Marine biomonitoring with eDNA: can metabarcoding of water samples cut it as a tool for surveying benthic communities?

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Abstract

In the marine realm, biomonitoring using eDNA of benthic communities requires destructive direct sampling or the setting-up of settlement structures. Comparatively much less effort is required to sample the water column, which can be accessed remotely. In this study we assess the feasibility of obtaining information from the eukaryotic benthic communities by sampling the adjacent water layer. We studied two different rocky-substrate benthic communities with a technique based on quadrat sampling. We also took replicate water samples at four distances (0, 0.5, 1.5, and 20 m) from the benthic habitat. Using broad range primers to amplify a ca. 313 bp fragment of the cytochrome oxidase subunit I gene, we obtained a total of 3,543 molecular operational taxonomic units (MOTUs). The structure obtained in the two environments was markedly different, with Metazoa, Archaeplastida and Stramenopiles being the most diverse groups in benthic samples, and Hacrobia, Metazoa and Alveolata in the water. Only 265 MOTUs (7.5%) were shared between benthos and water samples and, of these, 180 (5.1%) were identified as benthic taxa that left their DNA in the water.

Most of them were found immediately adjacent to the benthos, and their number decreased as we moved apart from the benthic habitat. It was concluded that water eDNA, even in the close vicinity of the benthos, was a poor proxy for the analysis of benthic structure, and that direct sampling methods are required for monitoring these complex communities via metabarcoding.

Keywords: eDNA, metabarcoding, marine, benthos, water, biomonitoring

Running title: eDNA from water as a proxy for benthos

Introduction

Metabarcoding is by now a well-established technique for assessing biodiversity in a variety of terrestrial, freshwater, and marine environments (reviewed in Bohmann et al., 2014; Creer et al., 2016; Cristescu, 2014; Deiner et al., 2017; Taberlet, Coissac, Pompanon, Bronchmann, & Willerslev, 2012). The wealth of published papers dealing with technical issues and generating new data with this method testifies to the widening scope of applications of metabarcoding. One such application, where metabarcoding is becoming a game-changer, is in the field of biomonitoring (Aylagas, Borja, Muxika, & Rodríguez-Ezpeleta, 2018; Hajibabaei, Baird, Fahner, Beiko, & Golding, 2016; Kelly, Port, Yamahara, & Crowder, 2014; Porter & Hajibabaei, 2018). Not in vain the use of DNA-based approaches for monitoring applications has been christened Biomonitoring 2.0 (Baird & Hajibabaei, 2012; Leese et al., 2018).

In the marine realm, all current policies, such as the European Union Marine Strategy Framework Directive, mandate comprehensive, community-wide approaches to monitoring (Danovaro et al., 2016; Goodwin et al., 2017; Hering et al., 2018; Leese et al., 2018). Metabarcoding provides a cost-effective, ecosystem-wide method for the assessment of biodiversity, which lies at the basis of all monitoring efforts (Aylagas et al., 2018; Krehenwinkel, Pomerantz, & Prost, 2019; Leray & Knowlton, 2016; Shaw, Weyrich, & Cooper, 2017). An ever widening range of ecological and socio-economic issues, such as invasive species management (Darling et al., 2017; Holman et al., 2019), marine protected areas design (Bani et al., 2020), pathogen monitoring (Peters et al., 2018), fisheries management (Zou et al., 2020), or deep-sea mining (Cowart, Matabos, Brandt, Marticorena, & Sarrazin, 2020), among others, require powerful and fast biomonitoring tools. Metabarcoding provides these tools at a pace, cost, and depth that are not achievable using conventional, morphology-based surveys (Porter & Hajibabaei, 2018). Alpha- and beta-diversity estimates, as well as biotic indices, can be reliably obtained using metabarcoding (Aylagas et al., 2018; Bani et al., 2020; Hering et al., 2018; Pawlowski et al., 2018). The amount of data typically generated in metabarcoding datasets allows also bioassessments based on taxonomy-free and

machine learning techniques (Cordier, & Pawlowski, 2018; Gerhard & Gunsch, 2019), or the analysis of diversity at the within-species level (Turon, Antich, Palacín, Præbel, & Wangensteen, 2020).

Of course, gaps and problems are also recognized in this burgeoning field (e.g. Alberdi, Aizpurua, Thomas, Gilbert, & Bohmann, 2018; Kelly, Shelton, & Gallego, 2019; McGee, Robinson, & Hajibabaei, 2019), among which the need to obtain better reference databases (Sinniger et al., 2016; Wangensteen, Palacin, et al., 2018; Weigand et al., 2019) and the need to standardize field and laboratory procedures (McGee et al., 2019; Weigand et al., 2019). Among the latter, the type of substrate sampled is of paramount importance (Koziol et al., 2019). In the sea, most studies to date have sampled either the sediment (e.g., Atienza et al., 2020; Brannock, Ortmann, Moss, & Halanych, 2018; Fonseca et al., 2014; Guardiola et al., 2016) or the water column (e.g., Brannock, Learman, Mahon, Santos, & Halanych, 2016; Fraija-Fernandez et al., 2019; Sigsgaard et al., 2019; Stefanni et al., 2018). Less effort has been devoted to the study of hard-substrate natural benthic communities. These have been analysed either using indirect methods based on deploying artificial substrates (Cahill et al., 2018; Leray & Knowlton, 2015; Pearman et al., 2019; Ransome et al., 2017), or by directly taking samples by scraping off standardized surfaces (Shum, Barney, O'Leary, & Palumbi, 2019; Wangensteen, Cebrian, Palacín, & Turon, 2018; Wangensteen, Palacín, Guardiola, & Turon, 2018) or using suction devices (Cowart et al., 2020; De Jode et al., 2019).

Either deploying settlement surfaces (that need to be recovered) or using direct collection methods, the sampling of benthic hard-bottom habitats requires direct access to the environment and involves more effort than sampling substrates such as water or sediment, which can be accessed remotely. In addition, direct methods are destructive, which is an inconvenience for the sustained sampling necessary for biomonitoring. It is, therefore, highly convenient to develop alternative methods for assessing benthic biodiversity, and an obvious choice would be to sample the water in the vicinity of the benthos to recover benthic DNA for metabarcoding applications. While water eDNA has been used for the study of protists, fito- and zooplankton or fish assemblages (e.g., Djurhuus et al, 2018; Massana et al., 2015; Shu, Ludwig, A., & Peng, 2020), its potential

utility to analyse benthic communities is much less understood. Some authors (Koziol et al., 2019; Rey, Basurko, & Rodriguez-Ezpeleta, 2020) compared eDNA from water, sediment and settlement plates in port environments, finding clearly distinct community profiles. Leduc et al. (2019) similarly found significant differences in community composition between eDNA from water samples and standard invertebrate collection methods in Arctic harbours. West et al. (2020) used surface water samples to assess coral reef community variation, but did not perform a comparison with the actual benthic communities. Alexander et al. (2020) used eDNA from surface waters to target scleractinian diversity, and found the method promising, albeit with notable differences with results from visual censuses. Stat et al. (2017) compared two different methods to study the eDNA from tropical marine reefs using shallow water and found eDNA metabarcoding more promising than the shotgun approach for assessing eukaryotic diversity.

The usefulness of DNA obtained from water samples as a proxy for benthic communities will depend on the many factors that affect DNA release, transport, and degradation (Barnes & Turner, 2016; Collins et al., 2018; Salter, 2018; Stewart, 2019). While some studies have assessed the spatial distribution of eDNA in coastal habitats, they have been done at scales too large to link water samples with particular benthic habitats. Bakker et al. (2019) analysed water eDNA from coastal shelf habitats spanning the Caribbean Sea. O'Donnell et al. (2017) found fine scale patterns in the distribution of water eDNA, but they used transects perpendicular to the shore spanning a few kilometres. Jeunen et al. (2019) analysed the vertical stratification of eDNA at the scale of metres, but did not focus on any relationship with benthic communities. Jacobs-Palmer et al. (2020) analysed eDNA from water taken in the vicinity (from 1 to 15 m) of the edges of *Zostera marina* patches, and could detect an inhibitory effect of the seagrass community on the dinoflagellate abundances in the plankton. To our knowledge, however, no study has assessed marine eDNA dynamics at the benthic boundary layer, which is the water immediately adjacent (from centimetres to metres) to the benthos, where steep gradients in abiotic and biotic parameters occur (Boudreau & Jorgensen, 2001). Only Hajibabaei et al. (2019) have compared, in freshwater

environments, the results from DNA obtained from matched water and benthic samples, and found water eDNA to be a poor surrogate for benthic community composition.

In this work, and using two hard-bottom communities on vertical walls in the NW Mediterranean, we compared the information obtained from analysing the DNA obtained from benthic (using direct methods as in Wangensteen, Palacin, et al., 2018) and water samples collected at increasing distances (from centimetres to metres) from these communities. We used metabarcoding of the COI gene with broad range primers as our focus was on recovering the taxonomically diverse eukaryotic communities present. Our goals were to assess the eDNA dynamics in the boundary layer of the benthos and to determine the feasibility of analysing benthic diversity by collecting water samples.

Material & Methods

Sample collection

In the present study samples were taken from two different hard-bottom communities, a shallower (photophilous) and a deeper (sciaphilous) communities found in the same vertical wall facing SSE, in the National Park of Cabrera Archipelago in the Balearic Islands (Western Mediterranean, 39°07′30.32″N, 2°57′37.14″E, Figure S1). The photophilous community at 10 m depth was dominated by the seaweeds *Padina pavonica* and *Dictyopteris membranacea*. In the sciaphilous community at 30 m depth, the seaweed *Halimeda tuna*, sponges and other invertebrates were the dominant biota. For more detailed information of these communities see Wangensteen, Palacin, et al. (2018).

Two different sampling methods were used in the present study. Benthic samples (3 replicates per community) were obtained by scraping to bare rock quadrats of 25x25 cm with hammer and chisel. All the material was collected underwater in plastic bags. Two divers performed the sampling, with one keeping the sample bag open just over the zone being scraped to avoid escape of small motile fauna. Water samples (4 replicates at each point) were obtained with 1.5 L bottles at different distances from the benthos (0

m, 0.5 m and 1.5 m) for each community. The sample labelled 0 m was obtained in the water layer just adjacent (ca. 5 cm) to the benthos. As an external pelagic control, water samples (3 replicates) of 1.5 L were obtained at 20 m from the benthos and at an intermediate depth (-20 m). The sampling design is sketched in Figure 1. Hereafter we will use the names photophilous and sciaphilous samples to designate both the benthic and the water samples ≤ 1.5 m from the wall at each of the two depth levels sampled, and the name pelagic samples to designate the water samples collected 20 m apart from the rocky wall at - 20 m. New, unopened mineral water plastic bottles were used for water collection, one per sample. They were first filled with sterilized water and, once in the collection point, they were held upside-down and water was displaced using air bubbled from a spare SCUBA regulator. The bottles were then righted and water from the exact point of collection was allowed to fill them.

Sample processing

Water samples were processed on site immediately after collection. The whole collected volume (1.5 L, comparable to other studies, e.g. Collins, Bakker, Wangensteen, Soto et al. 2019; Sales, Wangensteen, Carvalho, & Mariani, 2019) was pre-filtered with a 200 µm mesh to eliminate coarse particles and then filtered through 0.22 µm Sterivex™ millipore filters (Merck) using sterile, disposable syringes (a new syringe per sample). The filter cartridges were then stored at -20ºC in sterile plastic bags. Benthic samples were fixed with ethanol immediately after collection and kept at -20ºC until processed in the laboratory. Following Wangensteen & Turon, (2017), Wangensteen, Palacin, et al., (2018) and Wangensteen, Cebrian, et al. (2018), benthic samples were separated in the laboratory in three different size fractions (A: > 10mm; B: 1 – 10 mm; C: 63µm – 1mm) using a stainless steel mesh sieve column (Cisa S.L., www.cisa.net). Each fraction was homogenized with a blender and stored in ethanol at -20ºC until DNA extraction. All equipment was carefully bleached between samples.

Our sample dataset thus consisted of 18 benthic samples (2 communities * 3 replicates * 3 fractions) and 27 water samples (2 communities * 3 distances * 4 replicates + 3 pelagic samples).

DNA extraction

All procedures were made in a laminar flow cabinet sterilised with UV light between samples. DNA from benthic samples was extracted using 10 g of homogenized material and the DNeasy PowerMax Soil Kit (QIAGEN). The Sterivex filter cartridges were opened with sterile pincers in the cabinet and DNA from the filters was then extracted using the DNeasy PowerWater kit (QIAGEN). A Qubit fluorometer (ThermoFisher) was used to check the concentration of DNA (higher than 5 ng/μL in all cases).

PCR amplification and library preparation

A fragment of ca. 313 bp of the Cytochrome Oxidase 1 (COI) gene was amplified with a set of universal primers targeting eukaryotes. We used the Leray-XT primer set (Wangensteen, Cebrian, et al., 2018; Wangensteen, Palacin, et al., 2018): forward jgHCO2198 (Geller, Meyer, Parker, & Hawk, 2013): 5′-TAIACYTCIGGRTGICCRAARAAYCA-3′, reverse mlCOIintF-XT (Wangensteen, Palacin, et al., 2018): 5′-GGWACWRGWTGRACWITITAYCCYCC-3′. All primers had an 8-base specific tag attached. The tags had a minimum difference of 3 bases from each other, and were designed with the program Oligotaq (Boyer et al., 2016). Forward and reverse primers used for amplification of each sample had the same tag. A variable number of degenerate (N) bases (from two to four) were also attached to the forward and reverse primers to improve sequence diversity for illumina processing.

Three PCR replicates were performed for each DNA extraction. PCR conditions for COI amplification followed (Wangensteen, Palacin, et al., 2018). DNA was then purified and

concentrated using MinElute PCR Purification Kit (QIAGEN) and an electrophoresis gel was performed to check amplification success.

Amplification controls were added as follows: two PCR blanks were run by amplifying the PCR mixture without any DNA template. Negative controls were made for the benthic samples by processing triplicate sand samples that were charred in a furnace (400ºC for 24 h) and then sieved and processed as above. For the water samples we filtered *in situ* sterilized ultrapure water with three Sterivex filters that were then treated in the same manner as the seawater filters. Amplification products were pooled to build two Illumina libraries using Nextflex PCR-free library preparation kit (Perkin-Elmer). Both libraries were sequenced together in an Illumina MiSeq V3 run using 2x250 bp paired-end sequencing.

Bioinformatic analyses

The bioinformatic analyses followed the same pipeline of Atienza et al. (2020) with slight modifications. Most steps used the OBITools package (Boyer et al., 2016). Illuminapairedend was used to align paired-end reads and keep only those with >40 alignment quality score. Reads were demultiplexed using ngsfilter. Those with mismatched primer tags at any end were discarded. Obigrep and obiuniq were used to perform a length filter (retaining only those between 310-317 bp) and dereplicate sequences. Uchime-denovo algorithm from VSEARCH v2.7.1 was used to remove chimeric amplicons. The resulting read dataset in fasta format, with the abundances in each sample, was uploaded to the DRYAD repository (doi:10.5061/dryad.vt4b8gtq2).

Sequences were then clustered into Molecular Operational Taxonomic Units (MOTUs) with SWARM v2.1.7 using d=13 (Bakker et al., 2019; Siegenthaler, Wangensteen, Benvenuto, Campos, & Mariani, 2019). Singletons (MOTUs with just one read) were removed after this step to minimize data loss (Atienza et al., 2020). Taxonomic assignment was performed using ecotag and a custom database containing sequences from the EMBL nucleotide database and sequences obtained from the Barcode of Life

Database (BOLD), using a custom script to select the appropriate fragment (see details and a summary of the taxonomic groups represented in Wangensteen, Palacín, et al. 2018). This database contains 188,960 reference sequences covering most eukaryotic groups and is available from https://github.com/metabarpark/Reference-databases. Assignment of metazoan sequences was further improved by querying the BOLD database. Sequences with a species name assigned and with an identity match >95% in BOLD were kept, whereas matches below this threshold, even if assigned to species level by ecotag, were downgraded to genus level.

The final refining steps consisted of deleting any MOTU for which reads in blank or negative controls represented more than 10% of total reads for that MOTU in all samples. A minimum relative abundance filter was also applied, removing, for a given PCR replicate, the MOTUs that represented less than 0.005% of total reads of that replicate. We also removed MOTUs that had a combined total of <5 reads after the previous steps. Finally, all MOTUs that were not assigned to marine eukaryotes (i.e., MOTUs assigned to non-marine organisms, prokaryotes, or to the root of the Tree of Life) were eliminated. We then pooled the three PCRs of each sample. We used the higher classification of eukaryotes proposed by Guillou et al. (2013) at the super-group level, with one exception: Opisthokontha was split into Metazoa and Fungi.

Data analyses

Analyses were performed with the R package vegan (Oksanen et al., 2019). Rarefaction curves of the number of MOTUs obtained at an increasing number of reads were obtained with function rarecurve, separately for benthos and water samples. Likewise, MOTU accumulation curves with increasing numbers of samples were obtained for benthos and water with specaccum. MOTU richness values were compared with standard ANOVAs (factors community and sample type: benthos or water). Between-sample distances were computed using the Jaccard index based on presence/absence data of each MOTU per sample. These distances were then used to obtain ordinations of the samples in non-metric multidimensional scaling (nmMDS) representations using function metaMDS with 500 random starts. Permutational analyses of variance were

performed on Jaccard distances with function adonis to test differences between relevant factors: a one-way analysis was performed between benthos and water (all samples combined), a three-way analysis was done for the benthos with community and fraction as main factors and sample as a blocking factor nested in community. For the water, a two-way analysis was performed with community and distance to the wall (pelagic samples excluded as they were taken at an intermediate depth). Main factors were also tested for differences in multivariate dispersion (permdisp analysis using function betadisper) to check whether significant outcomes were a result of different multivariate heterogeneity (spread) or different centroid location of the groups. A Venn diagram was prepared with the VennDiagram package (Chen, 2018) to represent the degree of MOTU overlap between benthos and water. Upset diagrams were used to plot shared MOTUs at increasing distances of the benthic communities using package UpSetR (Conway, 2017).

Results

We obtained a total of 7,391,160 reads in total for the benthic samples (18 samples) and 13,652,493 reads for the water samples (27 samples). The controls had a negligible number of reads (85.29 ± 19.80, mean ± SE). After quality filtering, demultiplexing, dereplicating and chimera elimination we had a total 3,868,827 unique COI sequences. These were clustered into 15,954 non-singleton MOTUs. The final refining steps and, particularly, the elimination of MOTUs not assignable to marine eukaryotes using our reference database greatly reduced the dataset to a final list of 3,543 MOTUs. The impact of removing non-eukaryotic MOTUs was much greater in the water samples: only 14.35% of initial reads were retained at this step, while 99,36% were kept in the benthic samples. In the final dataset, benthic samples had 2,396 MOTUs, while water samples had 1,412 MOTUs. The final average number of eukaryotic reads in benthic samples was 233,957 ± 25,40 (mean ± SE) and in water samples was much lower, 34,708 ± 2,50, as a result of the elimination of non-eukaryotic MOTUs. Table S1

presents the final MOTU table with the taxonomic assignment and number of reads per sample. Rarefaction curves (Figure S2) showed that a plateau is reached in the number of MOTUs with the sequencing depth obtained in most samples from benthos and water (exceptions corresponded to some of the finer fractions in benthic samples). Likewise, MOTU accumulation curves (Figure S3) tended to saturate in water samples but not in benthic samples, so addition of more samples would likely increase the total number of MOTUs recovered from this habitat. In spite of the different number of total reads, we compared MOTU richness without rarefaction as in most samples the richness values plateaued at the sequencing depth obtained. Somewhat higher values were found in benthos (637.78 ± 59.00 and 420.34 ± 47.96 MOTUs in the photophilous and sciaphilous communities, respectively) compared to those in water at 0 to 1.5m of distance (541.58 ± 29.40 and 389.92 ± 20.58 MOTUs, respectively). A two-way ANOVA showed that the number of MOTUs was not significantly different between benthos and water samples, but it was significantly higher in the photophilous than in the sciaphilous community (community effect, $p<0.001$; sample type effect, $p=0.110$; interaction, $p=0.401$). The pelagic samples had 474.33 ± 28.50 MOTUs.

Taxonomic assignment revealed a total of 7 super-groups in the samples, of which the most diverse was Metazoa (996 MOTUs, 45.47% of reads, all samples combined) followed by Archaeplastida (351 MOTUs, 16.47% of reads, mostly belonging to Rhodophyta), and Stramenopiles (287 MOTUs, 3.25% of reads). A total of 1,565 eukaryotic MOTUs could not be assigned to a given super-group. They represent 32.25% of total reads, but the share of unassigned reads was highly uneven: 21.94% of reads in benthic samples, and 78.58% in water samples. Within metazoans we identified 15 phyla, of which the most diverse were Arthropoda (211 MOTUs, 2.17% of total reads, all samples combined), followed by Annelida (116 MOTUs, 1.71% of reads), Cnidaria (74 MOTUs, 11.65% of reads), Porifera (59 MOTUs, 6.35% of reads) and Mollusca (50 MOTUs, 1.20% of reads). Among metazoans, 382 MOTUs could not be assigned at phylum or lower levels. In addition, 165 MOTUs could be assigned at the species level by ecotag with more than 0.95 identity with the best match in the reference database.

The relative number of MOTUs as per super-group and metazoan phylum obtained in the benthos and water samples is shown in Figure 2. The general patterns recovered were notably different in the two habitats surveyed. Metazoa were markedly dominant in the benthos in terms of number of MOTUs, followed by Archaeplastida (mostly Rhodophyta). On the other hand, Hacrobia (mostly Haptophyta) had the highest diversity in water samples, where other important planktonic groups such as the Alveolata had a much higher representation than in the benthos. Nevertheless, Metazoa was the second most MOTU-rich group in the water. As for metazoan phyla, the distribution was more similar: Arthropoda was the most diverse group in both habitats, and Annelida, Cnidaria, Mollusca and Porifera (albeit in different order) came next. However, the picture is different considering the relative number of reads: Cnidaria were dominant in the benthos (26.05% of metazoan reads), where the abundance of Arthropoda was much lower (3.88%). Conversely, in the water Arthropoda was the most abundant by far in proportion of metazoan reads (46.70%).

The number of MOTUs of the main metazoan phyla, Arthropoda, Annelida, Cnidaria, and Mollusca was further assessed at lower taxonomic levels (Order) in Table S2. In arthropods, Amphipoda, Decapoda, Isopoda and Harpacticoida were highly diverse in the benthos but practically absent from water samples, which were dominated by planktonic groups such as Calanoida and Cyclopoida. In annelids, Sabellida and Sipuncula were the most diverse groups in the plankton, while the dominant group in benthos (Phyllodocida) was practically absent in water samples (only 4 MOTUs in total). Among Cnidaria, only hydrozoans (Trachymedusae, Siphonophora, and Leptothecata) are diverse in the plankton samples, with a negligible representation of anthozoan orders which, together with Leptothecata, dominate in the benthic samples. Among Mollusca, highly diverse groups in the benthos such as Mytiloida, or gastropoda in general (with the exception of the pelagic Pteropoda) were absent or poorly represented in water samples. This perusal indicates that we didn't capture in our samples planktonic stages of many benthic groups, and that the rates of DNA shedding from benthos to the water are in general low.

The sample ordination using the Jaccard index is shown in Figure 3A. A clear separation of benthic and water samples is evident, which is in agreement with one-way results comparing benthos and water, all samples pooled (PERMANOVA $p<0.001$, and permdisp $p<0.001$). In the benthos, the shallower and deeper communities formed clearly separated clusters. A PERMANOVA analysis on benthic samples alone showed a significant effect of community ($p<0.001$) and of the nested factor sample (within community); while fraction or the interaction between community and fraction were not significant (Table 1). The permdisp test showed that there was also a different dispersion of data in the two communities ($p<0.001$), which is also visible in the nmMDS. A second nmMDS was performed only with the water samples (Figure 3B), where a separation by communities can also be seen, albeit with some overlap. A PERMANOVA of water samples using community and distance to the wall as factors (pelagic samples were excluded in this analysis) showed a significant interaction term ($p=0.027$, Table 2), indicating different effects of the community with increasing distances. A comparison of the factor community at fixed distances showed that differences between photophilous and sciaphilous samples were significant at all distances (0, 0.5, and 1.5 m, all $p<0.031$), and this was not due to differences in heterogeneity (all permdisp tests not significant). Likewise, a comparison of the factor distance at each depth level showed that distance to the rocky wall didn't have a significant effect on the overall water assemblage composition ($p=0.063$ and 0.056 for the photophilous and sciaphilous communities, respectively).

Of the total 3,543 MOTUs, only 265 were shared between benthos and water (Figure 4, Tables S3-S4), which represented 11.06% of the MOTUs found in benthos. However, these 265 MOTUs accounted for 70.40% of the reads of the benthos, indicating that they correspond to abundant taxa. These same MOTUs accounted for 56,37% of the reads in the water samples. The MOTUs shared between benthos and water could be assigned to two main groups, those whose relative read abundance in the benthos was higher than in the water and those displaying the opposite pattern. We assume that the first group corresponds mainly to benthic MOTUs that left their DNA signature in the water (hereafter "shared benthic MOTUs" or SBM), while the second group likely corresponds to planktonic MOTUs (hereafter "shared pelagic MOTUs" or SPM). Only

388 one MOTU could not be assigned to any of these categories as it had the same number
389 of reads in both environments.

390 The first group (SBM) comprised 180 MOTUs (Table S3), which represented 7.51% and
391 70.33% of MOTUs and reads in the benthos, respectively, while they constituted 12.75
392 and 1.99% of the MOTUs and reads in the water. Of these MOTUs, almost half (84,
393 46.67%) belonged to metazoan groups, but only 7 of them were arthropods (the
394 dominant metazoan group in the plankton); the second most important group were the
395 red algae (a mostly benthic group), with 25 (13.89%) MOTUs. Of the dominant
396 planktonic groups, only 11 (6.11%) SBM were diatoms and 2 were dinoflagellates. The
397 taxonomic assignments were, therefore, mostly coherent with the idea that this subset
398 of MOTUs belong mainly to benthic groups (Table S3). A total of 45 SBM MOTUs (25%)
399 could not be assigned to any super-group.

400 The 84 shared pelagic MOTUs (SPM, Table S4) made up 3.51% of MOTUs but only
401 0.07% of reads in the benthos. On the other hand, while they comprised 5.95% of
402 pelagic MOTUs they accounted for 54.44% of pelagic reads. Their taxonomic
403 assignments showed that 22 (26.19%) MOTUs were metazoans, of which a majority
404 (17) were arthropods. On the other hand, 18 (21.43%) MOTUs belonged to typical
405 planktonic protists (diatoms, dinoflagellates, Hacrobia, Rhizaria) (Table S4). Finally, 42
406 (50%) SPM could not be assigned to any super-group. The higher number of
407 unassigned MOTUs and the taxonomic composition suggest a dominance of non-
408 benthic groups in the SPM subset.

409 When the distribution of the 180 shared benthic MOTUs was examined, they clearly
410 decreased with distance to the wall (Figure 5), with 135, 74, 24, and 15 MOTUs shared
411 between benthos and water samples at 0, 0.5, 1.5 and 20 m, respectively. Their
412 abundance in relative read numbers also decreased (from 0.056 to 0.002, Table S3),
413 which supports the idea of their benthic origin. This same general pattern was found
414 when both communities studied were analysed separately (Figures S4 and S5).

415 By contrast, the comparison of shared pelagic MOTUs did not show any clear trend with
416 distance to the wall (Figure 5): 72, 73, 66, and 67 at 0, 0.5, 1.5, and 20 m, respectively.

Neither was a trend found in relative read abundances per sample (between 0.570 and 0.526 irrespective of distance, Table S4). Again, this same general pattern was found in both communities separately (Figures S4 and S5).

Discussion

Metabarcoding of benthos and water samples, using a broad range eukaryotic marker (COI), retrieved clearly different communities. The patterns of MOTU richness and abundance of reads from the different environments were distinct, showing a dominance of taxa with important planktonic components (such as dinoflagellates, diatoms, and haptophytes) in the water samples, while metazoans and rhodophytes were the most diverse and abundant in the benthos. Metazoans, notwithstanding, were also well represented in water samples, with a dominance of arthropods (mostly calanoids and cyclopoids) in both number of MOTUs and reads. The rarefaction and MOTU accumulation curves showed that we captured adequately the richness present in the samples with our sequencing depth, and that the total eukaryotic diversity in the benthos was higher than that in the water. More replicates of benthic samples would be necessary to recover the overall MOTU richness of this habitat.

However, we acknowledge that the sampling methods used were different for benthos and plankton. We have used techniques currently applied to sample these environments. In complex communities such as the benthos, with organism sizes spanning several orders of magnitude, size-fractionation is necessary to recover the biodiversity present (Elbrecht, Peinert, & Leese, 2017; Wangensteen, Palacin, et al., 2018; Wangensteen & Turon, 2017). In addition, the mesh size used for the smallest sieve was 63 µm, meaning that most prokaryotes and a significant part of the smallest microeukaryotes were washed out, along with cell debris and extracellular DNA. In the filters, on the other hand, we retained everything down to colloidal level, thus the prokaryotic community, for instance, was captured in our samples. This explains the

amount of reads that had to be discarded in the water samples as not assignable to eukaryotes and, within eukaryotes, the high number of reads that could not be assigned to any supergroup (the smallest eukaryotes being the less represented in the reference database for COI). Our point was not to test both techniques or to compare their particularities, but rather to check if the information retrieved from currently established methods for the analysis of water DNA is comparable to that from current analytical techniques for benthos.

While the DNA obtained from the filters would be labelled as environmental DNA, the sampling from the benthos would be qualified as community or bulk DNA by many. Environmental DNA (eDNA) is defined as the DNA obtained from an environmental matrix such as water or sediment without isolating the organisms (Barnes & Turner, 2016; Creer et al., 2016; Stewart, 2019; Taberlet, Coissac, Hajibabaei, & Rieseberg, 2012); and is usually opposed to bulk or community DNA, referring to DNA obtained from organisms previously isolated from the environment (Andujar, Arribas, Yu, Vogler, & Emerson, 2018; Creer et al., 2016; Deiner et al., 2017). In a more restricted sense (e.g., Andujar et al., 2018; Cristescu & Hebert, 2018; Thomsen et al., 2012; Tsuji et al., 2019), the term eDNA is used as equivalent to trace DNA released from organisms (in the form of mucus, faeces, cells, hairs…), so when studying eDNA the organisms themselves are not in the sample. We consider, however, that eDNA should be used as a general term, to designate any DNA extracted from an environmental sample. It is commonly made up of a mix of intra-organismal (in the form of small organisms relative to the sample size) and extra-organismal or trace eDNA shed from large organisms (Creer et al., 2016; Pawlowski et al., 2018; Porter & Hajibabaei, 2018; Salter, 2018; Taberlet, Coissac, Hajibabaei, et al., 2012). The relative amount of both components is highly variable, though, and it depends on the sampling method and the target group, and hence the primers used. In our case, we used a broadly universal primer set for eukaryotes, capable of amplifying both intra-organismal and trace DNA from most eukaryotic taxa. So the benthic samples are more enriched in intra-organismal DNA (since most trace DNA was removed by sieving), while the water samples contain a mix of a high amount of intra-organismal DNA from planktonic microeukaryotes and a smaller fraction of extra-organismal DNA from larger organisms.

The ordination and PERMANOVA results confirmed the marked differentiation between the samples from both environments. An assessment at the Order level in the main metazoan phyla confirmed that the composition of the two environments is highly different. Moreover, the differences between the two depths sampled, which corresponded to two different communities (photophilous and sciaphilous) on precisely the same wall, were pronounced in the benthic samples, but were also significant in the water samples taken between 0 and 1.5 m of the rocky wall. Thus, the method is sensitive enough to detect ecological differences not just in the sessile communities, but also in the more dynamic planktonic habitat. This is in agreement with other studies that have also shown that the eDNA in seawater samples can detect differences in composition of several groups at relatively small scales (from meters to tens of meters, Jacobs-Palmer et al., 2020; Jeunen et al., 2019; Port et al., 2016).

A total of 3,543 eukaryotic MOTUs were detected in the whole dataset. In spite of the lower number of eukaryotic reads retrieved from the water (15% of those retrieved from the benthos), the number of eukaryotic MOTUs in the water was ca. 60% of those in the benthos (1,412 as compared to 2,396). Only 265 MOTUs were found to be shared between the benthos and the water samples. This represents only ca. 11% and 19% of the MOTUs in the two environments, respectively. In addition, a closer scrutiny allowed us to separate those shared MOTUs into those of possibly benthic origin (shared benthic MOTUs, SBM) and those of likely planktonic origin (shared pelagic MOTUs, SPM).

The 180 SBM comprised ca. 7.5% of the benthic MOTUs but represented ca. 70% or benthic reads (while only ca. 2% of water-derived reads), indicating that abundant benthic MOTUs are the ones more prone to leave their signature in the surrounding water. The 84 SPM accounted to ca. 6% of pelagic MOTUs but ca. 54% or eukaryotic pelagic reads (and only 0.07% of reads in the benthos), again indicating that the most abundant MOTUs are the ones that can be detected also in the other habitat.

The fine-scale distribution of the 180 SBM showed a clear trend: more MOTUs were shared in the immediate vicinity of the benthos (135 with water at 0 m), and the number

decreased with distance down to only 15 MOTUs shared with the water at 20 m. The shared MOTUs also represented a decreasing percent of reads in the water samples as we moved away from the rocky wall. On the other hand, there was no clear pattern of abundance changes with distance in the richness or amount of reads shared between benthos and water for the 84 PSM.

We found therefore evidence for DNA originating from the benthic communities being present in the adjacent water layer and, conversely, DNA of presumably pelagic origin could be detected in the benthos. The interest of this article was in detecting the presence of benthic DNA in the water column, of which only a modest amount could be retrieved. The form of this benthic DNA in the water cannot be assessed with our sampling design, but it likely includes naturally released meroplanktonic components, such as gametes (Tsuji & Shibata, 2020) or larvae, and degradation products in the form of fragments, mucus, cell aggregates, exudates, or extracellular DNA.

Our results clearly indicated that DNA from water samples is a poor surrogate for the analysis of benthic communities, as found previously in freshwater environments (Hajibabaei et al., 2019). Even in the water within a few centimetres from the benthos, only a modest portion (135) of the benthic MOTUs could be detected. In addition, we found that considering the relative number of reads of the shared MOTUs provided useful insights about the origin of the MOTUs and their dynamics as we move farther from the rocky wall. The lack of accordance between benthos and water is in agreement with previous comparisons of different substrates for eDNA made in port environments (e.g., Koziol et al., 2019; Rey et al., 2020) which found different community profiles in water and in sediments or settlement plates. We must keep in mind that we have used universal primers as we targeted the whole eukaryotic communities. With more specific targets, the results could be different. For instance, using vertebrate-specific primers to detect fish in the water has proved to be a sensitive method (e.g., Bakker et al., 2017; Sales et al., 2019; Salter, Joensen, Kristiansen, Steingrund, & Vestergaard, 2019; Sigsgaard et al., 2019; Thomsen et al., 2016), even at the intraspecific level (Sigsgaard et al., 2020), since it is possible to amplify selectively the DNA of the target group. Likewise, species-specific primers have been successfully used to detect particular

marine benthic species in the water column, usually as a means of monitoring invasive species (e.g. Pochon et al., 2013; Simpson, Smale, McDonald, & Wernberg, 2017; Von Ammon et al., 2019).

It seems reasonable to expect that DNA shedding rates from a highly diverse community such as sublittoral rocky bottom assemblages would be unbalanced between groups, and that this unevenness would hinder our ability to extract reliable monitoring information from seawater eDNA. This expectation is borne out by our results. Thus, albeit for group-specific or species-specific studies useful information from benthic groups may be gleaned from water DNA, the method is presently unsuitable for the community-wide diversity assessment required for many biomonitoring applications. New technologies affording much higher sequencing depth or metagenomic approaches (Singer, Fahner, Barnes, McCarthy, & Hajibabaei, 2019; Singer, Greg, Shekarriz, McCarthy, Fahner, & Hajibabaei, 2020) might improve our ability to extract information from water samples. But for the time being we must continue to rely on methods that can sample directly the benthos for reliable biodiversity assessment of these complex assemblages.

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Data Accessibility Statement

The original read dataset, with the abundances in each sample, was uploaded to the Dryad Data repository (https://doi.org/10.5061/dryad.vt4b8gtq2).

The final MOTU dataset has been uploaded as online supplementary material.

Author Contributions

AA, performed laboratory and bioinformatics work, prepared tables and figures and drafted the paper; CP, designed research, analysed data and revised the paper; EC,

898 performed field work, contributed funding and revised the paper; RG, performed field
899 work, analysed data and revised the paper; OSW, designed research, contributed
900 reagents and analytical tools, analysed data and revised the paper; XT, designed
901 research, performed field work, contributed funding, analysed data and revised the
902 paper.
903

Factor	DF	SS	F-statistic	P-value	Permdisp
Community	1	1.581	5.442	0.001*	0.001*
Fraction	2	0.731	1.258	0.140	0.869
Community*Fraction	2	0.653	1.124	0.267	
Sample(Community)	2	1.158	1.993	0.002*	
Residuals	10	2.905			

Table 1. Results of the PERMANOVA analysis performed on Jaccard distances among the samples collected in two benthic communities (photophilous and sciaphilous) and separated into three size classes (fractions). Sample was added as a nested factor within community. Columns are: degrees of freedom (DF), sum of squares (SS), F-statistic of the model, with its associated probability (P-value), and probability of the permdisp test of multivariate homogeneity of group dispersions (Permdisp). Significant values marked with asterisk.

914
915

Factor	DF	SS	F-statistic	P-value	Permdisp
Community	1	0.265	4.127	0.001*	0.216
Distance	2	0.166	1.293	0.129	0.940
Community*Distance	2	0.216	1.682	0.027*	
Residuals	18	1.157			

916

917 Table 2. Results of the PERMANOVA analysis performed on Jaccard distances among the
918 water samples collected in two communities (photophilous and sciaphilous) and at three
919 distances from the benthos (Distance factor: 0, 0.5 and 1.5 m). Columns are: degrees of
920 freedom (DF), sum of squares (SS), F-statistic of the model, with its associated probability (P-
921 value), and probability of the permdisp test of multivariate homogeneity of group dispersions
922 (Permdisp). Significant values marked with asterisk.
923

924 Figure Captions

925

926 Figure 1. Schema of the sampling design. We sampled two hard bottom communities
927 (green: photophilous; red: sciaphilous) at -10 and -30 m of depth, respectively, by
928 sampling quadrats of 25 x 25 cm (3 replicates each). Water samples (1.5 L) were
929 collected at different distances from each community (0 m, 0.5 m and 1.5 m, 4
930 replicates each). Pelagic samples were taken at intermediate (-20 m) depth and at 20 m
931 from the wall (3 replicates).

932

933 Figure 2. Barplot of relative MOTU richness of the super-groups (a) and metazoan phyla
934 (b) detected in benthic and water samples.

935

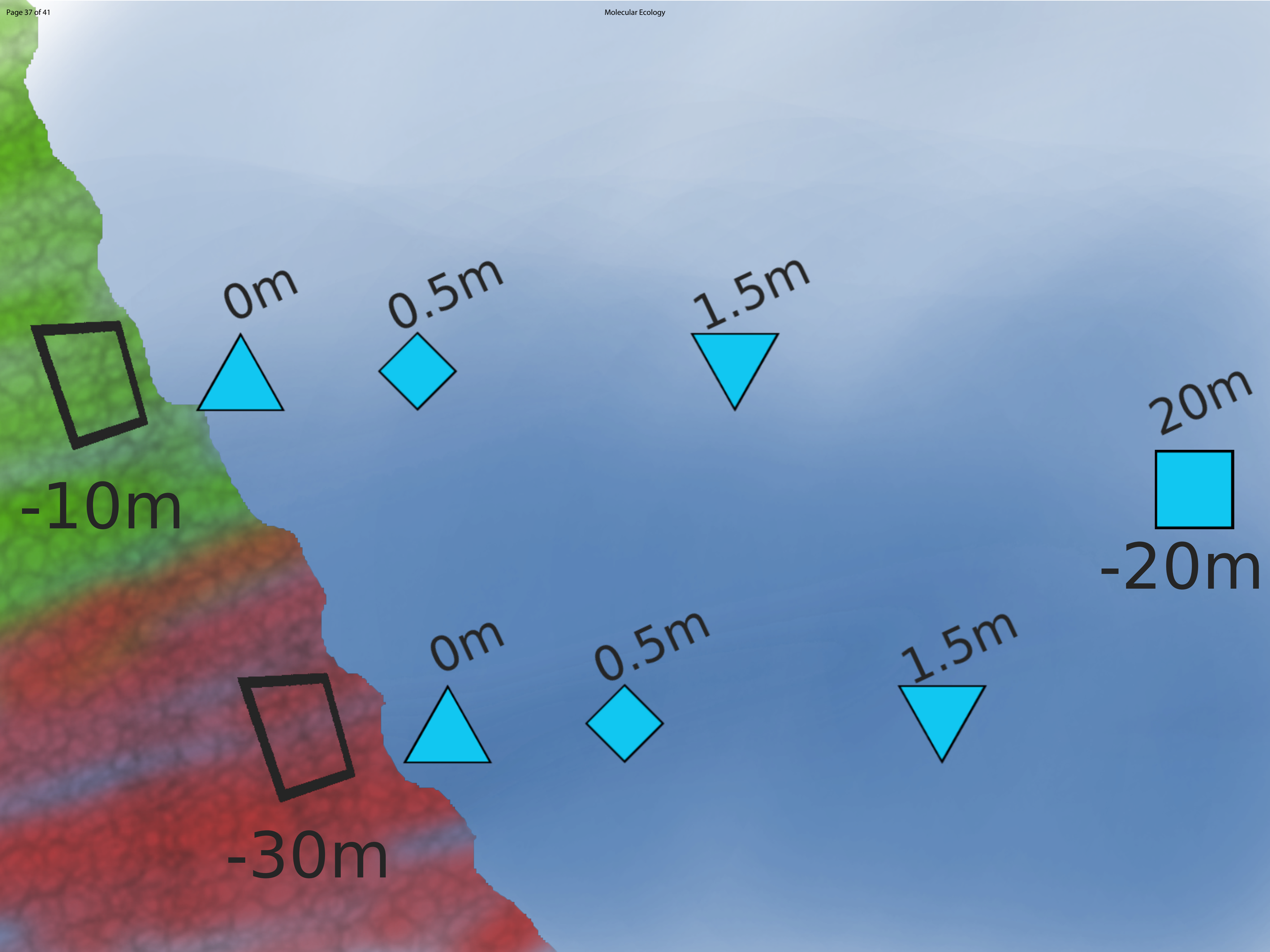
936 Figure 3. Non-metric Multidimentional Scaling representation of all samples (a) and only
937 water samples (b) using the Jaccard distance. Benthic samples (a) were separated in
938 three different size fractions: A (>10 mm), B (between 10 mm and 1 mm) and C
939 (between 1 mm and 63 µm). Communities are coded by colours and fractions (benthos)
940 and distances (water) by symbols.

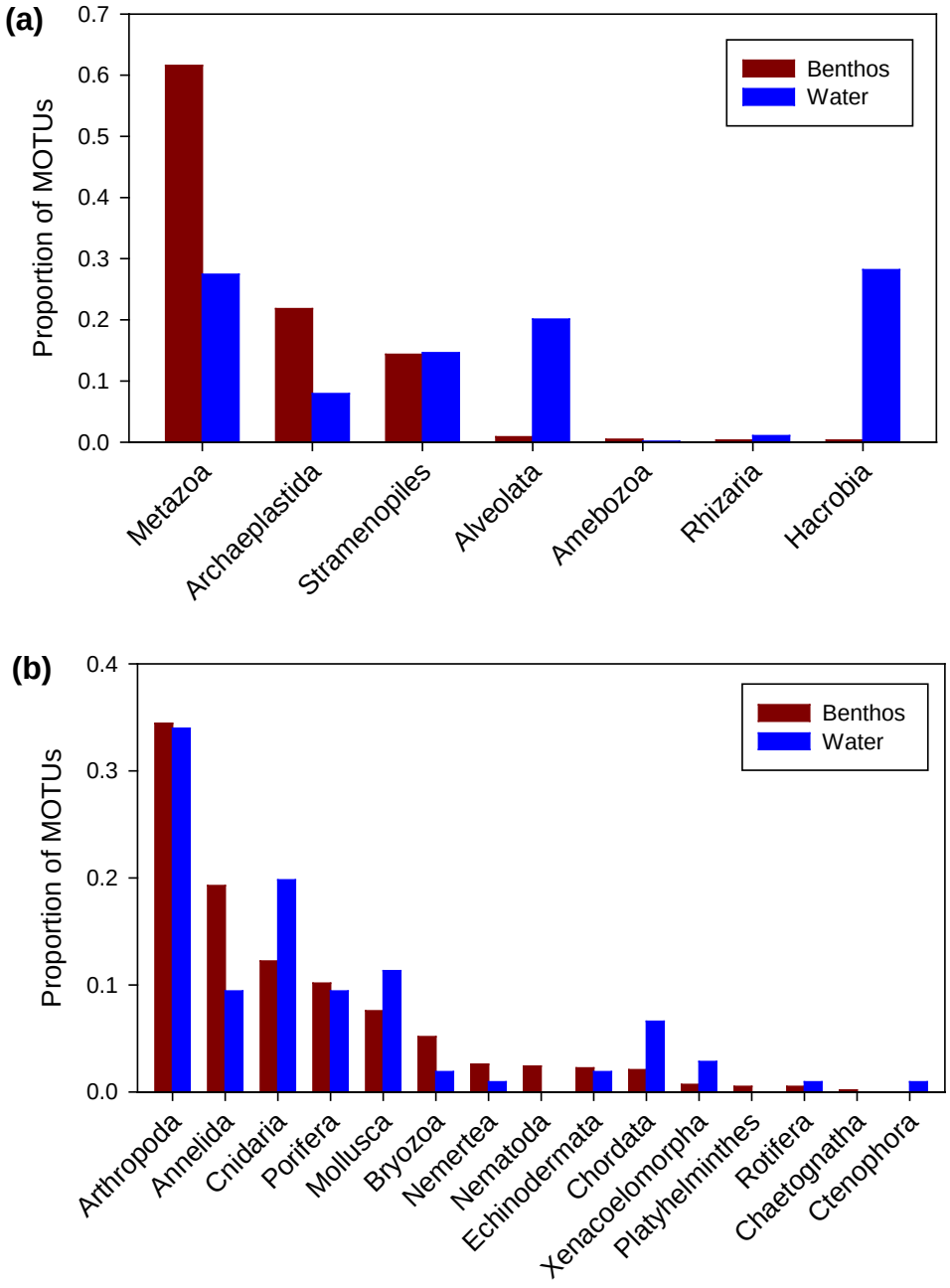
941

942 Figure 4. Venn diagram showing the overall MOTU overlap between the two types of
943 community considered.

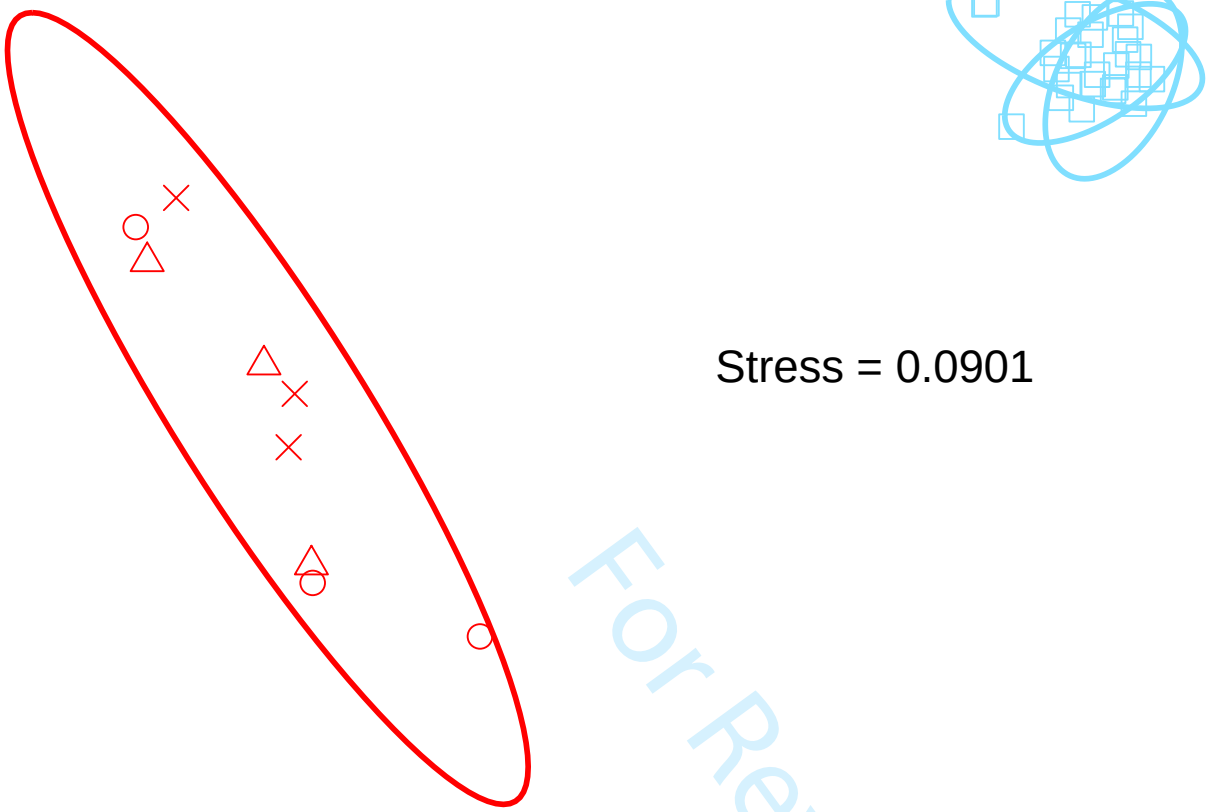
944

945 Figure 5. Upset plot with the number of shared MOTUs between the benthos and the
946 water samples and the total number of MOTUs detected. Shared benthic MOTUs (SBM)
947 are represented in pink and shared pelagic MOTUs (SPM) in light blue.


0m
0.5m
1.5m
20m
-10m
-20m
0m
0.5m
1.5m
-30m


(a)
Proportion of MOTUs
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Benthos
Water
Metazoa
Archaeplastida
Stramenopiles
Alveolata
Amebozoa
Rhizaria
Hacrobia
(b)
Proportion of MOTUs
0.0
0.1
0.2
0.3
0.4
Benthos
Water
Arthropoda
Annelida
Cnidaria
Porifera
Mollusca
Bryozoa
Nemertea
Nematoda
Echinodermata
Chordata
Xenacoelomorpha
Platyhelminthes
Rotifera
Chaetognatha
Ctenophora

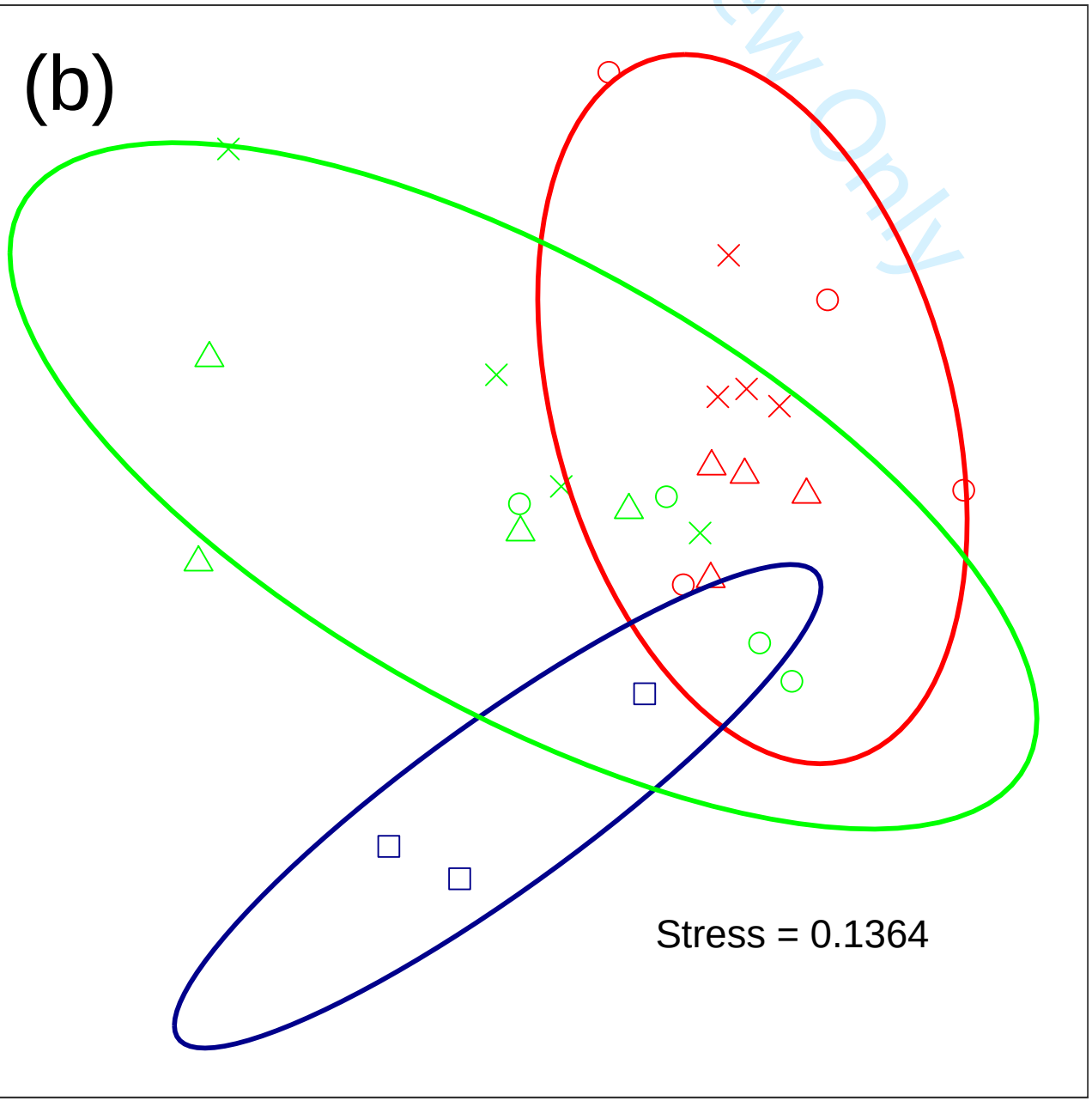
(a)



Fraction A
Fraction B
Fraction C
Water samples

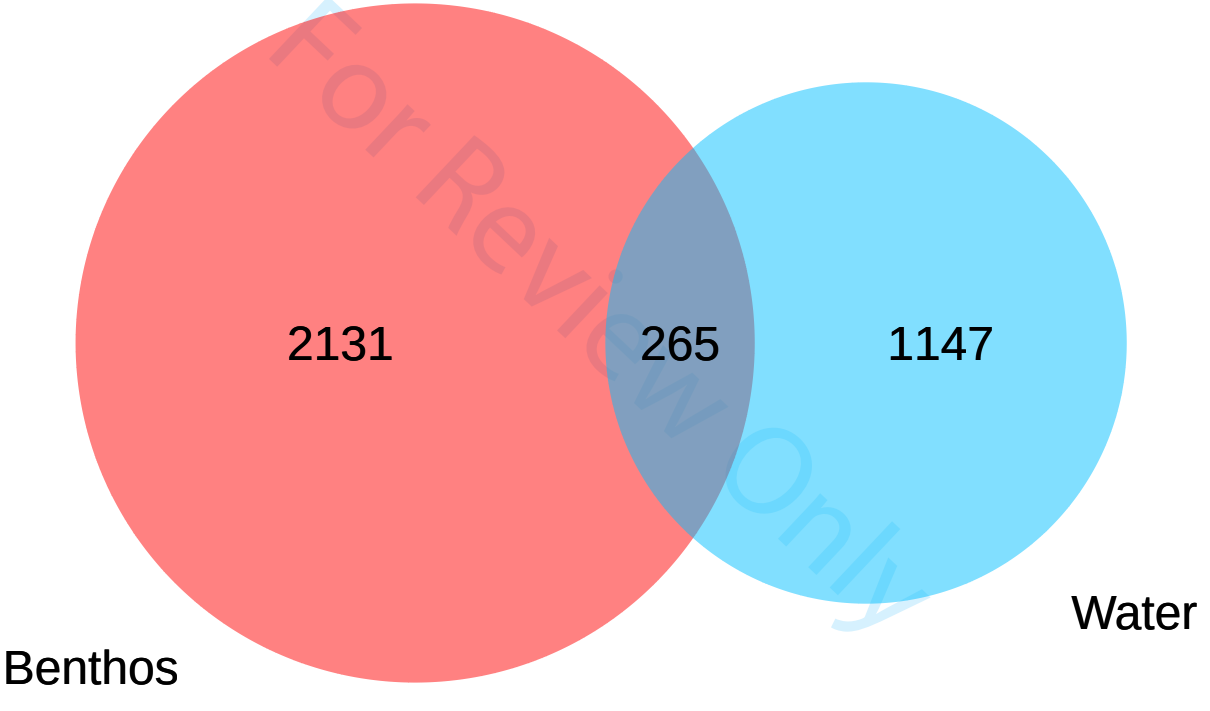
Benthos Photophilous
Benthos Sciaphilous
Water

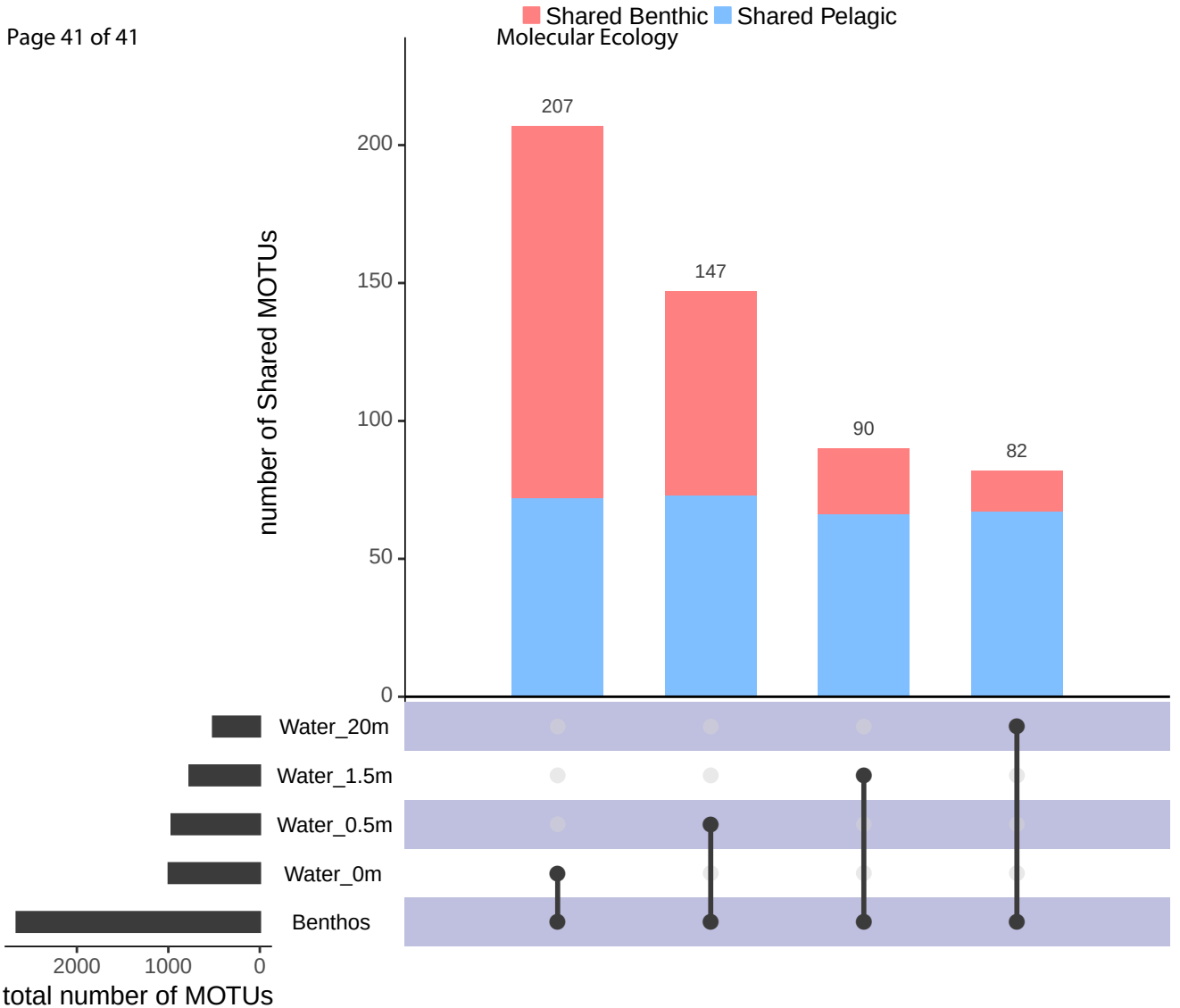
(b)



0m
0.5m
1.5m
20m

Photophilous
Sciaphilous
Pelagic




Shared Benthic
Shared Pelagic
number of Shared MOTUs
0
50
100
150
200
207
147
90
82
Water_20m
Water_1.5m
Water_0.5m
Water_0m
Benthos
2000
1000
0
total number of MOTUs