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Assessing without harvesting: Pros and cons of environmental DNA sampling and image analysis for marine biodiversity evaluation

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Abstract :

Marine stock assessments or biodiversity monitoring studies, which historically relied on extractive techniques (e.g., trawl or grab surveys), are being progressively replaced by non-extractive approaches. For instance, species abundance indices can be calculated using data obtained from high-definition underwater cameras that enable to identify taxa at low taxonomical level. In biodiversity studies, environmental DNA (eDNA) has proven to be a useful tool for characterising fish species richness. However, several marine phyla remain poorly represented in reference gene databases or release limited amounts of DNA, restricting their detection. The absence of amplification of some invertebrate taxa might also reflect primer bias. We here explore and compare the performance of eDNA and image data in describing the marine communities of several sites in the Bay of Biscay. This was achieved by deploying a remotely operated vehicle to both record images and collect seawater samples. A total of 88 taxa were identified from the eDNA samples and 121 taxa from the images. For both methods, the best characterised phylum was Chordata, with 29 and 27 Actinopterygii species detected using image versus eDNA, respectively. Neither Bryozoa nor Cnidaria was detected in the eDNA samples while the phyla were easily identifiable by imagery. Similarly, Asteroidea (Echinodermata) and Cephalopoda (Mollusca) were scarcely detected in the eDNA samples but present on the images, while Annelida were mostly identified by eDNA (18 taxa vs 7 taxa from imagery). The complementary community descriptions we highlight from these two methods therefore advocate for using both eDNA and imagery in tandem in order to capture the macroscopic biodiversity of bentho-demersal marine communities.

Highlights

► We compare the performance of eDNA and imagery in describing the marine communities. ► 88 taxa were identified from the eDNA samples and 121 taxa from the images. ► Actinopterygii species were the best characterised for both methods. ► Neither Bryozoa nor Cnidaria were detected in the eDNA samples contrary to Annelida. ► eDNA and imagery should be used in tandem to capture the biodiversity.

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Keywords : Photo, eDNA, Species richness, Bay of Biscay

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34 1. Introduction

35 Historically, research on species occurrence or abundance in marine systems has been carried out
36 using extractive methods such as trawl, grab, or trap surveys. Over time, scientists have grown
37 increasingly concerned about the environmental footprint associated with assessing marine stocks or
38 biodiversity (Trenkel et al., 2019). Low impact to non-invasive methods have thus been applied in the
39 marine realm to sample the occurrence and abundance of species (motile or sessile), reducing
40 damages on the seafloor and essential habitats (Bicknell et al., 2016). These technologies encompass,
41 among others, underwater visual census (UVC), remotely operated vehicles (ROVs), autonomous
42 underwater vehicles (AUVs) and acoustic methods. While such approaches are more environmentally
43 friendly, they have some drawbacks (Murphy and Jenkins, 2010). For instance, acoustic methods
44 provide information on fish size and abundance but at low taxonomic resolution, while ROVs, AUVs or
45 scuba divers might modify species behaviour as a result of their movement or noise (Lorance and
46 Trenkel, 2006; Watson and Harvey, 2007). Static underwater cameras avoid such issues by minimising
47 levels of disturbance related to the movement of the sampler (Bicknell et al., 2016). Still, underwater
48 cameras require the use of artificial light to work beyond a certain depth and at night, which can further
49 affect the species' behaviour and thus abundance estimates. As sensors' resolution keeps increasing,
50 it has become possible to identify and count species, analyse their behaviour and characterize the
51 surrounding environment (Mallet and Pelletier, 2014). Depending on environmental conditions,
52 however, factors such as turbidity or distance to the seafloor can impede precise species identification,
53 lessening the strength of such sampling method (Figueroa-Pico et al., 2020).

54 More recently, environmental DNA (eDNA) sampling has turned into a key method for studying marine
55 diversity (Afzali et al., 2020; Dalongeville et al., 2022; Polanco Fernández et al., 2020; Sanchez et al.,
56 2022; Stat et al., 2019). Extensive research has compared the performance of sampling with UVC and
57 trawling to eDNA analysis, but the results remain inconsistent. In some cases, eDNA analysis has
58 outperformed traditional methods: Afzali et al. (2020) identified 71 species using eDNA sampling *vs* 64
59 species with trawl surveys, and Boussarie et al. (2018) detected the presence of 44% more sharks
60 species with eDNA than with UVC or baited remote underwater video (BRUV). In contrast, other
61 research has found that trawling or video perform better such as Thomsen et al. (2016) that identified
62 37 fish species using eDNA analysis *vs* 49 species with trawling. Finally, Nguyen et al. (2020) identified
63 97 fish species using UVC and 79 species using eDNA.

64 The taxonomical resolution to be expected by eDNA sampling is conditioned by the availability of the
65 target species' markers (Cristescu, 2014; Schenekar et al., 2020) and the wholeness of reference
66 database. The larger availability of eDNA markers for fish (McClenaghan et al., 2020), which are less

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67 developed for phyla other than Chordata (de Jonge et al., 2021) led to focus most eDNA studies on
68 bony fishes while studies on other taxa remain rare (e.g., Boussarie et al., 2018 for sharks, Merten et
69 al., 2021 for cephalopods, Nguyen et al., 2020 and Antich et al., 2021 for benthic organisms). The more
70 comprehensive the reference database, the easier it is to precisely identify taxa using eDNA (e.g., down
71 to the species level for fish). However, when target taxa are not well characterized in databases, it
72 might result a low taxonomical assignment success (Alberdi et al., 2018).

73 Here, we aim at reducing the bias toward fish identification through eDNA sampling by comparing the
74 motile and sessile biodiversity obtained from underwater imaging and eDNA sampling. To address the
75 lack of information on species beyond fishes, we focus on the performance of both techniques to
76 describing an entire marine bentho-demersal community, including—but not limited to—fish and
77 benthos, to offering novel elements on the advantage and drawbacks of each method.

78

79 ## 2. Material & Methods

80 ### 2.1 Data collection

81 #### 2.1.1 Underwater video surveys

82 We studied nine sites at the limit between the Bay of Biscay and the Celtic Sea in June 2021
83 (Supplementary material 1), with a depth range from 83 to 401 m. We used the Hybrid ROV (HROV)
84 Ariane (DOI: 10.1109/OCEANSE.2019.8867102) operated by the French Oceanographic Fleet; it is
85 equipped with a camera (Nikon® D5500) pointing vertically toward the seabed. The HROV was kept 2
86 m above the seafloor at all times to allow for a detailed examination of the seabed (mean speed = 1 kt
87 ± 0.2). For each site, the total distance covered was 2,500 m with a mean width of 1.9 m (± 0.2 SD)
88 representing a mean number of pictures of 4092 (± 549 SD) per transect. Each transect was a series of
89 24Mpx photos (6000 x 4000 pixels) taken every two seconds, a frequency that ensures a large overlap
90 between successive photos. Each photo was analyzed by at least two qualified scientists, who
91 identified all the organisms seen to the lowest taxonomic level possible (hereafter referred as taxa):
92 species when possible, otherwise genus, family, or class.

93 #### 2.1.2 Environmental DNA surveys

94 **Sampling procedure**

95 Along each transect, seawater samples were collected in Niskin bottles using the HROV arm. Sampling
96 occurred 1 m above the seafloor at the beginning and end of each transect and during the transect
97 whenever possible. A total of 29 seawater samples of 2L were filtered using 0.45 um Sterivex filters

98 immediately once the HROV was back onboard. To prevent contamination, all the surfaces were
99 sterilized using PCR Clean™ Wipes before each sampling procedure. All the equipment were single use
100 sterile ones (gloves, masks, syringe, funnel, sample bag). Two filtered distilled water samples were also
101 taken as control. The control samples were taken at the beginning and end of the cruise. The Sterivex
102 filters were stored in a sterile sample bag at -20°C onboard the vessel then shipped to the lab for DNA
103 extraction.

104 **DNA extraction, PCR amplification and high-throughput sequencing**

105 The following steps were performed by ID-Gene Ecodiagnostics, a laboratory specialised in
106 environmental genomics. In the laboratory, bottom water eDNA was extracted (100 µl) using the
107 DNeasy Powerwater Sterivex kit (Quiagen), following manufacturer instructions. All DNA extracts were
108 stored at -20°C. All samples were then amplified using three mitochondrial markers: a 280bp long
109 fragment of the 16S rRNA gene specific to vertebrates and molluscs [16Smix F and R, degenerated
110 version of primers 16S from Kitano et al., (2007), reference herein], a 380bp long fragment of the COI
111 gene commonly used as barcode for animals [forward mlCOIintF from Leray et al., (2013), reverse
112 dgHCO-2198 from Meyer, (2003)], and a 220bp long fragment of the 16S gene specific to Decapods
113 (MiDeca-F from Komai et al. 2019, MiDecaMod-R modified from MiDeca-R form Komai et al. 2019,
114 reference herein) (see Supplementary material 2 for detailed information about the primers and PCR
115 conditions). For each sample and marker, seven PCR reactions and one negative control were
116 performed. A second round of amplifications (reamplification) was performed with DNA from the first
117 PCR as template for the MiDeca primer, in order to concentrate decapod DNA and facilitate detection.
118 For 16S and COI markers, 1 µl of DNA extract was used as template, and 2 µl for MiDeca primers.
119 Tagged primers bearing 8 or 9 nucleotides attached at each primer's 5'-extremity were used to enable
120 multiplexing of all PCR products in a unique sequencing library (Esling et al., 2015). The results of these
121 7 PCR reactions were pooled and quantified. These pools were then quantified with capillary
122 electrophoresis using QIAxcel instrument (Qiagen). Equimolar concentrations of PCR products were
123 pooled for each library and purified using High Pure PCR Product Purification kits (Roche Applied
124 Science). Library preparation was performed using Illumina TruSeq® DNA PCR-Free Library Preparation
125 Kit. The libraries were then quantified with qPCR using KAPA Library Quantification Kit and sequenced
126 on a MiSeq instrument using paired-end sequencing for 500 cycles (2 X 251bp) with Standard kit v2 for
127 each marker.

128 **High-throughput sequencing (HTS) data analysis**

129 Raw FASTQ reads were quality-filtered removing all sequences with ambiguous bases or any mismatch
130 in the tagged primer. This was done using the module DTD as implemented in SLIM pipeline (Dufresne
131 et al., 2019) using the parameters that do not allow any ambiguity in the sequence. Then, paired-end

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132 reads assembly, chimera removing and formation of the Amplicon Sequence Variant (ASV) were
133 performed using *dada2* R package (Callahan et al., 2016). The total reads/sample in original MiSeq
134 output, reads/sample after bioinformatic processing as well as number of reads/sample for each taxa
135 by markers are given in Supplementary material 3. The default parameters of dada1 were used for
136 quality filtration of reads using the function FilterAndTrim (maxN=0, truncQ=2, rm.phix=TRUE and
137 maxEE=2). A substantial proportion of reads were unassigned in some samples with all three markers.
138 Taxonomic assignment was then performed using curated database comprising all metazoan species
139 present in the studied area and whose sequences were available in GenBank using *decipher* R package
140 (Wright, 2016) and which assignment was verified by phylogenetic trees (See Supplementary material
141 4 for detailed information about the coverage of reference database).
142

143 2.3 Data analysis

144 Using the full list of identified taxa, a Wilcoxon test was performed to compare the per-sample
145 taxonomic richness obtained with the two sampling methods since normality assumptions were not
146 met for eDNA data (Shapiro tests, $p<0.05$).

147 To represent the match and mismatch of taxa identification by the eDNA and imaging methods, we
148 chose to focus on genera level to retain as much data as possible from the underwater video dataset
149 for which species identification was often limited. Using this restricted dataset, we performed a
150 taxonomic tree-like analysis and data visualization method (R package "*ape*", Paradis and Schliep 2019)
151 and a Venn diagram (*ggvenn* R package , Yan 2021).

152 All graphing and statistical analyses were performed in R (v.4.2.1), using the *tidyverse v*1.3.2 package
153 (Wickham et al., 2019) and *fishualise* v0.2.3 (Schiettekatte et al., 2019).

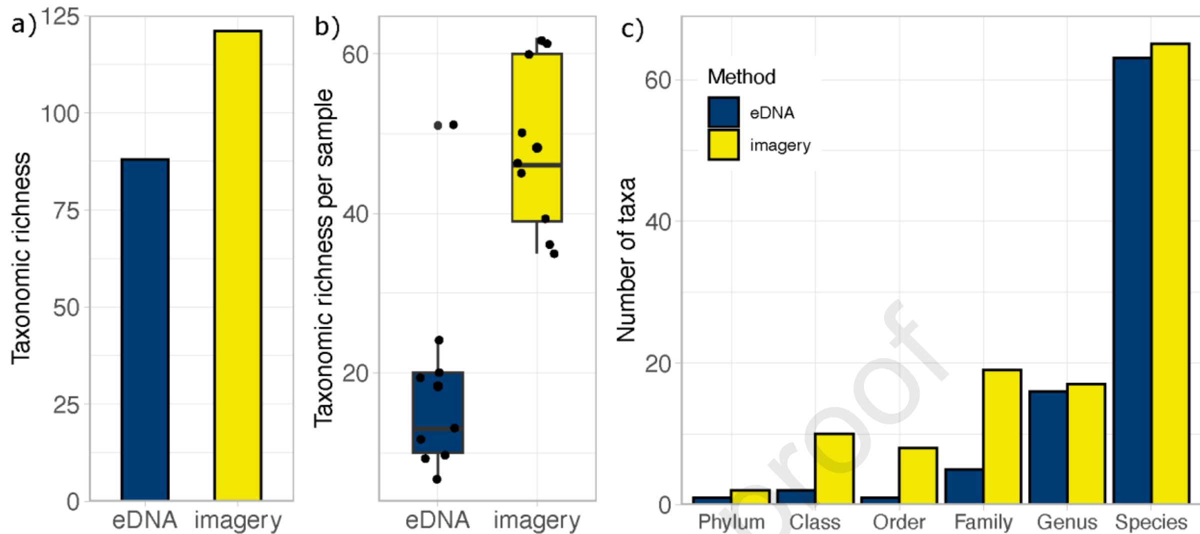
154

155 3. Results

156 3.1 Taxa detection by imagery and eDNA methods

157 A total of 88 and 121 taxa were identified in the eDNA sampling and images respectively (Fig. 1a). Mean
158 richness per sample (Fig. 1b) differed significantly between methods (Wilcoxon test, $V = 1$, p-value =
159 0.008). More specifically, compared to eDNA analysis, imaging detected over twice the number of taxa
160 per transect (mean richness ± SD = 48.2 ± 10.7 for images *vs* 18.3 ± 13.5 for eDNA; Fig. 1b). Both
161 methods had decent taxonomic resolution and yielded data that allowed more than half of the
162 organisms observed to be assigned to species (Fig. 1c), although eDNA performed better: 90% of
163 organisms could be identified at least to genus and 72% of organisms could be identified to species.

164 On contrast, underwater imaging allowed only 54% of organisms to be identified to species. The
165 remaining 46% could be classified to genus at best. In 10% of cases, only class or phylum could be
166 determined.



167

168 **Fig. 1** a) Overall taxonomic richness identified per method, b) Taxonomic richness per station for each
169 method. The boxplots show the median (horizontal line) and interquartile range (whiskers) of the
170 richness, and data distribution (points, with jitter on the x-axis), c) Number of taxa identified to a given
171 taxonomic level by each method.

172 For both methods, the most frequently represented phylum was Chordata where 29 and 27 members
173 of Actinopterygii were detected by imagery and eDNA sampling, respectively (Fig.2). Interestingly,
174 eDNA analysis failed to detect Cnidaria, Porifera and Bryozoa, taxa that were accurately described
175 using imagery. While images detected 5 orders in Cnidaria, 8 orders in Porifera, and 2 orders in
176 Bryozoa, eDNA only picked up on 3 orders in Porifera (Supplementary material 5). In contrast, eDNA
177 analysis comes out as the most efficient method to detect Annelida (18 taxa by eDNA *vs* 7 by imagery).
178 Interesting discrepancy was also observed with Asteroidea and Holothuridea (phylum Echinodermata)
179 that were easily described by imagery while poorly detected in seawater samples (Fig. 2). For Mollusca,
180 while imagery did a better job of identifying members of Bivalvia and Cephalopoda, eDNA performed
181 better with Gastropoda (Fig.2).

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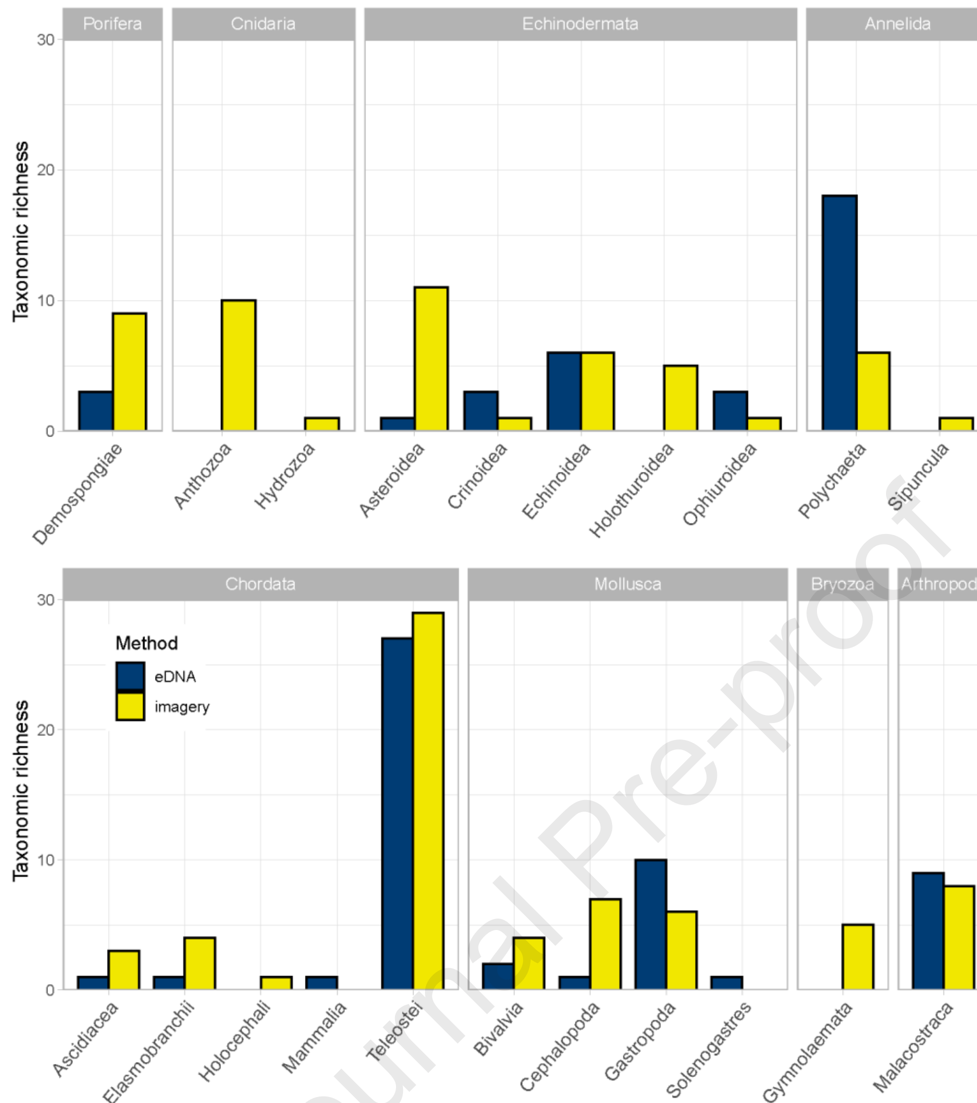

182

183 Fig. 2 Taxonomic richness identified per class by eDNA analysis (blue boxes) and imagery (yellow
184 boxes).

185 3.2 Match-mismatch between imagery and eDNA

186 A total of 54 and 55 genera were identified using eDNA sampling and UVC, respectively (Supplementary
187 material 6), with only 19 genera—including 12 fish—detected by both methods (Fig.3). Such
188 discrepancies are highlighted in the taxonomic tree-like representation, showing that only eDNA
189 identified certain fish taxa, among them several pelagic and mesopelagic genera (i.e. *Sardina*,
190 *Engraulis*, *Maurolicus*, *Scomber*), migratory taxa (*Euthynnus*), and one genus of small-sized fishes
191 (*Crystallogobius*). Of the 9 Asteroidae genera detected, 8 were found exclusively via imagery, with a
192 single genus found by both methods (*Luidia*). Overall, 10 genera in the phylum Porifera were detected:
193 eight were identified solely by imagery, one solely by eDNA (*Protosuberites*) and one by both methods
194 (*Mycale*; Fig.3).

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195

196 **Fig. 3** Taxonomic tree of genera identified via eDNA analysis (blue), underwater imagery (orange), or
197 both (green). Genus are gathered by phylum, illustrated by the grey shaded ring

198

199 4. Discussion

200 Our results revealed an unexpected limited overlap between eDNA and imagery sampling
201 methods to assess biodiversity in a Northeast Atlantic ecosystem. Both methods provide different
202 snapshots of the diversity and are thus complementary, while neither the imagery nor eDNA are
203 efficient at sampling biodiversity as a whole.

204 Nevertheless, the limited species detection of eDNA could result from several factors related
205 to the sampling method and analysis. For example, the volume of seawater is often seen as
206 determinant in our ability to detect species (Bessey et al., 2020). Here, the volume used falls within

207 the upper end of filtered seawater volumes considered in previous studies (e.g. 0.25L for Grey et al.,
208 (2018); 0.5L for Thomsen et al., (2012); 1-2L for Stat et al., (2017); Boussarie et al., (2018); Afzali et al.,
209 (2020); Stoeckle et al., (2021)); this factor may thus not have influenced the eDNA detection rate. The
210 position of the samplers may also have influenced our ability to detect species. We have sampled water
211 at 1 m above the seafloor, i.e. above benthic or sessile taxa, yet several benthic taxa were properly
212 detected. For example, eDNA was efficient at detecting annelid and gastropod molluscs, thus even if
213 the seawater was not taken at the surface of the sediment, it did allow sampling eDNA from species
214 buried in the sediment. Of no surprise, these species are hidden and were not detected by imagery.
215 Finally, the discrete sampling that we have used (i.e. sampling at the beginning and end of each
216 transect) could have led to miss some taxa in the eDNA samples. Other approaches undertake
217 continuous sampling (Maiello et al., 2022) or discrete samples combined (e.g. benthic, at 1m above
218 sediment and in the water column and process them all together) to prevent a sampling bias, although
219 the limited overlap between eDNA and imagery detections does not suggest such bias in the present
220 study.

221 Species detection from eDNA samples also relies on the completeness of gene database (Marques et
222 al., 2021) and on the type and number of primers used. As of today, the vast majority of eDNA research
223 in the marine realm has focused on fish (e.g. Afzali et al., 2020; Duhamet et al., 2023; McClenaghan et
224 al., 2020; Polanco Fernández et al., 2020; Stoeckle et al., 2021). Abundant information is thus largely
225 available for Chordata but not for other phyla. In our study, sampling failed to pick up on certain
226 groups, such as all of the bryozoans and cnidarians, and most of the poriferans. This could result from
227 the lack of data for certain taxa in the reference databases for metabarcoding. Second, there could
228 have been an effect of primer number and type. Past work indicates that taxonomic coverage can be
229 boosted by using multiple primers (Alberdi et al. 2018). In addition to 16S and COI, we used the MiDeca
230 marker to maximise our chance of detecting decapods. Other taxon-specific primers are available such
231 as Ceph18S for cephalopods (de Jonge et al., 2021; Merten et al., 2021), Shark COI-MINIR for sharks
232 (Fields et al., 2015) or other primers targeting specific taxonomic groups (e.g. Echinodermata,
233 Polychaeta or Mollusca; Taberlet et al. 2018). While their use would have increased our ability to pick
234 up on those taxa, it would have also greatly increased the cost of the analyses, given that we were
235 working at the community level.

236 The low detection rate of some species may also be related to species intrinsic factors, in particular
237 the amount of DNA released and its degradation in the seawater. It is noteworthy that species with
238 exoskeletons or shells (e.g. crustaceans or bivalves) release little DNA (Antich et al., 2021). The poor
239 detection of genera such as *Aequipecten*, *Atrina* or *Cancer* may thus result from to their lack of
240 signature in the eDNA rather than from missing primers. The few studies on taxa-specific DNA

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241 degradation rate in the marine realm revealed that DNA degradation could be rapid (e.g. between 9
242 and 17 hours for 3 fish species; Kirtane et al. 2021 and 14 hours for seastars; Kwong et al. 2021) and
243 may have impeded some fish and seastars species detection from seawater samples while observed
244 by imagery.

245 Our study revealed that imagery outperformed eDNA analysis for most taxonomical groups,
246 except Polychaeta that are buried in the sediment. Yet, no species of pelagic fish could be detected on
247 videos as the camera was oriented toward the seafloor whereas eDNA allowed to detect pelagic fish
248 (*Engraulis*, *Sardina* and *Scomber*). The noise and lights of the HROV may also have frightened mobile
249 species causing them to flee from the field of view (Lorance and Trenkel, 2006; Stoner et al., 2008;
250 Sward et al., 2019). Our study therefore shows that, as for eDNA, imagery is efficient at describing a
251 specific portion of the bentho-demersal marine community but also raises awareness regarding the
252 need for conducive conditions to reach optimal results. Indeed, the expected taxonomic resolution
253 from imagery is strongly driven by both environmental (e.g. water turbidity) and technical factors (e.g.
254 the distance of the camera to the seafloor, the camera resolution or the camera orientation; Mallet
255 and Pelletier, (2014)); combined, these factors may offer from optimal to prohibitive data for species
256 identification and are thus critical to the sampling success.

257 The last aspect to take into consideration is the time required for species-level identification from
258 imagery data. Even if imagery allows working with taxa that do not release DNA, a precise species
259 identification needs trained observers as the degree of taxonomic resolution is dependent on scientists
260 expertise (Ji et al., 2013). Regardless the training, the time needed to analyze the data of a single
261 transect requires up to tens of hours of work depending on the diversity and size of the species, but
262 also on the heterogeneity of the substrate. Altogether, here, the cost was lower for processing the
263 eDNA water samples with several primers than the time cost for the species identification from the
264 imagery data. For instance in this study, the cost of one eDNA analysis has an order of magnitude of
265 some hundreds of euros, whereas the cost of image analysis by a scientist was approximately 5 times
266 more expensive for one transect.

267 Given the current state of the eDNA reference databases and the lack of knowledge on the
268 species eDNA release and degradation rates, our study shows that neither eDNA or imagery alone
269 allows to comprehensively assess bentho-demersal marine biodiversity. Obviously, our results are
270 specific to the studied locations and could not be extrapolated to other regions where the eDNA
271 database are perhaps more complete. In the near future, with the developments engaged to obtain
272 species metabarcodes and with the democratization of portable eDNA sequencer (e.g. Srivathsan et
273 al. 2021), eDNA sampling will likely grow to outperform image analysis unless deep learning would

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274 become efficient enough to detect automatically species on video and forgo time-consuming manual
275 validation. For now however, we have demonstrated that each method contributes to different sets
276 of information about marine communities and still need to be used in tandem, or even with other
277 methods (e.g. acoustics for pelagic fishes) to capture an exhaustive description of marine communities.

278

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284

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- We compare the performance of eDNA and imagery in describing the marine communities
- 88 taxa were identified from the eDNA samples and 121 taxa from the images
- Actinopterygii species were the best characterised for both methods
- Neither Bryozoa nor Cnidaria were detected in the eDNA samples contrary to Annelida
- eDNA and imagery should be used in tandem to capture the biodiversity

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Dorothée Kopp: Conceptualization, Funding acquisition, Methodology; Project administration, Supervision, Validation, Writing - original draft. Robin Faillettaz: Methodology, Data analysis, Writing - review & editing. Anna Le Joncour: Methodology, Data analysis. Julien Simon: Methodology, Data analysis, Writing - review & editing. Fabien Morandeau: Methodology, Data analysis. Pierre Le Bourdonnec: Methodology, Data analysis. Ludovic Bouché: Methodology, Data analysis. Sonia Méhault: Conceptualization, Methodology, Project Administration, Validation, Writing - review & editing.

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Declaration of interests

☒ 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.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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