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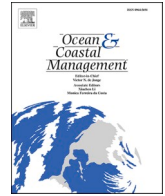
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# Coastal rocky reef fish monitoring in the context of the Marine Strategy Framework Directive: Environmental DNA metabarcoding complements underwater visual census

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## ABSTRACT

Efficient biodiversity monitoring programs are essential to assess the ecological status of marine ecosystems. In the context of the European Marine Strategy Framework Directive (MSFD), coastal rocky reef fish assemblages are most often surveyed using underwater visual census (UVC). While UVC is well-suited to monitor conspicuous fish, it underestimates hidden, vagrant and/or elusive fishes. Hence, complementary methods are needed to survey the whole fish assemblage. Environmental DNA (eDNA) metabarcoding is increasingly used in this context. Here, we evaluated the effectiveness of eDNA metabarcoding collected from water to provide sound and complementary diversity data to UVC in support of the MSFD. Both methods were used to examine coastal rocky reef fish assemblages in one bay of Brittany (France), along an inshore-offshore gradient and during two seasons. eDNA metabarcoding was carried out following different water sampling strategies: small water volumes (2L) from stationary sampling at the surface and bottom and large water volume (30L) filtered at the surface while moving in close vicinity of the sampling site. A total of 93 (including 57 species) and 33 (including 27 species) taxa were detected with eDNA metabarcoding and UVC, respectively. Eleven species were only recorded by UVC, a result explained for nine of them by a lack of taxonomic resolution of the markers used (12S and 16S) and/or the unavailability of reference sequences. eDNA metabarcoding allows to recover species expected to be present, such as demersal rocky specialist fishes, at a similar species richness level than UVC (14 species for UVC and 12 species for eDNA). It however also unveiled other taxa (pelagic, benthic-pelagic and others demersal fishes) inhabiting the bay and rarely reported through UVC (18 species for UVC and 45 species for eDNA). Despite these differences in species identified by UVC vs. eDNA, both survey methods were consistent in characterizing spatio-temporal variabilities of fish assemblages, highlighting strong site fidelity of eDNA to the source communities. In a well-mixed water column, the eDNA signal was very homogeneous, allowing the use of the cost-effective small water volume eDNA sampling method. eDNA metabarcoding is thus particularly promising to study coastal rocky reef assemblages, and when coupled with UVC, it improves monitoring surveys.

## 1. Introduction

Biodiversity surveys, not only inform on the environmental status of

marine ecosystems (Danovaro et al., 2016), but also on the efficiency of management and conservation actions (Perera-Valderrama et al., 2020).

In the marine realm, fish assemblages have a pivotal role on the

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structure and functioning of ecosystems (Villéger et al., 2017) and related ecosystems' services (Holmlund and Hammer, 1999). However, fish assemblages are impacted by a large array of anthropogenic pressures such as professional and recreational fishing, habitat degradation, spreads of non-indigenous species and climate change (Arthington et al., 2016). Being both essential to ecosystems and sensitive to anthropogenic pressures, it is not surprising that fish assemblages are considered as important targets in biodiversity surveys and included in diverse regulations and policies.

This is formalized in the European Union (EU) Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC), implementing a framework within which European Member States shall take the necessary measures to achieve or maintain good environmental status of the different ecosystems composing the marine environment. Therefore, European Member States must protect and preserve, prevent the deterioration or, restore adversely affected areas of marine ecosystems. Pressures on the marine environment, such as pollution, alien species, and human activities must be prevented and reduced to ensure that impacts on or risks to marine biodiversity remain non-significant on marine ecosystems, human health, or legitimate uses of the sea. The capacity of marine ecosystems to respond to human-induced changes should not be compromised, while enabling the sustainable use of marine goods and services by present and future generations. The MSFD framework is composed of 12 descriptors; four of which include fish as targets (D1 "biological diversity", D2 "non-indigenous species", D3 "commercially-exploited fish and shellfish" and D4 "food webs"). The global scope of these four descriptors is to assess fish ecological status, and the potentially impacting anthropogenic pressures at the levels of species, assemblages, and ecosystems. In a way to fulfil these requirements, fish monitoring programs are widely developed. However, fish assemblage monitoring is challenging as they are taxonomically and phylogenetically highly diversified (Hughes et al., 2018; Nelson et al., 2016). They also encompass a wide array of biological traits (Villéger et al., 2017), habitats and niches (Mora et al., 2008; Pörtner et al., 2010).

The performance and effectiveness of diverse traditional fish monitoring methods (e.g. fishery or non-extractive surveys, experimental fishing, video or acoustic methods) have been thoroughly reviewed and compared (Baker et al., 2016; Bosch et al., 2017; Franco et al., 2012; Pelletier et al., 2011; Pita et al., 2014). Most often when a single method is used, one has to trade-off between taxonomic resolution and coverage, qualitative and quantitative parameters estimation, spatial and temporal resolution and coverage (related to costs of replication) (Costello et al., 2017; Edgar et al., 2020; Franco et al., 2012; Livore et al., 2021). In subtidal rocky reefs, one of the most used non-destructive fish monitoring method is the underwater visual census (UVC) (Caldwell et al., 2016; Edgar et al., 2020). UVC is widely used to estimate abundance, size structure, and biomass of fishes, as well as to characterize and quantify the composition of the fish assemblage (i.e., species composition) (Caldwell et al., 2016). UVC has been shown to properly monitor necto-benthic fishes (i.e. conspicuous fishes swimming above the substrate) but the detection of nocturnal, elusive and/or crypto-benthic fishes remains limited (Boussarie et al., 2018; Pita et al., 2018; Thiriet et al., 2016). Additionally, UVC quality largely rely on the diver expertise (Thompson and Mapstone, 1997), local environmental parameters (e.g. turbidity (Figueroa-Pico et al., 2020)), habitat complexity (e.g. the presence of kelps (Thiriet et al., 2016)), and the sampling protocol (Pais and Cabral, 2018).

Metabarcoding of environmental DNA (eDNA), defined as DNA extracted from environmental samples, such as seawater, has been increasingly used for biodiversity surveys (Deiner et al., 2017). This method is based on the high-throughput sequencing of eDNA sequences targeted by genetic marker(s), and their taxonomic assignment using a reference database (Taberlet et al., 2012). Besides being non-destructive, this method has the potential to unveil otherwise difficult-to-detect species (e.g. Furlan et al., 2019). It is also a cost-effective survey method, allowing to increase both the frequency

and spatial coverage of surveys (Lamy et al., 2021). This survey method has been used to study fish diversity (Fraija-Fernandez et al., 2020; Gilbey et al., 2021; Juhel et al., 2020; Miya, 2022; Pont et al., 2021; Stoeckle et al., 2020a, 2020b) and holds great promise to better assess species distribution ranges and fine spatio-temporal changes in patterns of fish assemblages (Sigsgaard et al., 2020; West et al., 2021). For instance, eDNA metabarcoding of sharks, difficult to observe in the wild, outperformed both UVC and baited remote underwater video station in terms of species richness and level of detection (Boussarie et al., 2018). Besides that eDNA metabarcoding cannot evaluate abundance and density as done with UVC, previous studies focusing on coastal areas however often reported a moderate taxa overlap between eDNA metabarcoding and traditional surveys (Afzali et al., 2021; Andruszkiewicz et al., 2017; Jeunen et al., 2020; Juhel et al., 2020; Stat et al., 2019; Stoeckle et al., 2020a; Zou et al., 2020). More case studies are needed for determining the pros and cons of eDNA metabarcoding as compared to traditional surveys in documenting fish species richness and distribution (McElroy et al., 2020). In addition, coastal environments are very diverse in terms of habitat structure and distribution, as well as in their environmental characteristics. Many facets of eDNA metabarcoding, notably the sampling approach (Bessey et al., 2020) and its spatio-temporal representativeness (Harrison et al., 2019; West et al., 2020), await further testing to refine its applicability to diverse environments.

In this work, we focused on coastal areas and investigated i) the complementarity of eDNA metabarcoding and UVC to monitor fish assemblages in the MSFD context, ii) the spatio-temporal distinctiveness of eDNA metabarcoding, and iii) the impact of different eDNA sampling approaches (depth, volume and extent of sampling) on fish species detection and assemblage characterization. To our knowledge, the sampling depth (surface vs. above the seafloor) impacts on eDNA detection had mostly been studied in areas displaying thermoclines or haloclines (Andruszkiewicz et al., 2017; Jeunen et al., 2020; Sigsgaard et al., 2020; Stoeckle et al., 2020a; Yamamoto et al., 2017, 2016, but see DiBattista et al., 2019; Nester et al., 2020). Therefore, to enrich our understanding of the applicability of eDNA metabarcoding in different systems, our investigations focused on a bay characterized by a permanently homogenized and well-mixed water column.

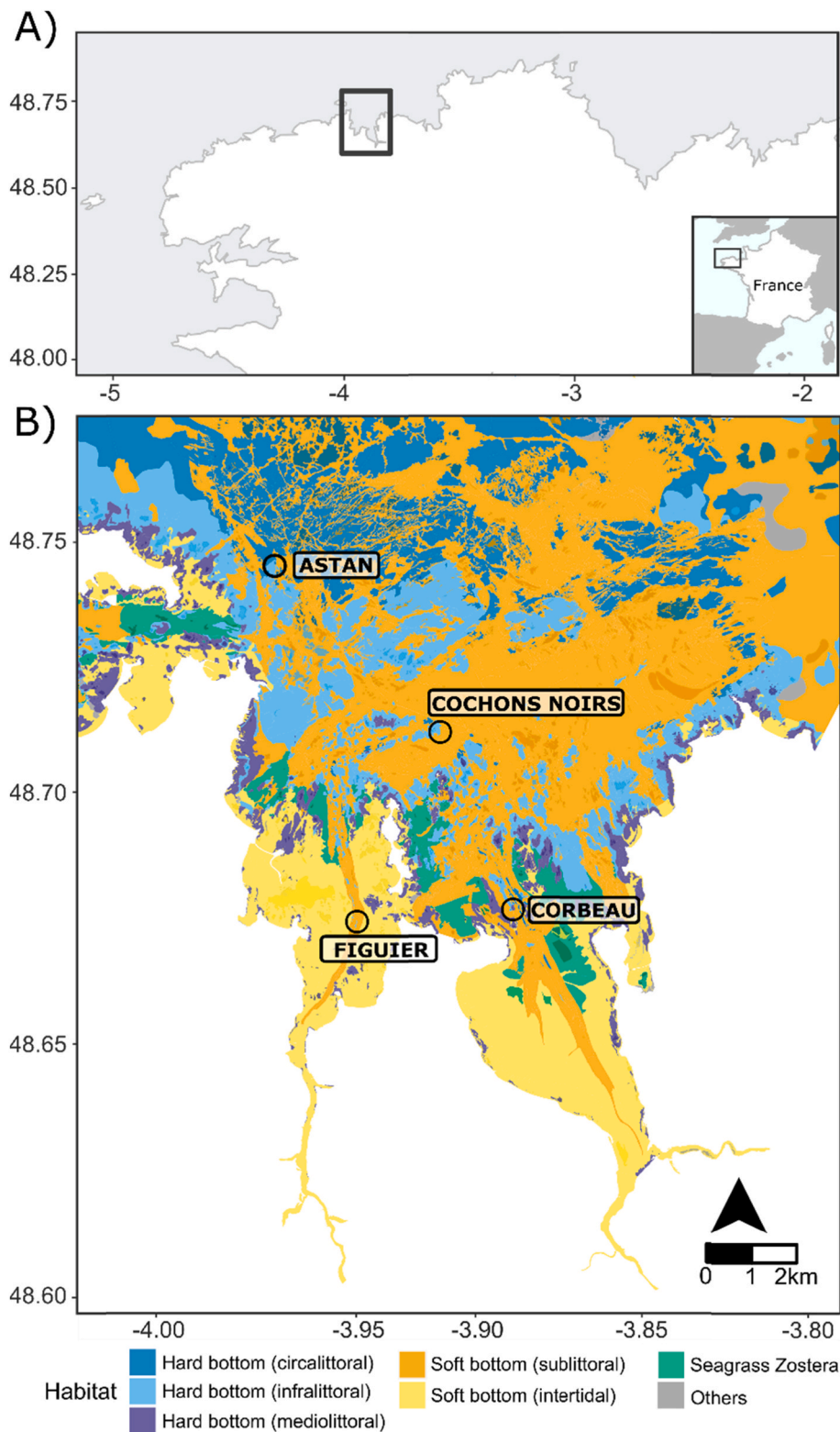
## 2. Materials and methods

### 2.1. Study sites, UVC surveys and water sampling

Fish UVC and water sampling were performed in spring and late summer 2018 at two inshore (Figuier and Corbeau), and two offshore (Astan and Cochons Noirs) sites in the bay of Morlaix, France (Fig. 1, Table A.1 in Appendix A). All sampling sites were subtidal and presented both infralittoral (in shallower depth) and circalittoral (in deeper depth) rocky habitats. Infralittoral habitats were largely dominated by *Laminaria* spp kelp forests, while circalittoral ones were composed of red algae and/or sessile fauna assemblages.

The UVC surveys and the three water sampling types were all done within a 2-h' time frame (Table 1).

The UVC survey was performed by a team of two divers, using open-circuit scuba following a timed swim protocol. The divers recorded all fishes observed around them during 8 min in the targeted depth strata ( $\pm 2$  m) following a transect of 20–40 m. At each sampling event, five depth strata (tide corrected depth: 3 m, -8 m, -13 m, -18 m, -23 m) were sampled once for Astan, Cochons Noirs and Corbeau. In the case of Figuier, only the -3 m and -8 m strata were sampled because of depth limit; those strata were sampled twice each. The bottom and top of the kelp forest of the infralittoral were sampled simultaneously by two different divers, while only the bottom was sampled for the circalittoral by the two divers. The UVC sampling unit is defined as the presence/absence of fish observed by the two divers over a period of 8 min at each depth strata replicate of the site.



**Fig. 1.** Maps of the sampling area showing the (A) location of the study bay (bay of Morlaix) in Brittany (France), and (B) the sampling sites with associated benthic habitats. Habitats' layers were retrieved and simplified from [Bajjouk et al. \(2019\)](#).

Water was sampled for eDNA metabarcoding with three sampling types. Two were stationary sampling, one taken below the surface (“2-L surface”) and the other at the deepest depth of the rocky habitat of the site (“2-L bottom”) which corresponds to the UVC deepest sampled depth for the site. The third sampling type (“30-L area”) consisted in a large volume of water filtered below the surface, over a 30 min period

with a small boat moving around the sampling rocky site in close vicinity (about up to 400 m; see [Figure B.1 in Appendix B](#)).

The sampling of 2-L water was done by the divers just before they started the UVC survey. Three replicates were retrieved both at the surface and bottom with disposable polyethylene sampling bags. These sampling bags are manufactured to come into contact human food (e.g.,

**Table 1**  
Sampling date and time per site and method.

Date	Site	Method	Sampling time (min)
June 08, 2018	Astan	UVC	08:34
		2L	08:34
September 17, 2018	Astan	UVC	10:27
		2L	10:22
		30L	11:39 and 12:26
June 04, 2018	Cochons Noirs	UVC	10:14
		2L	10:14
September 19, 2018	Cochons Noirs	UVC	09:54
		2L	09:54
		30L	09:06 and 09:52
June 06, 2018	Corbeau	UVC	09:57
		2L	09:57
September 18, 2018	Corbeau	UVC	10:12
		2L	10:12
		30L	10:20 and 11:08
June 05, 2018	Figuier	UVC	10:26
		2L	10:26
September 20, 2018	Figuier	UVC	15:14
		2L	15:14
		30L	14:16 and 15:02

treated for no BSE/TSE virus). They were used only once and for the first time for this study. They were kept unopened until their use in the field. Over the course of the UVC, lasting for 8–11 min, the closed bags of the 2-L bottom and 2-L surface samples were kept underwater at the bottom by divers for the 2-L bottom samples and attached to the boat for the 2-L surface samples. After the end of the UVC and until the way back to the laboratory (roughly 30 min), all samples were stored in a small tank filled with the local seawater (12–14 °C). Back to the laboratory, the sampling bags were stored at 4 °C for a maximum of 2 h before filtration. To standardize the volume across samples, 2-L of water per sample was transferred from the sampling bag to a 2-L beaker cleaned with a 12.5% commercial bleach solution (ca. 0.65% hypochlorite). A sterile plastic pipette was attached to a Masterflex L/S® Precision Pump Tubing (Tygon® E-Lab, L/S 25; 50 ft) linked to the 0.22 µm Sterivex® filter units and the filtering of 2-L was done using a Masterflex® L/S® economy drive peristaltic pump. After the filtration, each unit was filled with 2 mL of STE lysis buffer (0.75 nM sucrose, 0.05M Tris [pH 8], 0.04M EDTA) and stored at –20 °C.

The “30-L area” sampling was done by another team on another boat during the same period as the 2-L sampling and the UVC (Table 1). Two sampling trips were performed in a row to collect a total of four replicates of 30-L of water. At each trip, two replicates were taken thanks to the use of two waterproof peristaltic pumps (one pump attached on each side of the boat). The filtration device was composed of an Athena® peristaltic pump (Proactive Environmental Products LLC; nominal flow of 1 L/min), an enclosed VigiDNA® 0.2 µm cross-flow filtration capsule (SPYGEN) dedicated to large water volume filtrations, and disposable sterile tubing for each filtration capsule (Figure B.1 in Appendix B). The volume filtered was controlled by setting the pump parameters (filtering time and flow rate), following the manufacturer protocol. At the end of each filtration, the water inside the capsules was emptied and the capsules were filled with 80 ml of CL1 conservation buffer (SPYGEN) and stored at room temperature, until DNA extraction.

All DNA extractions occurred within four months after sampling (2L-Spring: 1 month, 2L-Autumn: 2 months, 30L: 4 months), as the CL1 conservation buffer reliably preserves eDNA for up to 5.5 months (Marques et al., 2021a). For the DNA extraction, all equipment, bench surfaces and consumables not sold as DNA-free were DNA decontaminated before use with a 12.5% commercial bleach solution (ca. 0.65% hypochlorite) and UV light. The DNA extraction of the 2-L water samples was performed at the Station Biologique de Roscoff, following the protocol detailed in Couton et al. (2022). Briefly, Sterivex® filter units were thawed and stored at 56 °C for 4 h with 100 µL of SDS (20%) and 100 µL of proteinase K (40 mg.mL<sup>-1</sup>) for the lysis step. Content of each

Sterivex® filter unit was pushed out in a 15 mL centrifuge tube and DNA was extracted using the NucleoSnap® Midi Finisher kit (Macherey-Nagel) following the manufacturer’s protocol except that an additional volume of 550 µL of PL3 buffer was added to each sample before adjusting the binding conditions. The elution was performed by adding 50 µL of elution buffer twice.

The DNA extraction of the 30-L samples were performed at SPYGEN in separate dedicated rooms equipped with positive air pressure, UV treatment, and frequent air renewal following the protocol described in Polanco Fernández et al. (2021a). Briefly, each filtration capsule was agitated with the CL1 conservation buffer and poured into two 50-ml tubes before being centrifuged. The supernatants were discarded, and 1.5 ml of 3 M sodium acetate and 33 ml of ethanol were added to each tube. The tubes were then stored at –20 °C for at least one night, after which, they were centrifuged for 15 min at 15,000 g at 6 °C, and the supernatants were discarded. After this step, 720 µL of ATL buffer from the DNeasy Blood & Tissue Extraction Kit (Qiagen GmbH) was added to each tube and the solution was transferred to a 2-ml tube containing 20 µL of proteinase K before incubation at 56 °C for 2 h. Subsequently, DNA extraction was performed using the NucleoSpin®Soil kit (Macherey-Nagel) following the manufacturer’s instructions. Two DNA extractions were carried out per filtration capsule. The elution was performed by adding 200 µL of SE buffer (5 mM Tris-HCl [pH 8.5]) twice. The two DNA samples for each filter were pooled together before the amplification step.

After the DNA extraction, all samples were tested for inhibition following the protocol described in Biggs et al. (2015). If a sample was considered inhibited, it was diluted fivefold before the amplification. DNA amplifications were performed in a final volume of 25 µL, using 3 µL of DNA extract as the template. Two markers were used to identify fish species: “Teleo” primers targeting a ~60 bp fragment of the 12S mitochondrial rRNA gene (Valentini et al., 2016) and the “Metaz” primers targeting a fragment of ~115 bp of the 16S mitochondrial rRNA gene (Kelly et al., 2016). The amplification mixture contained 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems), 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.2 µM of each primer, 4 µM human blocking primer (5'-ACCCTCCTCAAGTATACTTCAAAGGAC-SPC3-3') only for the “Teleo” primers, and 0.2 µg/µL bovine serum albumin (BSA, Roche Diagnostic). This step was done in a room dedicated to amplified DNA with negative air pressure, physically separated from the DNA extraction rooms (with positive air pressure). We performed 12 PCR replicates per filter sample. The forward and reverse primer tags were identical within each PCR replicate for “Teleo” primers and for the three PCR replicates for “Metaz” primers.

Two negative extraction controls for the 30-L samples and PCR negative controls (4 for the “Teleo” and 6 for the “Metaz” primers) were amplified and sequenced in parallel with samples. For the 2-L samples, two negative extraction controls were performed in spring 2018, by adding SDS and proteinase K in a Sterivex® unit extracted at the same time, as part of another project (Couton et al., 2022). They contained around 180 reads of laboratory contaminant (Fungi).

After amplification, the samples were titrated using capillary electrophoresis (QIAXcel; Qiagen) and purified using the MinElute PCR purification kit (Qiagen). Before sequencing, purified DNA was titrated again using capillary electrophoresis. The purified PCR products were then pooled in equal volumes to achieve an expected sequencing depth of 1,000,000 reads per sample and per marker before library preparation. Library preparation and sequencing were performed by Fasteris facilities (Geneva, Switzerland). In total, six libraries were prepared using a PCR-free library protocol (<https://www.fasteris.com/metafast>). Four libraries were sequenced using an Illumina HiSeq 2500 (2 × 125 bp) (Illumina) on HiSeq Rapid Flow Cell v2 (Illumina) with an HiSeq Rapid SBS Kit v2 (Illumina). For one of the HiSeq sequenced libraries, the 16S sequencing failed. Sequencing of the samples presents in this 16S library was then repeated on two libraries that were sequenced on

MiSeq (2 × 125 bp) (Illumina) using the MiSeq Flow Cell Kit Version3 (Illumina).

## 2.2. Reads processing

Details on software, commands and/or parameters are described in [Table A.2 in Appendix A](#). Briefly, paired-end raw reads were demultiplexed using Cutadapt ([Martin, 2011](#)), and processed with DADA2 ([Callahan et al., 2016](#)), for 12S and 16S separately, to retrieve a list of amplicon sequence variants (ASVs). To correct for index-jumping, which falsely assign ASVs to a given sample ([Schnell et al., 2015](#)), we applied a filtering threshold based on the ratio between the ASV abundance found in the blank samples and the total ASV abundance for each lane of the HiSeq run ([Figure B.2 in Appendix B](#)). For the 12S and 16S respectively obtained with HiSeq runs, only ASVs with a ratio superior to 0.006 for Lane 1 and to 0.005 for Lane 2, and to 0.0005 for Lane 1 and to 0.001 for Lane 2, were kept. Note that no index-jumping filtering was applied to the 16S sequences obtained with MiSeq runs. Because the 16S sequencing failed, one of the libraries sequenced on HiSeq had 2 to 3.5 times more reads than the three others for the 12S. The four libraries sequenced on HiSeq were thus rarefied to the smallest library size by randomly subsampling 100 times the libraries, with the R function *sample*, and by averaging the abundance of each ASV across these subsamples. Errors from the dataset were removed using the LULU post-clustering algorithm ([Frøslev et al., 2017](#)).

## 2.3. Taxonomic assignment and OTU clustering

A list of species potentially present in the English Channel and Northeast Atlantic was gathered based on expert knowledge. For 48 species with missing or incomplete sequences for the study region in the NCBI database, a local reference sequence could be obtained. Those sequences were obtained from DNA, extracted from tissues samples, which were then PCR amplified with the primers V05F\_898 and Teleo-R ([Thomsen et al., 2016](#)) targeting the mitochondrial rRNA 12S gene and the primers 16S\_MetaTabF and 16S\_MetaTabR ([Taberlet et al., 2018](#)) targeting the mitochondrial rRNA 16S gene. PCR products were sequenced with Sanger by Eurofins. A custom reference database was then created for each marker, by adding the references produced locally to sequences retrieved from NCBI (accessed in May 2020) targeting all Chordata and Metazoa sequences for 12S and 16S, respectively. A total of 141 (66.8%) and 166 (78.7%) species from the list had at least one unique sequence available for the 12S and 16S respectively ([Table A.3 in Appendix A](#)). A total of 42 (19.9%) and 139 (65.9%) species, for the 12S and 16S respectively, displayed at least one reference sequence 100% similar to reference sequence from other species. This result highlights lack of clear barcoding gap, especially for the 16S ([Figure B.3-B.4 in Appendix B](#)).

For each marker, ASVs retrieved from the DADA2 workflow were taxonomically assigned to species listed in the custom reference database using three methods: i) the RDP Classifier ([Wang et al., 2007](#)), a naïve Bayesian classifier providing a measure of confidence for each assignment, ii) BLAST ([Altschul et al., 1990](#)), a local alignment approach measuring the similarity between two sequences and iii) ECOTAG from OBITools ([Boyer et al., 2016](#)), a global alignment approach where ASVs are assigned to the lowest common ancestor of the sequences of the reference database that have the highest similarity. For BLAST, only alignments with a minimum query cover of 97% for 12S and of 98% for 16S were considered. For ASVs matching with several references for BLAST, the assignment was made to the species level with the highest identity percentage. In cases of similar identity to distinct taxa, the ASV was assigned to the upper common taxonomic level. Only ASVs assigned to Actinopterygii and Chondrichthyes were kept. The ASV was assigned based on consensus obtained by at least two methods. When none of the methods gave a similar assignment, the ASV was assigned to the upper common taxonomic level (except for three cases with 12S as detailed in

[Table A.4 in Appendix A](#)). Taxa absent from the North East Atlantic ([Froese and Pauly, 2019](#)) were considered as putative misidentifications (false positives) due to the incompleteness of databases, and assigned at the genus level ([Tables A.4-A.5 in Appendix A](#)). ASVs were clustered into OTUs using SWARM ([Mahé et al., 2015](#)). OTUs present only once in the entire dataset (in only one unique tag combination) were removed. Details on software, commands, parameters are described in [Table A.2 in Appendix A](#).

## 2.4. Habitat traits

In order to better interpret the results, habitat preference was defined for each species based on Fishbase ([Froese and Pauly, 2019](#)) and authors' personal observations ([Table A.6 in Appendix A](#)) as follows: *pelagic* (living and feeding in the water column, distant from the sea bed), *benthopelagic* (exhibiting regular/daily movements between pelagic and demersal zones), *demersal rocky specialist* (living and feeding on or near the sea bed, with exclusive affinity for rocky substrates), *demersal non rocky specialist* (living and feeding on or near the sea bed, without exclusive affinity for rocky substrates, including affinity for other substrate category(ies) like sandy bottoms and/or seagrass beds as well as absence of affinity for any specific substrate). The taxa not assigned at the species level were categorized under *no associated habitat*.

## 2.5. Statistical analyses

The effectiveness of the sampling effort for each survey method and type of water sample was analysed using rarefaction curves computed with the iNEXT R package ([Hsieh et al., 2016](#)). Presence-absence data were used to build community matrices transformed with Hellinger ([Legendre and Legendre, 2012](#)). This step was performed with the *decostand* function of the vegan R package ([Oksanen et al., 2013](#)). Variation in community composition and structure was visualized using Principal Component Analyses (PCA) of Hellinger-transformed OTU (metabarcoding) or taxa (UVC). The effect of the factors “types of water sample” (for metabarcoding only), “locality” and “sampling time” was assessed using Permutational Multivariate Analyses of Variance (PERMANOVA) of type III in order to consider for the unbalanced design, with PRIMER version 6 and PERMANOVA + B20 package ([Anderson et al., 2008](#); [Clarke and Gorley, 2006](#)). Additionally, a PERMDISP analysis (*betadisper* function of the vegan R package [Oksanen et al. \(2013\)](#)) was performed to determine if the significance of the factors was due to position and/or to heterogeneity of dispersion among groups. Similarities of the  $\beta$  diversity patterns between the type of water sampling for the 2-L sampling types only or between survey methods were assessed with co-inertia analyses, with RV coefficients (similar to a correlation coefficient; [Escoufier, 1973](#)) computed with the R package *ade4* ([Dray and Dufour, 2007](#)). To allow comparison at the sampling site level, co-inertia analyses were performed using PCAs of mean Hellinger-transformed OTUs (metabarcoding) or taxa (UVC) at the sampling site level. For each survey method, the contribution to  $\beta$  diversity of specific OTUs (metabarcoding) or taxa (UVC) was assessed with Species Contribution to Beta Diversity (SCBD; [Legendre and De Cáceres, 2013](#)) analyses, using the mean Hellinger-transformed OTU (metabarcoding) or taxa (UVC) with the R package *adespatial* ([Dray et al., 2018](#)). For the SCBD analysis, and to consider both spatial and temporal variations in communities, the data obtained with the water samples “30L area” were not used, as this type of sampling occurred only in autumn. All statistical analyses (except PERMANOVA) were conducted using R (R Core Team, 2020).

### 3. Results

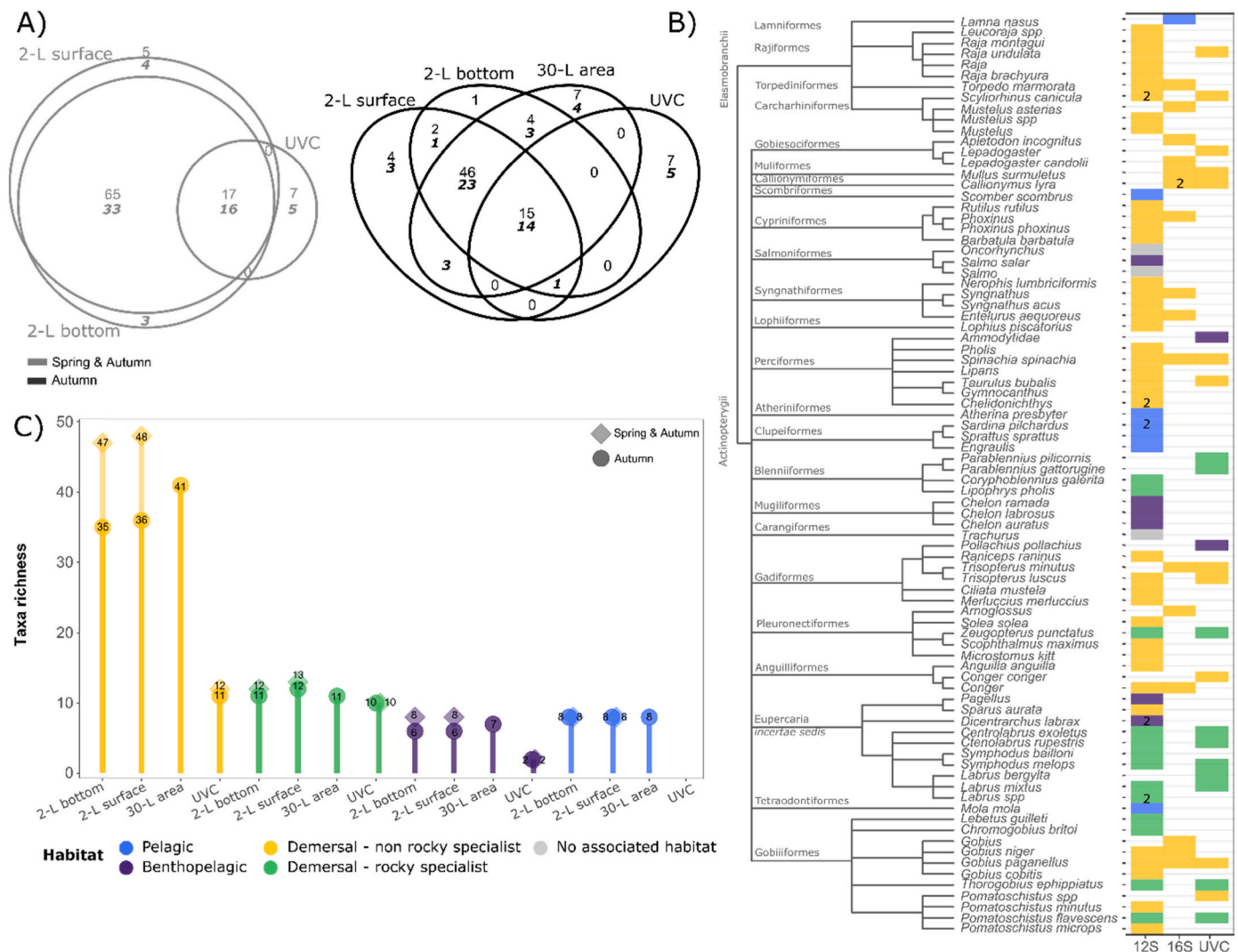
#### 3.1. Sequencing results, taxonomic assignment, and number of OTUs

A total of 252,129,702 paired-end reads were obtained after the demultiplexing step (Table A.7 in Appendix A). After all the processing steps (including index jumping, filtering and normalization between libraries), 18,867,477 and 33,213,663 reads belonging to Actinopterygii and Chondrichthyes were kept, corresponding to 775 and 125 ASVs for the 12S and 16S, respectively (Table A.7 in Appendix A). The mean number of reads per biological sample was 266,234 (1st quartile = 181,731 and 3rd quartile = 328,235) and 452,800 (1st quartile = 150,124 and 3rd quartile = 484,786) for the 12S and 16S, respectively. Most ASVs (632 (81.5%) and 108 (86.4%) for the 12S and 16S, respectively) showed a similar taxonomic assignment with at least two methods (Tables A.4-A.5 in Appendix A). For the remaining 143 and 17 ASVs, no consensus was reached between the assignment methods. Those ASVs were mostly (n = 115 and n = 16 for the 12S and 16S

respectively) assigned to high taxonomic level, such as Actinopterygii or Chordata with ECOTAG or RDP assignment methods.

The ASVs were clustered into 228 and 50 OTUs for the 12S and 16S, respectively, and after removal of OTUs present only once, 112 and 38 OTUs remained for 12S and 16S, respectively (Table A.7 in Appendix A). A total of 77 (50 at the species level) and 28 (12 at the species level) taxa were identified with the 12S and 16S, respectively. All species were represented by one OTUs, except four represented by two OTUs, (*Scyliorhinus canicula*, *Sardina pilchardus*, *Dicentrarchus labrax* for the 12S and *Callionymus lyra* for the 16S).

Only 13 taxa (five at the species level) were identified by both markers. In addition, while most of the fish diversity was detected with the 12S, 7 species were only detected with the 16S, including *Lepadogaster candolii*, *Mullus surmuletus*, *Trisopterus minutus* et *C. lyra*, also detected with UVC. The two markers had also different taxonomic resolution. For the 12S, more than 73% of ASVs were identified at the genus or species level, whereas for the 16S, it was only 30% of ASVs.



**Fig. 2.** Taxa identified according to the survey methods and type of water samples. (A) Venn diagram showing all taxa detected with each type of water sample and with UVC for spring and autumn and autumn period only (note that the 30L water samples were retrieved only in autumn). The total number of taxa is represented in association with the number of taxa assigned to the species (sp.) level (in bold and italic). (B) Taxonomic cladogram showing the taxa identified at the genus or species level with eDNA metabarcoding using the 12S marker (12S), the 16S marker (16S), and with UVC. One taxon assigned to the family level with UVC (Ammodytidae) was also identified with eDNA metabarcoding and added to the cladogram. Number of OTUs per taxon and marker are indicated after the scientific name only when it was superior to one OTU. Each colour represents the species-specific habitat when known. (C) Number of taxa per associated habitat identified at the species level found with each survey method and type of water sample. Dark circle symbol represents the dataset in autumn and light diamond symbol represents the dataset at the two sampling periods (spring and autumn).

### 3.2. Comparison between UVC and eDNA metabarcoding identification

Out of the 24 taxa observed with UVC, of which 21 were identified at the species level, 17 (16 species) were also detected with the 2-L water sampling methods for the two sampling seasons (Fig. 2A). Focusing on the autumn season, 15 (14 species) of the 23 taxa observed with UVC were detected with the three water sampling methods (Fig. 2A).

Five species identified with UVC were not recovered by metabarcoding (Fig. 2A). Two of them, *Pollachius pollachius* and *Labrus bergylta*, could not be assigned because the reference sequences were 100% identical to the one of another species for the two markers. For the other species, *Parablennius pilicornis*, *Parablennius gattorugine*, and *Conger conger*, the reference sequences were not available in the database used for taxonomic assignment with the 12S and were either unavailable or could not be inferred at the species level for the 16S.

eDNA metabarcoding detected 93 taxa with 76 of them not observed with UVC, including 41 assigned at the species level. A total of 91 and 73% taxa were observed through all water sampling methods respectively for autumn only and autumn and spring comparisons (Fig. 2A). Interestingly, when examining the species-specific habitats, pelagic species were only observed with eDNA metabarcoding (Fig. 2B and C), whereas both UVC and eDNA metabarcoding methods detected a similar number of species characterized as demersal rocky specialists (Fig. 2C). The number of demersal non rocky specialists detected by each water sampling method ( $35 < n < 41$  for autumn only and  $47 < n < 48$  for autumn and spring) was three times the one detected with UVC ( $n = 11$  for autumn only,  $n = 12$  for autumn and spring).

### 3.3. $\beta$ diversity patterns (presence-absence)

High RV coefficients ( $0.83 < RV < 0.90$ ) from the co-inertia analyses of presence-absence data revealed that  $\beta$  diversity patterns were very consistent at the site level i) between the two markers and ii) between the two types of 2-L water sampling (Table 2). In agreement with the co-inertia analysis, the PERMANOVA analysis revealed that the impact of the type of water sample on  $\beta$  diversity was weaker than the ones of season and site (Table A.8 in Appendix A). This is also observed with the principal component analyses (PCA) in which the type of water sample is not observed on the two first axes, representing 20% of the total variation (Fig. 3).

By contrast, when comparing the two survey methods (UVC vs eDNA metabarcoding), the RV coefficients were moderately high ( $0.58 < RV < 0.69$ ) highlighting that the  $\beta$  diversity patterns were moderately consistent. Indeed, as shown by the PCA analysis, the impact of seasons was stronger for the eDNA metabarcoding than for the UVC communities (Fig. 3). The PERMANOVA analyses highlighted significant effects of the sites and seasons, for the two survey methods, but the seasons had a Pseudo-F value higher than the site one with eDNA metabarcoding

**Table 2**

Similarity of  $\beta$  diversity patterns between the 12S and 16S datasets, the types of 2-L water sample, and survey methods, as assessed by the RV coefficients of co-inertia analyses. The co-inertia analyses were based on PCA of the presence-absence mean Hellinger transformed OTU (metabarcoding) or taxa (UVC) at the sampling site level ( $n = 8$ ). In order to account for both spatial and temporal variations in communities, the type of water sample 30L area was not used as the sampling occurred only in autumn.

Factors compared	Dataset	RV coefficient
<b>Markers</b>	12S vs 16S (2-L surface)	0.89
	12S vs 16S (2-L bottom)	0.89
<b>Water sampling</b>	2-L surface vs 2-L bottom (12S)	0.90
	2-L surface vs 2-L bottom (16S)	0.83
<b>Survey methods</b>	UVC vs 2-L surface (12S)	0.67
	UVC vs 2-L bottom (12S)	0.69
	UVC vs 2-L surface (16S)	0.58
	UVC vs 2-L bottom (16S)	0.66

while the reverse was observed for UVC (Table A.8 in Appendix A). Note however that the co-inertia analysis between the two survey methods revealed a consistent spatial pattern as retrieved from the two methods (Figure B.5 in Appendix B): on the first axis (57% of variation), the site Figui er, located inside the bay, is the most distinct to the sites Astan and Cochons Noirs, both located outside the bay, with Corbeau (inside the bay) being in intermediate position. In addition, the taxa shared by both survey methods generally showed similar variations across sites, mostly separating sites inside the bay from outside (Figure B.5 in Appendix B, lower panel).

### 3.4. Taxa contributing to presence-absence $\beta$ diversity

The contribution to  $\beta$  diversity, compared across species specific habitats, was similar between the types of 2-L water sample (Fig. 4). OTUs assigned to pelagic taxa had the highest species contribution to beta diversity (SCBD) scores, with *Atherina presbyter* displaying the highest score. OTUs assigned to demersal non rocky specialist taxa and with no associated habitat had the lowest SCBD median score, highlighting that many of these OTUs were rarely observed. Yet some OTUs from those habitats strongly contributed to  $\beta$  diversity such as the demersal non rocky specialist *Scyliorhinus canicula* or *Oncorhynchus* sp. (no associated habitat). When comparing the two survey methods (UVC vs DNA metabarcoding), the SCBD scores across species specific habitats were different. For instance, demersal rocky specialist taxa contributed the most to  $\beta$  diversity with UVC but not for any of the 2-L water samples. Nonetheless, some species contributed the most to  $\beta$  diversity with both survey methods, such as the demersal non rocky specialists *Gobius paganellus*, *Pomatoschistus* sp., *Trisopterus luscus*, and the demersal rocky specialist *Symphodus melops*.

### 3.5. Evaluation of sampling effort according to the survey method and sampling strategy

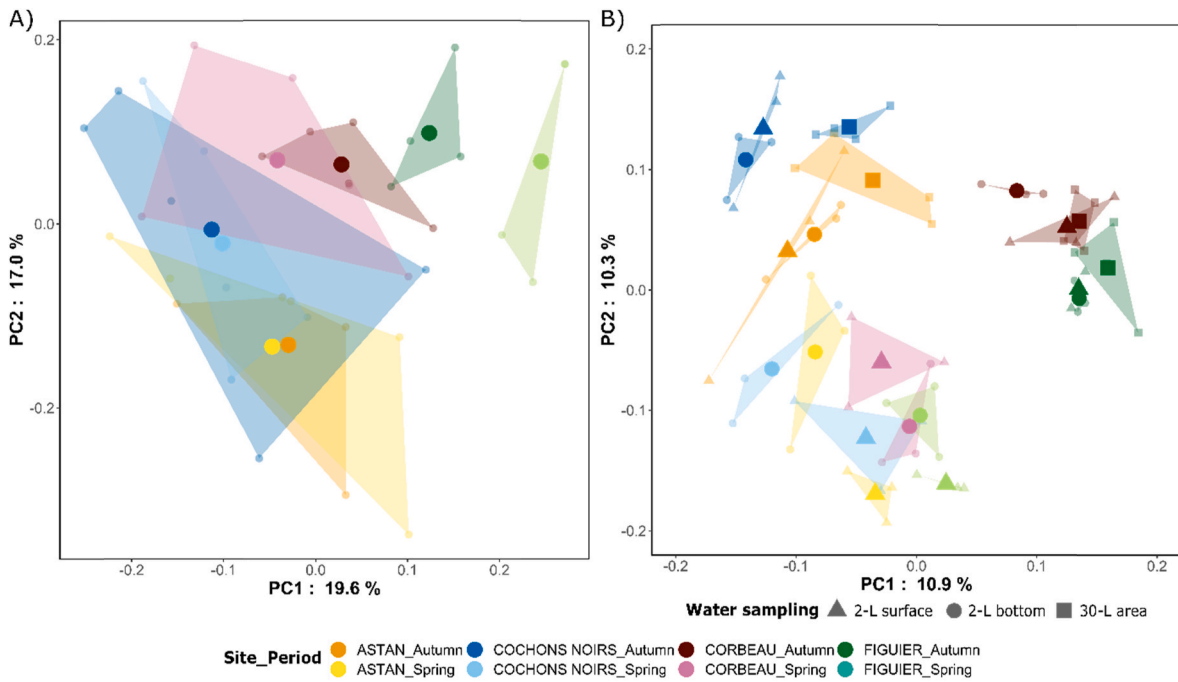
The three types of water sample accumulated OTUs at a very similar rate, at the different sampling sites, (Fig. 5A and Figure B.6 in Appendix B). No saturating level was reached with the 12S, whatever the water sampling type. It was however reached with the 16S for the 30-L area (Fig. 5A). No saturating level was observed with UVC either (Fig. 5B). When looking at the accumulation of OTUs in each site per species specific habitat, no particular type of water sample consistently detected more OTU than the others, except for the demersal non rocky specialist taxa, for which the 30-L area detected more taxa at three of the four sites (Figure B.7 in Appendix B). For both the 12S and 16S, almost all OTUs (roughly 98–99% of the OTUs) were found by each type of water sample (Fig. 5C and Figure B.6 in Appendix B). The ones not recovered by the three types of water sample represented between 0.1 and 1.7% of the total number of reads (Fig. 5C and Figure B.6 in Appendix B). The three types of water sample also recovered a similar taxonomic richness inhabiting the different habitats of the bay (Fig. 2C).

The frequency of occurrence of OTUs across replicates and sites was found to be quite similar, and with a significant relationship, between the types of water sample, especially for the 12S (Fig. 6 and Figure B.8 in Appendix B). Similar findings were observed when comparing the frequencies of occurrence across samples between the two survey methods (Figure B.9 in Appendix B). Some interesting discrepancies were nonetheless observed for *S. bailloni*, *D. labrax* and *T. marmorata*, rarely observed in UVC but commonly identified with DNA metabarcoding, irrespective of the types of water sample. Conversely, *Thorogobius ephippiatus* was observed in all sampled sites with UVC, whereas it was rarely detected with DNA metabarcoding.

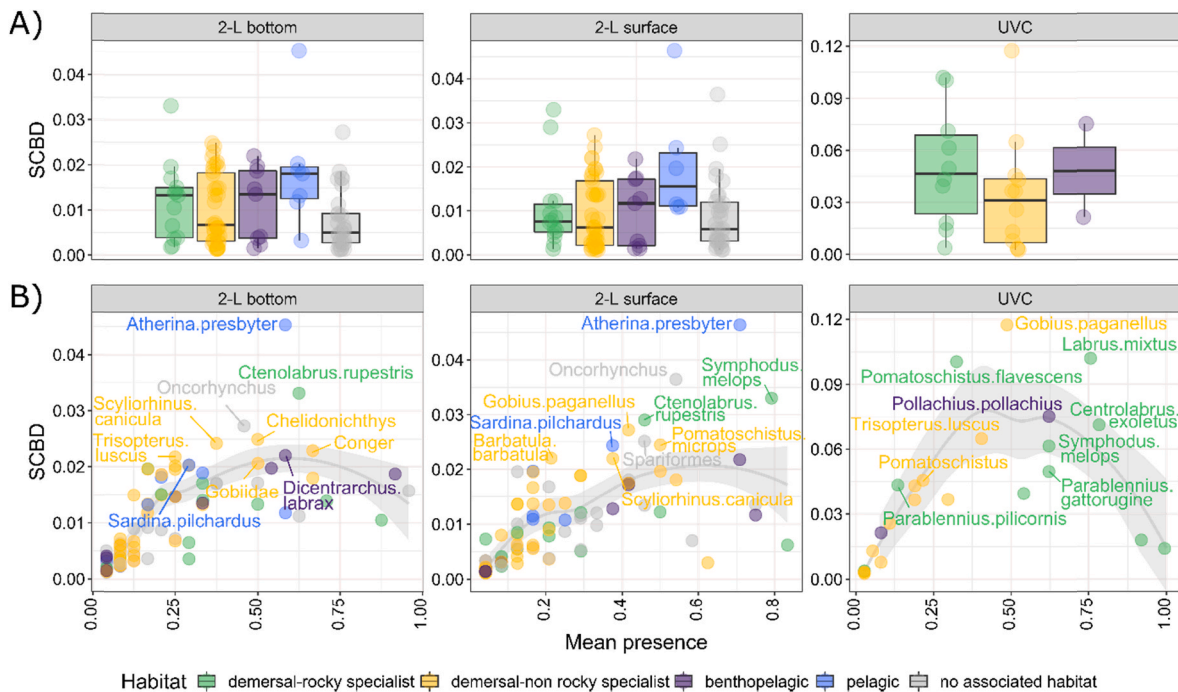
## 4. Discussion

In this study, we assessed the potential of eDNA metabarcoding to characterize the coastal rocky fish diversity and assemblage in a





**Fig. 3.** Spatio-temporal similarities between sites for each survey and sampling methods. The graphs are outcomes of Principal Component Analyses (PCAs) of presence-absence Hellinger transformed data of taxa for UVC (A) and OTUs for eDNA metabarcoding (12S marker) (B). PCAs are represented in scaling 1 preserving the distances among the sites. The smaller symbols and shading of the convex-hull polygons indicate the position of the sampling units (UVC, Taxa) and replicates (eDNA metabarcoding, OTUs) for each site at one period (UVC, Taxa) and also per type of water sample (eDNA metabarcoding, OTUs) and larger symbols indicate their centroid positions.



**Fig. 4.** Species contribution to  $\beta$  diversity (SCBD) for the 12S dataset for the 2-L water samples and the UVC dataset. SCBD was computed on the mean at the site level of the presence-absence Hellinger transformed OTUs (for water sample) and taxa (for UVC). (A) Boxplot of SCBD values for each OTU or taxa, for each associated habitat. (B) SCBD value as a function of the mean presence of each OTU or taxa over the total number of replicates (water samples) or transects (UVC). The 10 OTUs or taxa with the highest SCBD values are indicated in the plot.

temperate permanently well-mixed bay. As compared to UVC, and whatever the water sampling strategy, a higher number of taxa were reported through eDNA metabarcoding, encompassing different habitats of the study rocky sites and neighbouring habitats. In addition, it also

revealed a clear structure, like the one observed through UVC, of the fish assemblages across sites and seasons, highlighting its ability to characterize assemblages at a fine spatial and temporal scale.

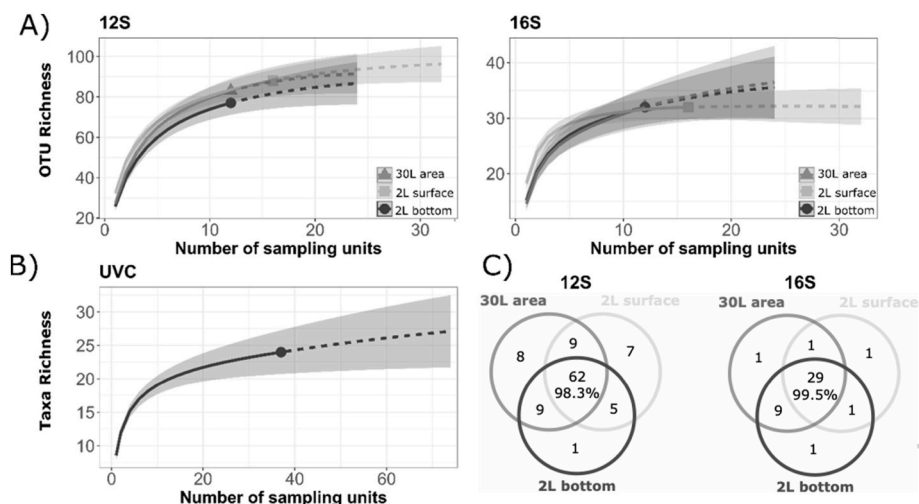


Fig. 5. Sample-size-based rarefaction (solid line) and extrapolation (dotted line) sampling curves with 95% confidence intervals (shaded areas) for estimated richness of (A) OTUs for each type of water sample (autumn) and (B) taxa for the UVC dataset. (C) Venn diagrams of OTUs found with each of the three types of water sampling (autumn), showing the number of OTUs found by one or two types of water sampling or shared among the three sampling (with percentage of shared reads).

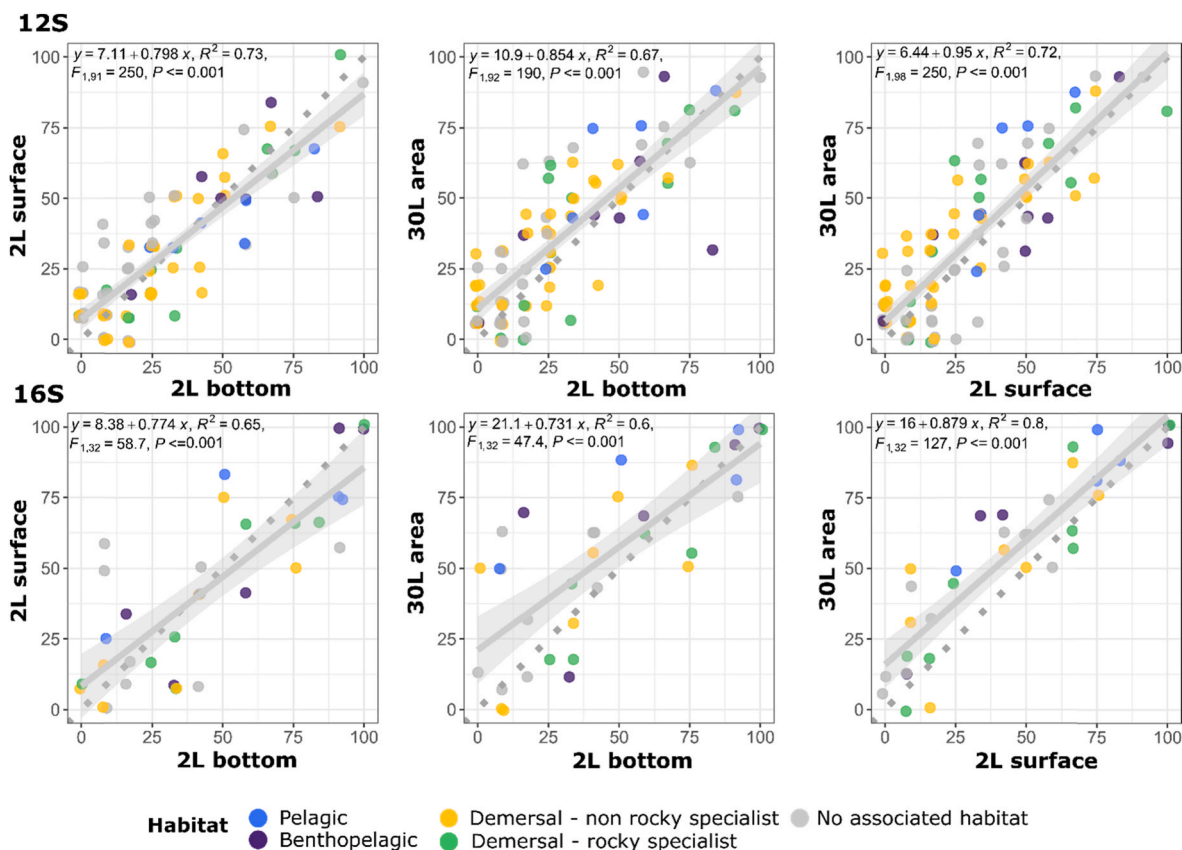


Fig. 6. Water sample comparisons in terms of OTU occurrence. Graphs show the relationships between the frequencies of OTU occurrence (computed across all replicates) between the three types of water sample for the autumn season. Each dot represents one OTU, coloured according to its specific habitat. Solid line represents the regression line ( $y \sim x$ ) with the 95% confidence interval. The dotted line represents the 1:1 line along which the frequency of OTU occurrence would be equal in both types of water sample.

#### 4.1. eDNA metabarcoding informs on coastal rocky fish assemblages

The eDNA metabarcoding survey detected taxa specific to demersal rocky habitats as well as taxa living in the water column or associated to adjacent demersal habitats of the study bay. Along the same line,  $\beta$  diversity patterns revealed with eDNA metabarcoding were driven by

OTUs assigned to taxa inhabiting those non-rocky habitats. The benthopelagic European sea bass (*D. labrax*), frequently detected in eDNA metabarcoding samples, and very rarely observed in UVC transects, is a noteworthy example. Therefore, eDNA metabarcoding increases the detectability of mobile and elusive species, living in or nearby rocky habitats, otherwise generally underestimated with UVC (Aglieri et al.,

2020; Thanopoulou et al., 2018). Our findings are in agreement with previous studies, comparing eDNA metabarcoding and traditional coastal fish survey methods (observational and fishery catches data), which showed that eDNA metabarcoding detected broader phylogenetic and functional diversities and least redundant fish assemblages than traditional survey methods (Aglieri et al., 2020; Marques et al., 2021a; Polanco Fernández et al., 2021a).

When focusing on demersal rocky specialist species, as targeted by the UVC survey, eDNA metabarcoding and UVC revealed similar richness at the bay level. In the case of Labridae, a family frequently observed in North East Atlantic rocky fish assemblages (Jackson et al., 2006; Pita et al., 2018), eDNA metabarcoding successfully detected five (*Labrus mixtus*, *Ctenolabrus rupestris*, *Centrolabrus exoletus*, *S. melops*, *S. bailloni*) of the six species observed with UVC. The sixth species, *L. bergylta*, was only identified to the genus level (see 4.4). In addition, the five species were observed within the same range of occurrence frequency between UVC and eDNA metabarcoding, except for *S. bailloni*, undetected through UVC, while observed in 19 of the 64 eDNA samples. Several studies, and notably two of them in the English Channel, have warned about the under detection of this species with UVC because of recurrent misidentification with *S. melops* (Dunn and Brown, 2003; Göktürk et al., 2012; Maran and Chanet, 2011). These studies concluded that *S. bailloni* is likely the most abundant *Symphodus* species in the area and common in estuarine habitats, encompassing several types of bottom substrates (rocky, muddy, sandy and sea-grass beds). Thus, the fact that *S. bailloni* was not detected in our UVC dataset, is likely because of misidentification, or simply because it was absent from the site at the time of sampling. Its detection through eDNA metabarcoding could mean that this species is indeed present in surrounding habitats, which were not monitored by UVC. This particular example highlights the great potential of eDNA metabarcoding to increase knowledge on species distribution, particularly for species supposedly infrequently observed through traditional surveys (Stoeckle et al., 2020b).

This is also especially the case for species only present in certain habitat at some time of the year, or in very dynamic environments. Indeed, local diversity and fish assemblage can be captured at a fine spatio-temporal scale, through eDNA metabarcoding, even in very dynamic marine coastal environments (Collins et al., 2018; Jeunen et al., 2019; Lamy et al., 2021; Oka et al., 2021; Port et al., 2016). While transport of DNA over small distances from neighbouring habitats cannot be excluded (Jeunen et al., 2019), it is noteworthy in our study that both sampling seasons and coastal gradient explained the  $\beta$  diversity patterns revealed by eDNA metabarcoding, similar to those detected with UVC. For instance, *A. presbyter* was mainly detected in spring samples, probably because spawning occurs in this season for this species (Henderson et al., 1984; Moreno et al., 2005). A positive correlation between eDNA concentration and fish counts during spawning events has been recently highlighted with ddPCR (Thalinger et al., 2019) and qPCR (Tsuji and Shibata, 2021) approaches. These studies and our results suggest that eDNA metabarcoding could be suitable to detect seasonal movement and spawning events of coastal and estuarine species (Sigsgaard et al., 2017; Stoeckle et al., 2017).

#### 4.2. Water sampling strategy for coastal fish diversity monitoring

The presence and persistence through time of DNA in the water column depend on habitats (e.g. pelagic vs demersal), environmental characteristics (e.g. seasonality, tide, vertical stratification, temperature), and species ecological characteristics (e.g. spawning period/location, behaviours) (Bracken et al., 2019; Collins et al., 2018; Kelly et al., 2018; Sassoubre et al., 2016; Thomsen et al., 2012). Consequently, those factors, among others, need to be appreciated when selecting a water sampling protocol (e.g. volume, replicability, spatial cover, depth). In our study, the distance from the seafloor (surface vs bottom), spatial covering and filtered volume (2-L stationary vs 30-L in the close

vicinity of the sampling point), had very low impact on the observed taxonomic richness, OTU detectability, accumulated OTU diversity and overall  $\beta$  diversity patterns (sample dissimilarity and OTU contribution to  $\beta$  diversity). Other studies, highlighting differences in observed fish diversity between the surface and bottom of coastal areas, suggested that the DNA transport is limited because the thermoclines or haloclines prevent the vertical mixing of water layers (Andruszkiewicz et al., 2017; Jeunen et al., 2020; Sigsgaard et al., 2020; Stoeckle et al., 2020a; Yamamoto et al., 2016, 2017). Our study site, the bay of Morlaix, is characterized by strong tides, with no seasonal thermocline, resulting in a water column permanently well-mixed (Wafar et al., 1983). This characteristic of the southern part of the western English Channel could explain the absence of differences between samples taken at the surface and bottom.

Similarly, there are currently no common guideline or recommendations on the volume of filtered water, even if 1–2L is often preferred as a trade-off between quantity and filtering feasibility (Shu et al., 2020). However, other studies demonstrated that large water volumes collected alongside longitudinal transects, or large number of small volume water samples are necessary for reliable fish biodiversity assessment in coastal areas (Bessey et al., 2020; Juhel et al., 2020; Stat et al., 2019; Stauffer et al., 2021; Rozanski et al., 2022). In our study, we did not observe substantial changes in the detectability of taxa between samples of 30-L covering a small area around the study sites and samples of 2-L collected at a stationary point. The only exception was for demersal non rocky taxa, for which the 30-L water samples detected higher richness in the inner sites of the bay than at other sites. One of the main aims of our study was to evaluate the capacity of eDNA metabarcoding to detect spatial variations of fish assemblage in sites close of few kilometres. We thus deliberately decided to not perform an integrative sampling alongside a transect of several kilometres, as done in other marine eDNA studies performing large volume sampling (Boulanger et al., 2021; Marques et al., 2021a; Polanco Fernández et al., 2021a). Clearly, for fish biodiversity surveys across different habitats, large volumes collected over a wide area (several kilometres) might be preferred to increase the detectability of species. Yet, in a context of optimizing sampling protocol of monitoring programmes, our results stress the need for additional tests to unveil the relative importance of spatial covering in relation with filtered volume.

#### 4.3. Molecular limitations of eDNA metabarcoding of coastal fishes

Our study agrees with a plethora of studies using eDNA metabarcoding for fish monitoring in that this method detects more species than traditional ones but is paradoxically not able to recover all the species reported by traditional methods. This is mostly due to limitations in taxonomic resolution and/or lacks of reference data (Aglieri et al., 2020; DiBattista et al., 2017; Eble et al., 2020; Juhel et al., 2020; Nester et al., 2020). Here we showed that the two markers used failed to identify seven of the 11 species observed with UVC only because of these two limitations. In the case of *L. bergylta* and *C. conger*, for which reference sequences were 100% identical to other species of the same genus (*Labrus viridis*, *Labrus merula* and *Conger oceanicus*, respectively), OTUs could only be assigned at the genus level. It is therefore tempting to assign them to the two species reported through UVC, as the other species matching at 100% with our sequence are not present in the study area (Iglesias, 2020). This is however at risk if some of these species have recently migrated in the area.

In order to develop common standard procedures to integrate eDNA metabarcoding in future legislative frameworks monitoring, the choice of markers and primers remains critical. In our study, the use of primers targeting 12S allowed for a better species-level identification than 16S. The latter is indeed characterized by the absence of clear barcoding gap explaining its weak taxonomic discriminating power. However, the reference sequences targeting 16S are more numerous in public databases. The trade-off between sequence availability, the marker

taxonomic discriminating power and the use of primers targeting a wide range of taxa had been scrutinized for marine and freshwater fishes and the 12S marker is becoming more and more popular for fishes (Collins et al., 2019; Polanco Fernández et al., 2021b). Here, we worked with the Teleo primers (Valentini et al., 2016) because of their great ability to detect and identify both actinopterygians and elasmobranchs (Collins et al., 2019). However, their taxonomic resolution can be limited and the use of MiFish primers (Miya et al., 2015) in parallel targeting a longer fragment of the same 12S marker would have potentially increase the detection of species observed in UVC, in particular for *P. pollachius*, *P. pagrus* and *M. surmuletus* (Polanco Fernández et al., 2021b). The case of *P. pollachius*, which is a conspicuous species in the rocky habitat of the study bay, illustrates how the choice of primers is crucial. Therefore, we recommend a multi-marker and multi-primers approach, as well as an evaluation of markers and primers upstream of future monitoring surveys to increase the benefit of eDNA metabarcoding. To ease such evaluation, future studies should benefit from the easy-to-use application GAPeDNA (Marques et al., 2021b) indicating the availability of references according to primers and marine bioregion and the pipeline Meta-Fish-Lib (Collins et al., 2021) creating a curated reference database for fishes from public databases.

#### 4.4. eDNA metabarcoding as a complementary fish monitoring tool for the Marine Strategy Framework Directive

Coastal areas are composed of multiple patchy adjacent habitats requiring the use of various survey methods (e.g. bottom trawling for demersal soft-bottom, UVC for demersal rocky bottom, echosounder or fishing net for pelagic habitat) to monitor fishes in support of the MFSD (Baudrier et al., 2018). Many species utilize those different habitats depending on their life-stages and seasons (e.g. reproduction, nursery, feeding) and are consequently sampled with different survey methods. Relying on observational data from different survey methods can complicate their combination to monitor the species at an inter-habitat level. In this context, the use of eDNA metabarcoding, by potentially encompassing the coastal diversity across different habitats, could complement and better link monitoring programs developed separately for each habitat of the MFSD. While traditional survey methods offer a fine scale monitoring focused on a target habitat, eDNA metabarcoding provides an integrated view of the coastal habitats. It is therefore expected to be beneficial for the MFSD descriptors D2 “Non-Indigenous Species” and D4 “food webs” as it can provide data on the composition and structure of the species assemblage in terms of taxonomy, phylogeny and ecological functions. It could also complement traditional survey methods for the D1 “biodiversity” especially regarding the species distributional range (D1C4). Yet, other criteria relying on abundance (D1C2) and individual size spectra (D1C3) need to be informed by traditional survey methods.

### 5. Concluding remarks and future directions

Coastal rocky habitats are integrating vertically (demersal to pelagic) and horizontally (from inside the reef to neighbouring habitats) diverse fish assemblages (Harmelin, 1987; Pita et al., 2018). Our study showed that the use of eDNA metabarcoding can ease the monitoring of coastal rocky fishes. By grasping fish diversity at a fine spatio-temporal scale, eDNA metabarcoding can enhance the assessment of taxonomic, phylogenetic and functional diversities of coastal rocky fish assemblages. This is thus an added-value over traditional survey methods not designed to capture a wide biodiversity spectrum (Aglieri et al., 2020). Yet, no single survey method will be self-sufficient. Indeed, eDNA metabarcoding uncertainties in providing a quantitative assessment of the species present in the community (Gilbey et al., 2021; Lamb et al., 2019; Ruppert et al., 2019), as well as in identifying different life-stages of a species, limit its monitoring applications. Nonetheless, further efforts are needed to better integrate eDNA metabarcoding in current

monitoring programs. For that purpose, the use of multi-species occupancy models holds great promise to account for differences in taxa detectability between eDNA metabarcoding and traditional survey methods (Doi et al., 2019; McClenaghan et al., 2020; McColl-Gausden et al., 2020). While many challenges are still ahead (Ruppert et al., 2019), there are growing evidences that integrating eDNA metabarcoding into monitoring programs will be beneficial to the conservation of coastal fish diversity.

#### Authorship contribution statement

F.V., E.C. and P.T. conceived the study and obtained fundings. F.V. and P.T. conducted fieldwork. F.V. conducted laboratory work until DNA extraction for the 2L water samples and A.L. conducted laboratory work to produce the local reference sequences. A.R. conducted the analyses, with advice from E.C. and F.V. regarding the bioinformatics steps, and from P.T. regarding the statistical analyses. A.V. coordinated the DNA extraction for the 30L water samples and the analyses for all eDNA. A.R. wrote a first draft with substantial contribution from F.V., A. L. and P.T. All authors made critical editing of the drafts and gave final approval for publication.

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#### Declaration of competing interest

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

#### Data availability

Reference sequences locally are available in GenBank (OM509528 to OM509567, OM470917 to OM470931). All sequencing raw data and scripts are available at <https://doi.org/10.5281/zenodo.6091488>

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ocecoaman.2023.106625>.

## References

- Afzali, S.F., Bourdages, H., Laporte, M., Mérot, C., Normandeau, E., Audet, C., Bernatchez, L., 2021. Comparing environmental metabarcoding and trawling survey of demersal fish communities in the Gulf of St. Lawrence, Canada. *Environ. DNA* 3, 22–42. <https://doi.org/10.1002/edn3.111>.
- Aglieri, G., Baillie, C., Mariani, S., Cattano, C., Calò, A., Turco, G., Spatafora, D., Franco, A.D., Lorenzo, M.D., Guidetti, P., Milazzo, M., 2020. Environmental DNA effectively captures functional diversity of coastal fish communities. *Mol. Ecol.* 30, 3127–3139. <https://doi.org/10.1111/mec.15661>.
- Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. *Permanova+ for Primer: a guide to software and statistical methods*. PRIMER-E Ltd. Plymouth, pp. 214. <https://www.worldcat.org/title/permanova-for-primer-guide-to-software-and-statistical-methods/oclc/841889821>.
- Andruszkiewicz, E.A., Starks, H.A., Chavez, F.P., Sassoubre, L.M., Block, B.A., Boehm, A.B., 2017. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. *PLoS One* 12, e0176343. <https://doi.org/10.1371/journal.pone.0176343>.
- Arthington, A.H., Dulvy, N.K., Gladstone, W., Winfield, I.J., 2016. Fish conservation in freshwater and marine realms: status, threats and management. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 26, 838–857. <https://doi.org/10.1002/aqc.2712>.
- Bajjouk, T., Hamdi, A., Maud, B., 2019. Map of intertidal habitats for the natura 2000 FR05300015 site – Morlaix bay. *Ifremer*. <https://doi.org/10.12770/57a9280a-caca-4e89-b2a0-52a2b85f8d5a>.
- Baker, D.G., Eddy, T.D., McIver, R., Schmidt, A.L., Thériault, M.-H., Boudreau, M., Courtenay, S.C., Lotze, H.K., 2016. Comparative analysis of different survey methods for monitoring fish assemblages in coastal habitats. *PeerJ* 4, e1832. <https://doi.org/10.7717/peerj.1832>.
- Baudrier, J., Lefebvre, A., Galgani, F., Sarau, C., Doray, M., 2018. Optimising French fisheries surveys for Marine Strategy Framework Directive integrated ecosystem monitoring. *Mar. Pol.* 94, 10–19. <https://doi.org/10.1016/j.marpol.2018.04.024>.
- Bessey, C., Jarman, S.N., Berry, O., Olsen, Y.S., Bunce, M., Simpson, T., Power, M., McLaughlin, J., Edgar, G.J., Keesing, J., 2020. Maximizing fish detection with eDNA metabarcoding. *Environ. DNA* 2, 493–504. <https://doi.org/10.1002/edn3.74>.
- Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R.A., Foster, J., Wilkinson, J.W., Arnell, A., Brotherton, P., others, 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biol. Conserv.* 183, 19–28. <https://doi.org/10.1016/j.biocon.2014.11.029>.
- Bosch, N., Gonçalves, J., Erzini, K., 2017. “How” and “what” matters: sampling method affects biodiversity estimates of reef fishes. *Ecol. Evol.* 7, 4891–4906. <https://doi.org/10.1002/ece3.2979>.
- Boulanger, E., Loiseau, N., Valentini, A., Arnal, V., Boissery, P., Dejean, T., Deter, J., Guellati, N., Holon, F., Juhel, J.-B., Lenfant, P., Manel, S., Mouillot, D., 2021. Environmental DNA metabarcoding reveals and unpacks a biodiversity conservation paradox in Mediterranean marine reserves. *Proc. R. Soc. B Biol. Sci.* 288, 20210112. <https://doi.org/10.1098/rspb.2021.0112>.
- Boussarie, G., Bakker, J., Wangensteen, O.S., Mariani, S., Bonnin, L., Juhel, J.-B., Kiszka, J.J., Kulbicki, M., Manel, S., Robbins, W.D., Vigliola, L., Mouillot, D., 2018. Environmental DNA illuminates the dark diversity of sharks. *Sci. Adv.* 4, eaap9661. <https://doi.org/10.1126/sciadv.aap9661>.
- Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., Coissac, E., 2016. obitools: a unix-inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.* 16, 176–182. <https://doi.org/10.1111/1755-0998.12428>.
- Bracken, F.S.A., Rooney, S.M., Kelly-Quinn, M., King, J.J., Carlsson, J., 2019. Identifying spawning sites and other critical habitat in lotic systems using eDNA “snaphots”: a case study using the sea lamprey *Petromyzon marinus* L. *Ecol. Evol.* 9 (1), 553–567. <https://doi.org/10.1002/ece3.4777>.
- Caldwell, Z.R., Zgliczynski, B.J., Williams, G.J., Sandin, S.A., 2016. Reef fish survey techniques: assessing the potential for standardizing methodologies. *PLoS One* 11, e0153066. <https://doi.org/10.1371/journal.pone.0153066>.
- 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. *Nat. Methods* 13, 581–583. <https://doi.org/10.1038/nmeth.3869>.
- Clarke, K.R., Gorley, R.N., 2006. *PRIMER v6: User Manual/Tutorial* (Ed. Plymouth Marine Laboratory), Plymouth, UK.
- Collins, R.A., Bakker, J., Wangensteen, O.S., Soto, A.Z., Corrigan, L., Sims, D.W., Genner, M.J., Mariani, S., 2019. Non-specific amplification compromises environmental DNA metabarcoding with COI. *Methods Ecol. Evol.* 10, 1985–2001. <https://doi.org/10.1111/2041-210X.13276>.
- Collins, R.A., Trauzzi, G., Maltby, K.M., Gibson, T.I., Ratcliffe, F.C., Hallam, J., Rainbird, S., Maclaine, J., Henderson, P.A., Sims, D.W., Mariani, S., Genner, M.J., 2021. Meta-Fish-Lib: a generalised, dynamic DNA reference library pipeline for metabarcoding of fishes. *J. Fish. Biol.* 99, 1446–1454. <https://doi.org/10.1111/jfb.14852>.
- Collins, R.A., Wangensteen, O.S., O’Gorman, E.J., Mariani, S., Sims, D.W., Genner, M.J., 2018. Persistence of environmental DNA in marine systems. *Commun. Biol.* 1, 1–11. <https://doi.org/10.1038/s42003-018-0192-6>.
- Costello, M.J., Basher, Z., McLeod, L., Asaad, I., Claus, S., Vandepitte, L., Yasuhara, M., Gislason, H., Edwards, M., Appeltans, W., Enevoldsen, H., Edgar, G.J., Miloslavich, P., De Monte, S., Pinto, I.S., Obura, D., Bates, A.E., 2017. Methods for the study of marine biodiversity. In: Walters, M., Scholes, R.J. (Eds.), *The GEO Handbook on Biodiversity Observation Networks*. Springer International Publishing, Cham, pp. 129–163. [https://doi.org/10.1007/978-3-319-27288-7\\_6](https://doi.org/10.1007/978-3-319-27288-7_6).
- Couton, M., Lévêque, L., Daguin-Thiébaud, C., Comtet, T., Viard, F., 2022. Water eDNA metabarcoding is effective in detecting non-native species in marinas, but detection errors still hinder its use for passive monitoring. *Biofouling* 38, 367–383. <https://doi.org/10.1080/08927014.2022.2075739>.
- Danovaro, R., Carugati, L., Berzano, M., Cahill, A.E., Carvalho, S., Chenuil, A., Corinaldesi, C., Cristina, S., David, R., Dell’Anno, A., Dzhenbekova, N., Garcés, E., Gasol, J.M., Goela, P., Féral, J.-P., Ferrera, I., Forster, R.M., Kurekin, A.A., Rastelli, E., Marinova, V., Miller, P.L., Moncheva, S., Newton, A., Pearman, J.K., Pitois, S.G., René, A., Rodríguez-Ezpeleta, N., Saggiomo, V., Simis, S.G.H., Stefanova, K., Wilson, C., Lo Martire, M., Greco, S., Cochrane, S.K.J., Mangoni, O., Borja, A., 2016. Implementing and innovating marine monitoring approaches for assessing marine environmental status. *Front. Mar. Sci.* 3, 213. <https://doi.org/10.3389/fmars.2016.00213>.
- Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D.M., de Vere, N., Pfrender, M.E., Bernatchez, L., 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Mol. Ecol.* 26, 5872–5895. <https://doi.org/10.1111/mec.14350>.
- 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, 1245–1252. <https://doi.org/10.1007/s00338-017-1618-1>.
- DiBattista, J.D., Reimer, J.D., Stat, M., Masucci, G.D., Biondi, P., De Brauwier, M., Bunce, M., 2019. Digging for DNA at depth: Rapid universal metabarcoding surveys (RUMS) as a tool to detect coral reef biodiversity across a depth gradient. *PeerJ* 7, e6379. <https://doi.org/10.7717/peerj.6379>.
- Doi, H., Fukaya, K., Oka, S., Sato, K., Kondoh, M., Miya, M., 2019. Evaluation of detection probabilities at the water-filtering and initial PCR steps in environmental DNA metabarcoding using a multispecies site occupancy model. *Sci. Rep.* 9, 3581. <https://doi.org/10.1038/s41598-019-40233-1>.
- Dray, S., Blanchet, G., Borcard, D., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Madi, N., Wagner, H.H., 2018. ade4: Multivariate Multiscale Spatial Analysis. R package version 0. <https://CRAN.R-project.org/package=ade4spatial>.
- Dray, S., Dufour, A.-B., 2007. The ade4 package: implementing the duality diagram for ecologists. *J. Stat. Software* 22, 1–20. <https://doi.org/10.18637/jss.v022.i04>.
- Dunn, M.R., Brown, M.J., 2003. The occurrence of *Symphodus bailloni* on the south coast of England. *Mar. Biol. Assoc. U. K. J. Mar. Biol. Assoc. U. K.* 83, 875. <https://doi.org/10.1017/S002531540300794X>.
- Eble, J.A., Daly-Engel, T.S., DiBattista, J.D., Koziol, A., Gaither, M.R., 2020. Chapter two - marine environmental DNA: approaches, applications, and opportunities. In: Sheppard, C. (Ed.), *Advances in Marine Biology*. Academic Press, pp. 141–169. <https://doi.org/10.1016/bs.amb.2020.01.001>.
- Edgar, G.J., Cooper, A., Baker, S.C., Barker, V., Barrett, N.S., Becerro, M.A., Bates, A.E., Brock, D., Ceccarelli, D.M., Clausius, E., Davey, M., Davis, T.R., Day, P.B., Green, A., Griffiths, S.R., Hicks, J., Hinojosa, I.A., Jones, B.K., Kininmonth, S., Larkin, M.F., Lazzari, N., Lefcheck, J.S., Ling, S.D., Mooney, P., Oh, E., Pérez-Matus, A., Pocklington, J.B., Riera, R., Sanabria-Fernandez, J.A., Seroussi, Y., Shaw, I., Shields, D., Shields, J., Smith, M., Soler, G.A., Stuart-Smith, J., Turnbull, J., Stuart-Smith, R.D., 2020. Reef Life Survey: establishing the ecological basis for conservation of shallow marine life. *Biol. Conserv.* 252, 108855. <https://doi.org/10.1016/j.biocon.2020.108855>.
- Escoufier, Y., 1973. Le Traitement des Variables Vectorielles. *Biometrics* 29, 751–760. <https://doi.org/10.2307/2529140>.
- Figuerola-Pico, J., Carpio, A.J., Tortosa, F.S., 2020. Turbidity: a key factor in the estimation of fish species richness and abundance in the rocky reefs of Ecuador. *Ecol. Indic.* 111, 106021. <https://doi.org/10.1016/j.ecolind.2019.106021>.
- Fraija-Fernandez, N., Bouquieaux, M., Rey, A., Mendibil, I., Cotano, U., Irigoien, X., Santos, M., Rodríguez-Ezpeleta, N., 2020. Marine water environmental DNA metabarcoding provides a comprehensive fish diversity assessment and reveals spatial patterns in a large oceanic area. *Ecol. Evol.* 10, 7560–7584. <https://doi.org/10.1002/ece3.6482>.
- Franco, A., Pérez-Ruzafa, A., Drouineau, H., Franzi, P., Koutrakis, E.T., Lepage, M., Verdiell-Cubedo, D., Bouchoucha, M., López-Capel, A., Riccato, F., Sapounidis, A., Marcos, C., Oliva-Paterna, F.J., Torralva-Foreror, M., Torricelli, P., 2012. Assessment of fish assemblages in coastal lagoon habitats: effect of sampling method. *Estuar. Coast. Shelf Sci., Assessing Ecological Quality in Estuarine and Coastal Systems – Functional Perspective* 112, 115–125. <https://doi.org/10.1016/j.ecss.2011.08.015>.
- Froese, R., Pauly, D., 2019. FishBase. World Wide Web electronic publication. [www.fishbase.org](http://www.fishbase.org).
- Frøsløv, T.G., Kjølner, 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. *Nat. Commun.* 8, 1188. <https://doi.org/10.1038/s41467-017-01312-x>.
- Furlan, E.M., Gleeson, D., Wisniewski, C., Yick, J., Duncan, R.P., 2019. eDNA surveys to detect species at very low densities: a case study of European carp eradication in Tasmania, Australia. *J. Appl. Ecol.* 56, 2505–2517. <https://doi.org/10.1111/1365-2664.13485>.
- Gilbey, J., Carvalho, G., Castilho, R., Coscia, I., Coulson, M., Dahle, G., Derycke, S., Francisco, S., Helyar, S., Johansen, T., Junge, C., Layton, K., Martinsohn, J.,

- Matejusova, I., Robalo, J., Rodriguez-Ezpeleta, N., Silva, G., Strammer, I., Vasemägi, A., Volckaert, F., 2021. Life in a drop: sampling environmental DNA for marine fishery management and ecosystem monitoring. *Mar. Pol.* 124, 104331 <https://doi.org/10.1016/j.marpol.2020.104331>.
- Göktürk, D., Karakulak, F.S., Ünsal, N., Kahraman, A.E., 2012. A new record for occurrence of *Symphodus bailloni* (osteichthyes: perciformes: Labridae) in the western black sea coast of Turkey. *Sci. World J.* 2012, 615318 <https://doi.org/10.1100/2012/615318>.
- Harmelin, J.-G., 1987. Structure et variabilité de l'ichtyofaune d'une zone rocheuse protégée en Méditerranée (Pare national de Port-Cros, France). *Mar. Ecol.* 8, 263–284. <https://doi.org/10.1111/j.1439-0485.1987.tb00188.x>.
- Harrison, J.B., Sunday, J.M., Rogers, S.M., 2019. Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proc. R. Soc. B Biol. Sci.* 286, 20191409 <https://doi.org/10.1098/rspb.2019.1409>.
- Henderson, P.A., Turnpenny, A.W.H., Bamber, R.N., 1984. Long-term stability of a sand smelt (*Atherina presbyter* Cuvier) population subject to power station cropping. *J. Appl. Ecol.* 21, 1–10. <https://doi.org/10.2307/2403034>.
- Holmlund, C.M., Hammer, M., 1999. Ecosystem services generated by fish populations. *Ecol. Econ.* 29, 253–268. [https://doi.org/10.1016/S0921-8009\(99\)00015-4](https://doi.org/10.1016/S0921-8009(99)00015-4).
- Hsieh, T.C., Ma, K.H., Chao, A., 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods Ecol. Evol.* 7, 1451–1456. <https://doi.org/10.1111/2041-210X.12613>.
- Hughes, L.C., Ortí, G., Huang, Y., Sun, Y., Baldwin, C.C., Thompson, A.W., Arcila, D., Betancur-R., Li, C., Becker, L., Bellora, N., Zhao, X., Li, X., Wang, M., Fang, C., Xie, B., Zhou, Z., Huang, H., Chen, S., Venkatesh, B., Shi, Q., 2018. Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transcriptomic and genomic data. *Proc. Natl. Acad. Sci. USA* 115, 6249–6254. <https://doi.org/10.1073/pnas.1719358115>.
- Iglesias, S., 2020. Piscibus Marinis - Guide des poissons marins, Europe et eaux adjacentes. Archimer. <https://archimer.ifremer.fr/doc/00660/77177/>.
- Jackson, E.L., Attrill, M.J., Rowden, A.A., Jones, M.B., 2006. Seagrass complexity hierarchies: influence on fish groups around the coast of Jersey (English Channel). *J. Exp. Mar. Biol. Ecol.* 330, 38–54. <https://doi.org/10.1016/j.jembe.2005.12.016>.
- Jeunen, G.-J., Knapp, M., Spencer, H.G., Lamare, M.D., Taylor, H.R., Stat, M., Bunce, M., Gemmill, N.J., 2019. Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. *Mol. Ecol. Resour.* 19, 426–438. <https://doi.org/10.1111/1755-0998.12982>.
- Jeunen, G.-J., Lamare, M.D., Knapp, M., Spencer, H.G., Taylor, H.R., Stat, M., Bunce, M., Gemmill, N.J., 2020. Water stratification in the marine biome restricts vertical environmental DNA (eDNA) signal dispersal. *Environ. DNA* 2, 99–111. <https://doi.org/10.1002/edn3.49>.
- Juhel, J.-B., Utama, R.S., Marques, V., Vimono, I.B., Sugeha, H.Y., Kadarusman, K., Pouyaud, L., Dejean, T., Mouillot, D., Hocdé, R., 2020. Accumulation curves of environmental DNA sequences predict coastal fish diversity in the coral triangle. *Proc. R. Soc. B Biol. Sci.* 287, 20200248 <https://doi.org/10.1098/rspb.2020.0248>.
- Kelly, R., Gallego, R., Jacobs-Palmer, E., 2018. The effect of tides on nearshore environmental DNA. *PeerJ* 6, e4521. <https://doi.org/10.7717/peerj.4521>.
- Kelly, R.P., O'Donnell, J.L., Lowell, N.C., Shelton, A.O., Samhoury, J.F., Hennessey, S.M., Feist, B.E., Williams, G.D., 2016. Genetic signatures of ecological diversity along an urbanization gradient. *PeerJ* 4, e2444. <https://doi.org/10.7717/peerj.2444>.
- Lamb, P.D., Hunter, E., Pinnegar, J.K., Creer, S., Davies, R.G., Taylor, M.I., 2019. How quantitative is metabarcoding: a meta-analytical approach. *Mol. Ecol.* 28, 420–430. <https://doi.org/10.1111/mec.14920>.
- Lamy, T., Pitz, K.J., Chavez, F.P., Yorke, C.E., Miller, R.J., 2021. Environmental DNA reveals the fine-grained and hierarchical spatial structure of kelp forest fish communities. *Sci. Rep.* 11, 14439 <https://doi.org/10.1038/s41598-021-93859-5>.
- Legendre, P., Cáceres, M.D., 2013. Beta diversity as the variance of community data: dissimilarity coefficients and partitioning. *Ecol. Lett.* 16, 951–963. <https://doi.org/10.1111/ele.12141>.
- Legendre, P., Legendre, L., 2012. Numerical Ecology. Elsevier. <https://shop.elsevier.com/books/numerical-ecology/legendre/978-0-444-53868-0>.
- Livore, J.P., Mendez, M.M., Miloslavich, P., Rilov, G., Bigatti, G., 2021. Biodiversity monitoring in rocky shores: challenges of devising a globally applicable and cost-effective protocol. *Ocean Coast Manag.* 205, 105548 <https://doi.org/10.1016/j.ocecoaman.2021.105548>.
- 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://doi.org/10.7717/peerj.1420>.
- Maran, V., Chanet, B., 2011. Observations du crénilabre de Baillon *Symphodus bailloni* (Valenciennes, 1839), Labridae, dans et autour de la rade de Brest (Nord Bretagne, France). *Bull. Société Sci. Nat. Ouest Fr.* 33, 57–60. <https://www.researchgate.net/publication/233842670-Observations-de-crenilabre-de-Baillon-Symphodus-bailloni-Valenciennes-1839-Labridae-dans-et-autour-de-la-rade-de-Brest-Nord-Bretagne-France>.
- Marques, V., Castagné, P., Polanco Fernández, A., Borrero-Pérez, G.H., Hocdé, R., Guérin, P.-É., Juhel, J.-B., Velez, L., Loiseau, N., Letessier, T.B., Bessudo, S., Valentini, A., Dejean, T., Mouillot, D., Pellissier, L., Villéger, S., 2021a. Use of environmental DNA in assessment of fish functional and phylogenetic diversity. *Conserv. Biol.* 35, 1944–1956. <https://doi.org/10.1111/cobi.13802>.
- Marques, V., Milhau, T., Albouy, C., Dejean, T., Manel, S., Mouillot, D., Juhel, J.-B., 2021b. GAPeDNA: assessing and mapping global species gaps in genetic databases for eDNA metabarcoding. *Divers. Distrib.* 27, 1880–1892. <https://doi.org/10.1111/ddi.13142>.
- Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet journal* 17, 10–12. <https://doi.org/10.14806/ej.17.1.200>.
- McClenaghan, B., Compson, Z.G., Hajjibabaei, M., 2020. Validating metabarcoding-based biodiversity assessments with multi-species occupancy models: a case study using coastal marine eDNA. *PLoS One* 15, e0224119. <https://doi.org/10.1371/journal.pone.0224119>.
- McColl-Gausden, E.F., Weeks, A.R., Coleman, R.A., Robinson, K.L., Song, S., Raadik, T.A., Tingley, R., 2020. Multispecies models reveal that eDNA metabarcoding is more sensitive than backpack electrofishing for conducting fish surveys in freshwater streams. *Mol. Ecol.* 30, 3111–3126. <https://doi.org/10.1111/mec.15644>.
- McElroy, M., Dressler, T., Titcomb, G., Wilson, E., Deiner, K., Dudley, T., Eliason, E., Evans, N., Gaines, S., Lafferty, K., Lamberti, G., Li, Y., Lodge, D., Love, M., Mahon, A., Pfrender, M., Renshaw, M., Selkoe, K., Jerde, C., 2020. Calibrating environmental DNA metabarcoding to conventional surveys for measuring fish species richness. *Front. Ecol. Evol.* 8, 276. <https://doi.org/10.3389/fevo.2020.00276>.
- Miya, M., 2022. Environmental DNA metabarcoding: a novel method for biodiversity monitoring of marine fish communities. *Ann. Rev. Mar. Sci.* 14, 161–185. <https://doi.org/10.1146/annurev-marine-041421-082251>.
- Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J.Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., others, 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *R. Soc. Open Sci.* 2, 150088 <https://doi.org/10.1098/rsos.150088>.
- Mora, C., Tittensor, D.P., Myers, R.A., 2008. The completeness of taxonomic inventories for describing the global diversity and distribution of marine fishes. *Proc. R. Soc. B Biol. Sci.* 275, 149–155. <https://doi.org/10.1098/rspb.2007.1315>.
- Moreno, T., Castro, J.J., Socorro, J., 2005. Reproductive biology of the sand smelt *Atherina presbyter* Cuvier, 1829 (pisces: atherinidae) in the central-East Atlantic. *Fish. Res.* 72, 121–131. <https://doi.org/10.1016/j.fishres.2004.06.016>.
- Nelson, J.S., Grande, T.C., Wilson, M.V.H., 2016. Fishes of the World. John Wiley & Sons, Inc, Hoboken, NJ, USA. <https://doi.org/10.1002/9781119174844>.
- Nester, G.M., Brauwer, M.D., Kozioł, 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). *Environ. DNA* 2, 614–626. <https://doi.org/10.1002/edn3.93>.
- Oka, S., Doi, H., Miyamoto, K., Hanahara, N., Sado, T., Miya, M., 2021. Environmental DNA metabarcoding for biodiversity monitoring of a highly diverse tropical fish community in a coral reef lagoon: estimation of species richness and detection of habitat segregation. *Environ. DNA* 3, 55–69. <https://doi.org/10.1002/edn3.132>.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. Package 'vegan.' community ecology. Package Version 2, 1–295. <http://CRAN.Rproject.org/packge=vegan>.
- 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. <https://doi.org/10.7717/peerj.5378>.
- Pelletier, D., Leleu, K., Mou-Tham, G., Guillemot, N., Chabanet, P., 2011. Comparison of visual census and high definition video transects for monitoring coral reef fish assemblages. *Fish. Res.* 107, 84–93. <https://doi.org/10.1016/j.fishres.2010.10.011>.
- Perera-Valderrama, S., Cerdeira-Estrada, S., Martell-Dubois, R., Rosique-de la Cruz, L., Caballero-Aragón, H., Valdez-Chavarín, J., López-Perea, J., Ressler, R., 2020. A new long-term marine biodiversity monitoring program for the knowledge and management in marine protected areas of the Mexican caribbean. *Sustainability* 12, 7814. <https://doi.org/10.3390/su12187814>.
- Pita, P., Fernández-Márquez, D., Freire, J., 2018. Spatiotemporal variation in the structure of reef fish and macroalgal assemblages in a north-east Atlantic kelp forest ecosystem: implications for the management of temperate rocky reefs. *Mar. Freshw. Res.* 69, 525–541. <https://doi.org/10.1071/MF17193>.
- Pita, P., Fernández-Márquez, D., Freire, J., 2014. Short-term performance of three underwater sampling techniques for assessing differences in the absolute abundances and in the inventories of the coastal fish communities of the Northeast Atlantic Ocean. *Mar. Freshw. Res.* 65, 105–113. <https://doi.org/10.1071/MF12301>.
- Polanco Fernández, A., Marques, V., Fopp, F., Juhel, J.-B., Borrero-Pérez, G.H., Cheutin, M.-C., Dejean, T., González Corredor, J.D., Acosta-Chaparro, A., Hocdé, R., 2021a. Comparing environmental DNA metabarcoding and underwater visual census to monitor tropical reef fishes. *Environ. DNA* 3, 142–156. <https://doi.org/10.1002/edn3.140>.
- Polanco Fernández, A., Richards, E., Flück, B., Valentini, A., Altermatt, F., Brosse, S., Walser, J.-C., Eme, D., Marques, V., Manel, S., Albouy, C., Dejean, T., Pellissier, L., 2021b. Comparing the performance of 12S mitochondrial primers for fish environmental DNA across ecosystems. *Environ. DNA* 3, 1113–1127. <https://doi.org/10.1002/edn3.232>.
- Pont, D., Valentini, A., Rocle, M., Maire, A., Delaigue, O., Jean, P., Dejean, T., 2021. The future of fish-based ecological assessment of European rivers: from traditional EU Water Framework Directive compliant methods to eDNA metabarcoding-based approaches. *J. Fish. Biol.* 98, 354–366. <https://doi.org/10.1111/jfb.14176>.
- 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. *Mol. Ecol.* 25, 527–541. <https://doi.org/10.1111/mec.13481>.
- Pörtner, H.O., Schulte, P.M., Wood, C.M., Schiemer, F., 2010. Niche dimensions in fishes: an integrative view. *Physiol. Biochem. Zool.* 83, 808–826. <https://doi.org/10.1086/655977>.
- Rozanski, R., Trenkel, V.M., Lorange, P., Valentini, A., Dejean, T., Pellissier, L., Ecme, D., Albouy, C., 2022. Disentangling the components of coastal fish biodiversity in

- southern Brittany by applying an environmental DNA approach. *Envir. DNA* 4, 920–933. <https://doi.org/10.1002/edn3.305>.
- Ruppert, K.M., Kline, R.J., Rahman, M.S., 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: a systematic review in methods, monitoring, and applications of global eDNA. *Glob. Ecol. Conserv.* 17, e00547 <https://doi.org/10.1016/j.gecco.2019.e00547>.
- Sassoubre, L.M., Yamahara, K.M., Gardner, L.D., Block, B.A., Boehm, A.B., 2016. Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. *Environ. Sci. Technol.* 50, 10456–10464. <https://doi.org/10.1021/acs.est.6b03114>.
- Schnell, I.B., Bohmann, K., Gilbert, M.T.P., 2015. Tag jumps illuminated—reducing sequence-to-sample misidentifications in metabarcoding studies. *Mol. Ecol. Resour.* 15, 1289–1303. <https://doi.org/10.1111/1755-0998.12402>.
- Shu, L., Ludwig, A., Peng, Z., 2020. Standards for methods utilizing environmental DNA for detection of fish species. *Genes* 11, 296. <https://doi.org/10.3390/genes11030296>.
- Sigsgaard, E.E., Nielsen, I.B., Carl, H., Krag, M.A., Knudsen, S.W., Xing, Y., Holm-Hansen, T.H., Møller, P.R., Thomsen, P.F., 2017. Seawater environmental DNA reflects seasonality of a coastal fish community. *Mar. Biol.* 164, 128. <https://doi.org/10.1007/s00227-017-3147-4>.
- Sigsgaard, E.E., Torquato, F., Frøslev, T.G., Moore, A.B.M., Sørensen, J.M., Range, P., Ben-Hamadou, R., Bach, S.S., Møller, P.R., Thomsen, P.F., 2020. Using vertebrate environmental DNA from seawater in biomonitoring of marine habitats. *Conserv. Biol.* 34, 697–710. <https://doi.org/10.1111/cobi.13437>.
- Stat, M., John, J., DiBattista, J.D., Newman, S.J., Bunce, M., Harvey, E.S., 2019. Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Conserv. Biol.* 33, 196–205. <https://doi.org/10.1111/cobi.13183>.
- Stauffer, S., Jucker, M., Keggins, T., Marques, V., Andrello, M., Bessudo, S., Cheutin, M.-C., Borrero-Pérez, G.H., Richards, E., Dejean, T., Hocdé, R., Juhel, J.-B., Ladino, F., Letessier, T.B., Loiseau, N., Maire, E., Mouillot, D., Mutis Martinezguerra, M., Manel, S., Polanco Fernández, A., Valentini, A., Velez, L., Albouy, C., Pellissier, L., Waldoock, C., 2021. How many replicates to accurately estimate fish biodiversity using environmental DNA on coral reefs? *Ecol. Evol.* 11, 14630–14643. <https://doi.org/10.1002/ece3.8150>.
- Stoeckle, M.Y., Adolf, J., Charlop-Powers, Z., Dunton, K.J., Hinks, G., VanMorter, S.M., 2020a. Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. *ICES J. Mar. Sci.* 78, 293–304. <https://doi.org/10.1093/icesjms/fsaa225>.
- Stoeckle, M.Y., Das Mishu, M., Charlop-Powers, Z., 2020b. Improved environmental DNA reference library detects overlooked marine fishes in New Jersey, United States. *Front. Mar. Sci.* 7, 226. <https://doi.org/10.3389/fmars.2020.00226>.
- Stoeckle, M.Y., Soboleva, L., Charlop-Powers, Z., 2017. Aquatic environmental DNA detects seasonal fish abundance and habitat preference in an urban estuary. *PLoS One* 12, e0175186. <https://doi.org/10.1371/journal.pone.0175186>.
- Taberlet, P., Bonin, A., Zinger, L., Coissac, E., 2018. *Environmental DNA: for Biodiversity Research and Monitoring*. Oxford University Press, Oxford, UK. <https://doi.org/10.1093/oso/9780198767220.001.0001>.
- Taberlet, P., Coissac, E., Hajibabaei, M., Rieseberg, L.H., 2012. Environmental DNA. *Mol. Ecol.* 21, 1789–1793. <https://doi.org/10.1111/j.1365-294X.2012.05542.x>.
- Thalinger, B., Wolf, E., Traugott, M., Wanzenböck, J., 2019. Monitoring spawning migrations of potamodromous fish species via eDNA. *Sci. Rep.* 9, 15388. <https://doi.org/10.1038/s41598-019-51398-0>.
- Thanopoulou, Z., Sini, M., Vatikiotis, K., Katsoupis, C., Dimitrakopoulos, P.G., Katsanevakis, S., 2018. How many fish? Comparison of two underwater visual sampling methods for monitoring fish communities. *PeerJ* 6, e5066. <https://doi.org/10.7717/peerj.5066>.
- Thiriet, P.D., Franco, A.D., Cheminée, A., Guidetti, P., Bianchimani, O., Basthard-Bogain, S., Cottalorda, J.-M., Arceo, H., Moranta, J., Lejeune, P., Francour, P., Mangialajo, L., 2016. Abundance and diversity of crypto- and necto-benthic coastal fish are higher in marine forests than in structurally less complex macroalgal assemblages. *PLoS One* 11, e0164121. <https://doi.org/10.1371/journal.pone.0164121>.
- Thompson, A.A., Mapstone, B.D., 1997. Observer effects and training in underwater visual surveys of reef fishes. *Mar. Ecol. Prog. Ser.* 154, 53–63. [https://www.google.com/url?sa=t&trct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKewj\\_wpmU3Kv\\_AhWWTKQEhX0iDXQQgAmoAHOeCAMQAw&url=https%3A%2F%2Fscholar.google.fr%2Fscholar\\_url%3Furl%3Dhttps%3A%2F%2Fwww.int-res.com%2Farticles%2Fmeps%2F154%2Fm154p053.pdf%26hl%3Den%26sa%3DX%26ei%3D5Z19ZoATB5CTy9YPnOes8AE%26scisig%3DAGIGAw9pE-DRbtYg\\_Ja1iYDy9f9Z%26oi%3Dscholar&usg=AOvVaw2wxuoaihdu-BHQL0pgcuel](https://www.google.com/url?sa=t&trct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKewj_wpmU3Kv_AhWWTKQEhX0iDXQQgAmoAHOeCAMQAw&url=https%3A%2F%2Fscholar.google.fr%2Fscholar_url%3Furl%3Dhttps%3A%2F%2Fwww.int-res.com%2Farticles%2Fmeps%2F154%2Fm154p053.pdf%26hl%3Den%26sa%3DX%26ei%3D5Z19ZoATB5CTy9YPnOes8AE%26scisig%3DAGIGAw9pE-DRbtYg_Ja1iYDy9f9Z%26oi%3Dscholar&usg=AOvVaw2wxuoaihdu-BHQL0pgcuel).
- 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, e41732. <https://doi.org/10.1371/journal.pone.0041732>.
- Thomsen, P.F., Møller, P.R., Sigsgaard, E.E., Knudsen, S.W., Jørgensen, O.A., Willerslev, E., 2016. Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. *PLoS One* 11, e0165252. <https://doi.org/10.1371/journal.pone.0165252>.
- Tsuji, S., Shibata, N., 2021. Identifying spawning events in fish by observing a spike in environmental DNA concentration after spawning. *Environ. DNA* 3, 190–199. <https://doi.org/10.1002/edn3.153>.
- Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G.H., Geniez, P., Pont, D., Argillier, C., Baudoin, J.-M., Peroux, T., Crivelli, A. J., Olivier, A., Acqueberge, M., Le Brun, M., Møller, P.R., Willerslev, E., Dejean, T., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol.* 25, 929–942. <https://doi.org/10.1111/mec.13428>.
- Villéger, S., Brosse, S., Mouchet, M., Mouillot, D., Vanni, M.J., 2017. Functional ecology of fish: current approaches and future challenges. *Aquat. Sci.* 79, 783–801. <https://doi.org/10.1007/s00027-017-0546-z>.
- Wafar, M.V.M., Le Corre, P., Birrien, J.L., 1983. Nutrients and primary production in permanently well-mixed temperate coastal waters. *Estuar. Coast Shelf Sci.* 17, 431–446. [https://doi.org/10.1016/0272-7714\(83\)90128-2](https://doi.org/10.1016/0272-7714(83)90128-2).
- Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73, 5261–5267. <https://doi.org/10.1128/AEM.00062-07>.
- West, K., Travers, M.J., Stat, M., Harvey, E.S., Richards, Z.T., DiBattista, J.D., Newman, S.J., Harry, A., Skepper, C.L., Heydenrych, M., Bunce, M., 2021. Large-scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical north-western Australia. *Divers. Distrib.* 27, 1942–1957. <https://doi.org/10.1111/ddi.13228>.
- West, K.M., Stat, M., Harvey, E.S., Skepper, C.L., DiBattista, J.D., Richards, Z.T., Travers, M.J., Newman, S.J., Bunce, M., 2020. eDNA metabarcoding survey reveals fine-scale coral reef community variation across a remote, tropical island ecosystem. *Mol. Ecol.* 29, 1069–1086. <https://doi.org/10.1111/mec.15382>.
- Yamamoto, S., Masuda, R., Sato, Y., Sado, T., Araki, H., Kondoh, M., Minamoto, T., Miya, M., 2017. Environmental DNA metabarcoding reveals local fish communities in a species-rich coastal sea. *Sci. Rep.* 7, 40368. <https://doi.org/10.1038/srep40368>.
- Yamamoto, S., Minami, K., Fukaya, K., Takahashi, K., Sawada, H., Murakami, H., Tsuji, S., Hashizume, H., Kubonaga, S., Horiuchi, T., Hongo, M., Nishida, J., Okugawa, Y., Fujiwara, A., Fukuda, M., Hidaka, S., Suzuki, K.W., Miya, M., Araki, H., Yamanaka, H., Maruyama, A., Miyashita, K., Masuda, R., Minamoto, T., Kondoh, M., 2016. Environmental DNA as a 'snapshot' of fish distribution: a case study of Japanese jack mackerel in maizuru bay, sea of Japan. *PLoS One* 11, e0149786. <https://doi.org/10.1371/journal.pone.0149786>.
- Zou, K., Chen, J., Ruan, H., Li, Z., Guo, W., Li, M., Liu, L., 2020. eDNA metabarcoding as a promising conservation tool for monitoring fish diversity in a coastal wetland of the Pearl River Estuary compared to bottom trawling. *Sci. Total Environ.* 702, 134704. <https://doi.org/10.1016/j.scitotenv.2019.134704>.