis, E. (2020). PEMA: a flexible Pipeline for Environmental DNA Metabarcoding Analysis of the 16S/18S ribosomal RNA, ITS, and COI marker genes. *GigaScience*, *9*(3), giaa022.) https://github.com/hariszaf/pema	• OTU clustering (VSEARCH) and ASV inference (Swarm) • supports **downstream ecological analysis** of the profiles retrieved, facilitated by the phyloseq R package. REMARKS: PEMA only supports **COI** and **18S rRNA** (metazoa)
10	**VSEARCH** (Rognes T, Flouri T, Nichols B, Quince C, Mahé F. (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584.) https://github.com/torognes/vsearch	**CLUSTERING ALGORITHM** • Clustering • Chimera detection • Dereplication. • Sorting. • Subsampling. • FASTQ file processing (i.e. merging paired-ends reads).
11	**SWARM** (Mahé, F., Rognes, T., Quince, C., De Vargas, C., & Dunthorn, M. (2015). Swarm v2: highly-scalable and high-resolution amplicon clustering. *PeerJ*, *3*, e1420.) https://github.com/frederic-mahe/swarm/wiki/Fred's-metabarcoding-pipeline	**CLUSTERING ALGORITHM** • Clustering. • Dereplication. • Outputs OTU representatives in fasta format.

12	**DADA2** (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) https://benjjneb.github.io/dada2/	**CLUSTERING ALGORITHM** • Clustering • Filtering. • Dereplication. • Chimera identification • Merging of paired-end reads.
	SINGLE-STEP TOOLS FOR PRE-PROCESSING	
13	**Cutadapt** (Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal*, *17*(1), 10-12.) **https://cutadapt.readthedocs.io/en/stable/**	**CLEANNING** Finds and removes adapter sequences, primers, poly-A tails and other types of unwanted sequence from your high-throughput sequencing reads.
14	**FASTQC** **https://www.bioinformatics.babraham.ac.uk/projects/fastqc/**	**QUALITY CONTROL OF FASTQ SEQUENCES**
15	**FASTP (**Shifu Chen, Yanqing Zhou, Yaru Chen, Jia Gu; fastp: an ultra-fast all-in-one FASTQ preprocessor, Bioinformatics, Volume 34, Issue 17, 1 September 2018, Pages i884–i890) **https://github.com/OpenGene/fastp**	**PREPROCESSING FOR FASTQ FILES** • **Cleaning** • **Quality control** • **Trimming** • **…**
16	**FASTQX-toolkit** (http://hannonlab.cshl.edu/fastx_toolkit/	**PREPROCESSING FOR FASTQ FILES** • **Cleaning** • **Quality control** • **Trimming** • **…**
17	**PANDAseq** (Masella, A. P., Bartram, A. K., Truszkowski, J. M., Brown, D. G., & Neufeld, J. D. (2012). PANDAseq: paired-end assembler for illumina sequences. *BMC bioinformatics*, *13*(1), 1-7.) https://github.com/neufeld/pandaseq	**ASSEMBLES PAIRED-END READS**

18	**PEAR** (Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*, *30*(5), 614-620.) https://cme.h-its.org/exelixis/web/software/pear/doc.html	**ASSEMBLES PAIRED-END READS**
19	**Flash** (Magoč, T., & Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics*, *27*(21), 2957-2963.) http://www.cbcb.umd.edu/software/flash	**ASSEMBLES PAIRED-END READS**
20	**Flexbar (**Dodt, M., Roehr, J. T., Ahmed, R., & Dieterich, C. (2012). FLEXBAR—flexible barcode and adapter processing for next-generation sequencing platforms. *Biology*, *1*(3), 895-905.) https://guix.gnu.org/packages/flexbar-3.4.0/	**DEMULTIPLEX** It demultiplexes barcoded runs and removes adapter sequences.
21	**Sabre** (https://github.com/najoshi/sabre)	**DEMULTIPLEX**
22	**Deblur** https://telatin.github.io/microbiome-bioinformatics/Metabarcoding-deblur/	**DENOISE PAIRED AND SINGLE-READ SEQUENCES**
23	**MBRAVE** (Ratnasingham, S. (2019). mBRAVE: The Multiplex Barcode Research And Visualization Environment. *Biodiversity Information Science and Standards*, (2)) http://www.mbrave.net/	**DATA MANAGEMENT**
24	**microDecon R package (**McKnight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R. (2019). microDecon: A highly accurate read-subtraction tool for the post-sequencing removal of contamination in metabarcoding studies. *Environmental DNA*, *1*(1), 14-25.) https://github.com/donaldtmcknight/microDecon	**REMOVING CONTAMINATION**
	SINGLE-STEP TOOLS FOR PROCESSING (CLUSTERING AND TAXONOMIC ASIGNMENTS)	
25	**CROP** (Hao, X., Jiang, R., & Chen, T. (2011). Clustering 16S rRNA for OTU prediction: a method of unsupervised Bayesian clustering. *Bioinformatics*, *27*(5), 611-618.) https://github.com/tingchenlab/CROP	**CLUSTERING ALGORITHM**

26	**LULU** (Frøslev, T. G., Kjøller, R., Bruun, H. H., Ejrnæs, R., Brunbjerg, A. K., Pietroni, C., & Hansen, A. J. (2017). Algorithm for post-clustering curation of DNA amplicon data yields reliable biodiversity estimates. Nature Communications, 8(1), 1188.) https://github.com/tobiasgf/lulu	**CORRECTING OTUs**
27	**Meta-Fish-Lib** (Collins, R. A., Trauzzi, G., Maltby, K. M., Gibson, T. I., Ratcliffe, F. C., Hallam, J., ... & Genner, M. J. (2021). Meta-Fish-Lib: A generalised, dynamic DNA reference library pipeline for metabarcoding of fishes. *Journal of Fish Biology*, *99*(4), 1446-1454.) https://github.com/genner-lab/meta-fish-lib.	**FOR REFERENCE DATABASES BUILDING** Multi-locus mitochondrial DNA reference library dataset for fish species
28	**BOLDSYSTEMS** (Hebert, P. D. N., & Ratnasingham, S. (2007). BOLD: the barcode of life data system. *Molecular Ecology Notes*, *7*, 355-364.) http://www.boldsystems.org/	**FOR REFERENCE DATABASES BUILDING** COI sequences
29	**MitoAnnotator (**Sato, Y., Miya, M., Fukunaga, T., Sado, T., & Iwasaki, W. (2018). MitoFish and MiFish pipeline: a mitochondrial genome database of fish with an analysis pipeline for environmental DNA metabarcoding. *Molecular biology and evolution*, *35*(6), 1553-1555.) http://mitofish.aori.u-tokyo.ac.jp	**FOR REFERENCE DATABASES BUILDING** Fish mitochondrial sequences
30	**NCBI nucleotide DataBase** https://www.ncbi.nlm.nih.gov/	**FOR REFERENCE DATABASES BUILDING** All sequences
31	**PHYLOFISH (**Pasquier, J., Cabau, C., Nguyen, T., Jouanno, E., Severac, D., Braasch, I., ... & Bobe, J. (2016). Gene evolution and gene expression after whole genome duplication in fish: the PhyloFish database. *BMC genomics*, *17*(1), 1-10.) http://phylofish.sigenae.org/index.html	**FOR REFERENCE DATABASES BUILDING** Transcriptome sequences of fish species
32	**MITOFISH (**Sato, Y., Miya, M., Fukunaga, T., Sado, T., & Iwasaki, W. (2018). MitoFish and MiFish pipeline: a mitochondrial genome database of fish with an analysis pipeline for environmental DNA metabarcoding. *Molecular biology and evolution*, *35*(6), 1553-1555.) http://mitofish.aori.u-tokyo.ac.jp	**REFERENCE DATABASES** Fish mitochondrial sequences

33	**ITS2-dbase** (Ankenbrand, M. J., Keller, A., Wolf, M., Schultz, J., & Förster, F. (2015). ITS2 database V: Twice as much. *Molecular Biology and Evolution*, *32*(11), 3030-3032.) http://its2.bioapps.biozentrum.uni-wuerzburg.de/	**REFERENCE DATABASES** ITS
34	**SILVA** (Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic acids research*, *35*(21), 7188-7196.) https://www.arb-silva.de/	**REFERENCE DATABASES** rRNA sequences
35	**pplacer** (Matsen, F. A., Kodner, R. B., & Armbrust, E. (2010). pplacer: linear time maximum-likelihood and Bayesian phylogenetic placement of sequences onto a fixed reference tree. *BMC bioinformatics*, *11*(1), 1-16.) https://github.com/matsen/pplacer	**TAXONOMIC ASSIGNEMENT** Phylogenetic placement and downstream analysis
36	**phyloseq R package** https://joey711.github.io/phyloseq/	**TAXONOMIC ASSIGNEMENT** Import, store, analyze, and graphically display complex phylogenetic sequencing data that has already been clustered into Operational Taxonomic Units (OTUs)
37	**EPA-ng** (Barbera, P., Kozlov, A. M., Czech, L., Morel, B., Darriba, D., Flouri, T., & Stamatakis, A. (2019). EPA-ng: massively parallel evolutionary placement of genetic sequences. *Systematic biology*, *68*(2), 365-369.) https://github.com/Pbdas/epa-ng	**TAXONOMIC ASSIGNEMENT** Likelihood-based phylogenetic placement of genetic sequences on a user-supplied reference tree and alignment
38	**BLAST** (Basic Local Alignment Search Tool) and **MOLE-BLAST** https://blast.ncbi.nlm.nih.gov/Blast.cgi	**TAXONOMIC ASSIGNEMENT** **BLAST** finds regions of similarity between biological sequences, and **MOLE-BLAST** experimental tool to establish taxonomy from eDNA
39	**IDTAXA (**Murali, A., Bhargava, A., & Wright, E. S. (2018). IDTAXA: a novel approach for accurate taxonomic classification of microbiome sequences. *Microbiome*, *6*(1), 1-14.)	**TAXONOMIC ASSIGNEMENT** Taxonomic classifier

	http://www2.decipher.codes/Classification.html	
	SINGLE-STEP TOOLS FOR POST-PROCESSING	
40	**TaxonTableTool**s (TTT; Macher, T. H., Beermann, A. J., & Leese, F. (2021). TaxonTableTools: A comprehensive, platform-independent graphical user interface software to explore and visualise DNA metabarcoding data. *Molecular Ecology Resources*, *21*(5), 1705-1714.) https://github.com/TillMacher/TaxonTableTools	**DIVERSITY ANALYSES** **RAREFACTION CURVES**
41	**Vegan R package (**Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927-930.) https://www.rdocumentation.org/packages/vegan/versions/2.4-2	**DIVERSITY ANALYSES**
	ABUNDANCE ESTIMATES eDNA METABARCODING	
42	**eDNAPLUS** (Diana, A., Matechou, E., Griffin, J., Yu, D., Luo, M., Tosa, M., ... & Griffiths, R. (2022). eDNAPlus: A unifying modelling framework for DNA-based biodiversity monitoring. *arXiv preprint arXiv:2211.12213*). https://blogs.kent.ac.uk/edna/metabarcoding-data/download/	**BIOMASS ESTIMATES FOR EDNA METABARCODING**
	ABUNDANCE ESTIMATES eDNA TARGETED	
43	**qPCR_LOD_CALC** (Merkes, C., Klymus, K., Allison, M., Goldberg, C., Helbin, C., Hunter, M., Jackson, C., Lance, R., Mangan, A., Monroe, E., Piagio, A., Stokdyk, J., Wilson, C., and Richter, C., 2019, Reporting the limits of detection (LOD) and quantification (LOQ) for environmental DNA assays: Data: U.S. Geological Survey data release) https://github.com/cmerkes/qPCR_LOD_Calc	**SPECIES DETECTION PROBABILITIES** LOD & LOQ estimates
44	**eDNA SHINY APP** (Diana, A., Matechou, E., Griffin, J. E., Buxton, A. S., & Griffiths, R. A. (2021). An RShiny app for modelling environmental DNA data: accounting for false positive and false negative observation error. *Ecography*, *44*(12), 1838-1844). https://seak.shinyapps.io/eDNA/ https://blogs.kent.ac.uk/edna/qpcr-data/	**SPECIES DETECTION PROBABILITIES** Occupancy modelling
45	**EDNAOCCUPANCY** (Dorazio, R. M., & Erickson, R. A. (2018). ednaoccupancy: An r package for multiscale occupancy modelling of environmental DNA data. *Molecular ecology resources*, *18*(2), 368-380) https://rdrr.io/github/RobertDorazio/eDNAoccupancy/	**SPECIES DETECTION PROBABILITIES** Occupancy modelling