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Different approaches to estimate benthic metazoan diversity associated with free-living macroalgae (*Fucus vesiculosus*) on shallow soft sediments

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ABSTRACT

Habitat complexity can boost biodiversity by providing a wide range of niches allowing species co-existence. Baltic Sea benthic communities are characterised by low species diversity. Thus the occurrence of the habitat forming macroalga *Fucus vesiculosus* may influence benthic communities and promote diversity. Here biodiversity estimates were obtained through conventional and eDNA approaches for the benthic assemblages associated with free-living *Fucus* and the adjacent bare-sediment habitats at six sites from the Northern Baltic Proper and the Gulf of Finland. Free-living *F. vesiculosus* habitats are heterogeneous with biodiversity estimates varying considerably among sites. The additional habitat complexity provided by *F. vesiculosus* tended to improve taxa richness as a result of additional epifauna assemblages, although macroinfaunal taxa richness and abundance was often reduced. Consequently the complex habitats provided by free-living *F. vesiculosus* often improve biodiversity, yet alters the composition of assemblages in soft sediment habitats and consequential ecosystem functioning. The study emphasised the disparity in biodiversity estimates achieved when employing different biodiversity approaches. Biodiversity estimates were more similar within approaches compared to between habitat types, with each approach detecting exclusive taxa. Consequently, biodiversity estimates can benefit from a multi-approach design where both conventional and eDNA approaches are employed in complement.

1. Introduction

Habitat complexity influences biodiversity and associated ecosystem functioning (Kovalenko et al., 2012). In many ecosystems higher habitat complexity will attract more associated species (Johnson and Agrawal, 2005) by promoting species coexistence through providing a wide range of niches, thereby reducing niche overlap and increasing diversity (Huston and DeAngelis, 1994; Levins, 1979). The macroalgae genus *Fucus* represents important foundation species within northern hemisphere coastal environments. Within the Baltic Sea, *Fucus vesiculosus* (herein *Fucus*), is one of only a few large, perennial macroalgae forming structurally complex canopies in the coastal photic zone, supporting numerous associated organisms (Henseler et al., 2019; Kraufvelin and Salovius, 2004; Wikström and Kautsky, 2007). Attached *Fucus* canopies are some of the most highly productive habitats within the Baltic Sea (Attard et al., 2019).

Alongside the typical attached form, a free-living form is common throughout the Baltic Sea on any substrate type, although most frequently on soft sediments, in more sheltered areas (Preston et al., 2022a, 2022b). Consequently the free-living form can form stable, perennial mats in locations where attached algae would otherwise be absent. These free-living mats create three-dimensional habitats of varying heights and densities that are comparable to the interstitial space of sediments (HELCOM, 2013). Thus free-living *Fucus* likely provide high complexity habitats alongside also influencing the normally associated fauna of soft sediment habitats. In fact algal mats are often associated with high biodiversity (El-Khaled et al., 2022; Rossbach et al., 2021, 2022). As Baltic Sea benthic habitats are characterised by exceptionally low species diversity (Kotta and Orav, 2001) the addition of algal cover may provide several conditions, including increasing food resources (Arroyo et al., 2006; Norkko et al., 2000) and providing protection from predation (Aarnio and Mattila, 2000; Norkko et al., 2000),

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which may in turn boost biodiversity. Although algal cover may also contribute to hypoxic conditions within the sediment resulting in faunal reductions (Everett, 1994; Norkko and Bonsdorff, 1996a, 1996b; Rabalais et al., 2010).

Baseline biodiversity estimates for benthic habitats can be obtained through conventional approaches whereby samples are collected by sampling devices (e.g. cores, quadrats, scuba diving), sorted and individually taxonomically identified. This approach is often time-consuming and requires specialist taxonomic expertise (Kim and Byrne, 2006; Port et al., 2016). Increasingly, DNA-based molecular methods are being applied to assess biodiversity (Zaiko et al., 2018). Environmental DNA (eDNA) originates from living organisms, dead cells and extracellular DNA present within the sample (Levy-Booth et al., 2007; Pietramellara et al., 2009; Taberlet et al., 2012). DNA sequence information from the pool of genetic material within the environmental sample is used to determine the taxonomic identification within the sample. This study employed these two approaches to provide biodiversity estimates for two habitat types: *Fucus* associated soft sediments and the adjacent bare-sediment. Firstly the study used two conventional sampling approaches to identify macroinfaunal and epifaunal benthic assemblages and secondly an eDNA approach on sediment samples to identify metazoan benthic assemblages. The aims of the study were to (i) investigate the influence of free-living *Fucus* on soft sediment faunal benthic assemblages, including the spatial variability among sites and countries, and (ii) evaluate the discrepancies between approaches in generating biodiversity estimates.

2. Methods

2.1. Sample locations

The Baltic Sea is a semi-enclosed brackish system with a defined north-south salinity gradient (Furman et al., 2014; Lüning, 1990; Zillén et al., 2008). Surface salinity ranges from 8 to 10 in the southern Baltic, 7–8 in the Baltic Proper and down to 3–6 in the Gulfs of Bothnian and Finland (Lüning, 1990; Matthäus, 2006). Species diversity is low, having approximately 10 times fewer species compared to the neighbouring North Sea (Elmgren and Hill, 1997; Johannesson et al., 2011). Sampling was performed at two locations approximately 340 km apart: Askö in the Northern Baltic Proper and Tvärminne in the Gulf of Finland (Fig. 1). Three sites were selected per location (Table 1). All sites were within close proximity of the shore, in shallow, sheltered embayments associated with *Phragmites australis* reed beds. The bottoms at all sites were soft, being either clay, sandy or mixed substrata. Free-living *Fucus* was

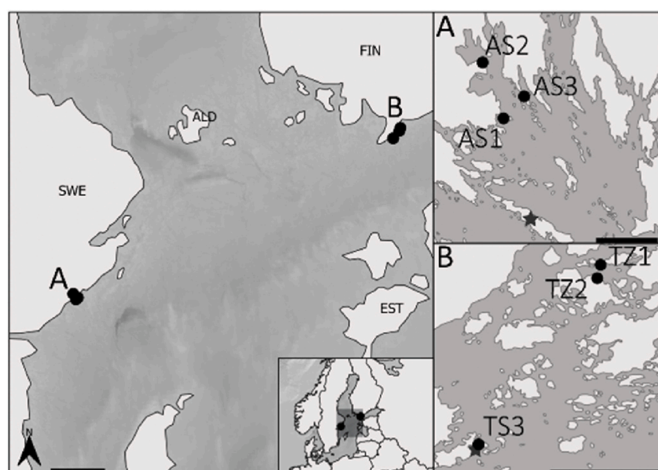

Fig. 1. Locations of the sampling sites. A: Askö, B: Tvärminne. Scale bars represent 50 km in the main map and 5 km in the inset maps (A and B). Star symbols represents field stations.

the dominant macroalga within these locations. At sites AS1 and TZ1 the thalli were entangled within *P. australis*. Salinity at the sites ranged from c. 5.9–6.0 at Askö and c. 5.9–6.1 at Tvärminne whilst maximum depth ranged from 1.9 to 3.4 m at Askö and 2.5–3.2 m at Tvärminne.

2.2. Sample collection and sorting

Sampling was performed in June 2019 from two habitat types: soft sediments associated with free-living *Fucus* and the adjacent bare-sediment (Table 1). Samples from both habitats were collected from all sites. *Fucus* samples were taken from stable, localised mats of free-living *Fucus* covering several square metres at points with 100% algae coverage. The bare-sediment samples were taken from surrounding sediment lacking any form of vegetation. Adjacent bare-sediment areas were in close proximity (<10 m) to the boundary of algal cover and represented a large area of notably changed habitat. Samples were collected through SCUBA diving at depths ranging from 1.5 to 3.4 m. The conventional approach incorporated two sampling methods to capture the macroinfauna (cores) and epifauna (quadrats) assemblages (Supplementary material S1). At each site three 20 × 20 cm quadrats with <1 mm mesh bags were randomly placed. Within the frame all vegetation, including epifauna, were collected. Eight benthic cores (5.6 cm diameter, 10 cm deep) were randomly collected per site (4 per habitat type). *Fucus* sediment cores were collected from underneath the free-living *Fucus* mats whilst bare-sediment cores were taken adjacent to the mats. Subsections of all four cores were removed for the eDNA approach. The 2 ml sediment subsamples for eDNA analysis were transferred to individual microcentrifuge tubes and stored at −20 °C. From the same four cores per habitat type, three were used for the conventional approach. The conventional macroinfaunal and epifaunal samples were run through sieves of 0.5 mm and 0.8 mm, respectively, prior to fixing in 70% ethanol. Faunal samples were sorted and identified to species level or lowest feasible taxonomic ranking. Specimens that were unable to be identified by the available taxonomic expertise were recorded as unclassified (<0.1% of total detected taxa).

2.3. eDNA processing & bioinformatics

DNA extraction was performed using the DNeasy powersoil kit (Qiagen, 12888-100) following the standard kit protocol and stored at −20 °C. Purification of 100 µl of each DNA extract was performed using the Dneasy Powerclean Pro Cleanup Kit (Qiagen, 12997-50). DNA was quality checked on a NanoDrop™ (Thermo Scientific™) and diluted to a concentration of ~2.5–25 ng/µl. Purified samples with low yields were not diluted. Negative reactions (controls) of MQH_2O and buffers from DNA extraction and purification were also processed alongside the samples. Primers TAReuk454FWD1 and TAReukREV3 (Stoeck et al., 2010) targeting the 18 S nSSU gene region were used yielding fragments between 231 and 401 bp not including adaptors or barcodes. Duplicate PCR amplification was performed in 20 µl reaction mixes, with each reaction containing 10 µl Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific™, F548S), 2 µl each of forward and reverse primers (10 µM), 2 µl DNA and MQH_2O to make up to 20 µl. Reactions were prepared on ice. The thermocycler program consisted of an initial denaturation step of 98 °C for 10 s, 10 cycles of denaturation at 98 °C for 1 s, annealing at 57 °C for 5 s and extension at 72 °C for 15 s, then 25 cycles of denaturation at 98 °C for 1 s, annealing at 47 °C for 5 s and extension at 72 °C for 15 s. A final extension of 72 °C for 2 min was performed before samples were held at 4 °C. Thermocycler programs were run on a Veriti 96-Well (Applied Biosystems). PCR products were checked by gel electrophoresis then duplicate reactions were pooled. Samples were further processed and run on Miseq (Illumina) at the DNA Sequencing and Genomics Lab, Institute of Biotechnology, Helsinki Institute of Life Science, University of Helsinki, using the 600-cycle V3 Illumina MiSeq sequencing kit.

Primers were removed from the raw amplicon reads, using cutadapt

Table 1
Sampling site information.

Site	Subbasin	Country	Location	Coordinates (decimal degrees)	Date	Salinity	Max depth (m)
AS1	Northern Baltic Proper	Sweden	Askö	58.89455, 17.62786	13/06/19	6.1	2.5
AS2	Northern Baltic Proper	Sweden	Askö	58.93688, 17.60736	13/06/19	6.1	3.2
AS3	Northern Baltic Proper	Sweden	Askö	58.90913, 17.66022	13/06/19	5.9	3.2
TZ1	Gulf of Finland	Finland	Tvärminne	59.90994, 23.38147	01/06/19	6	1.9
TZ2	Gulf of Finland	Finland	Tvärminne	59.90469, 23.37602	01/06/19	6	2
TZ3	Gulf of Finland	Finland	Tvärminne	59.84587, 23.25200	02/06/19	5.9	3.4

v2.1 (Martin, 2011). Then, the reads were processed with the R package DADA2 1.18 (Callahan et al., 2016) in R 4.1.2 (R Core Team, 2021). The quality parameters in DADA2 were adjusted based on the quality profile of the sequencing run, and were 3 maximum expected errors, 0 ambiguous bases, truncation after quality score of 13, maximum length of forward reads 211 bases, maximum length of reverse reads 201 bases, minimum overlap of 11 bases in merging and chimeric sequences were searched in consensus mode. Taxonomic affiliations of the generated ASVs (Amplicon Sequence Variants) were identified in several steps to remove unassigned and other remaining spurious ASVs. First, taxonomic affiliations were identified, using DADA2 with PR2 reference database (Guillou et al., 2013). Secondly, the PR2 database was searched, using blastn in BLAST+ 2.6.0 (Zhang et al., 2000) to identify ASVs that had low match percentage (<97%) or low query coverage (<80%) to a reference sequence in the PR2 database. Thirdly, NCBI GenBank was searched (May 23, 2022), using blastn. Taxonomic affiliations of the GenBank search were parsed using the weighted lowest common ancestor algorithm in MEGAN 6.22.2 (Huson et al., 2016) with minimum bit score 600, top percentage 1.0 and minimum support 1. In the end, only ASVs that were identified to family, genus or species level were kept. Metazoan taxonomic affiliations were based on the GenBank search while all other affiliations were based on the DADA2 assignment using the PR2 database. If blastn search of the PR2 database gave low match or coverage, GenBank affiliation was used instead if family, genus or species level GenBank match was found. In addition, the ASVs that were identified to genus or family level were clustered into 97-% OTUs to represent proxies of species using vsearch v2.14.1 (Rognes et al., 2016). Finally, terrestrial species of Metazoa and Embryophyta were removed from the dataset. Four out of the eight control samples (negative reactions of MQH_2O and buffers from DNA extraction and purification) did not include any good-quality reads while three of the eight control samples included reads of species not present in the other samples (yeast *Malassezia restricta*, spider *Oecobius* sp., mite *Gamasina* sp. and an annelid worm). These species were removed from the dataset. One control sample included a single read of *Sabateria* sp. (Nematoda) that was abundant in the other samples as well, showing very low rate of tag jumps in the dataset. At site AS2 a single replicate per habitat recorded no sequences and thus these replicates were discarded. Sequence reads were normalized to relative abundance per sample.

2.4. Statistical tests

Counts from the conventional approach were converted into abundance (per m^2) and then standardised as relative abundances. In the eDNA approach only metazoan taxa were included. Sequence reads were converted to relative abundances. Taxa were first standardised to phylum or class level to allow direct comparisons. All analyses were performed in R 4.0.3 (R Core Team, 2021), unless expressly mentioned. Comparisons of sampling methods were made through Venn diagrams, drawn using ggVennDiagram (Gao et al., 2021). Replicates per sites were grouped and data were converted to presence/absence format. Within the Venn diagrams taxa were classified to class level. For assemblage compositions replicates per sites were grouped and plotted using ggplot2 (Wickham, 2016) with taxa classified to phylum level. Multiple Response Permutation Procedure (MRPP) ordination was used to analyse the differences between assemblages across country and site using the vegan package (Oksanen et al., 2022). As direct comparison between the separate approaches were not performed, taxa were classified down to lowest available taxonomic classification. Species and environmental variables (depth [m], salinity, and average *Fucus* thalli width [cm], height [cm] and wet weight [g]) with a significance level set at 0.05 were included within the plot. *Fucus* morphological measurements were acquired from the open access dataset: 10.6084/M9.FIGSHARE.19690930 (Preston and Rodil, 2022). Examination of the habitat (fixed factor, *Fucus* vs Bare) dissimilarity of the macroinfauna assemblages (i.e. species-specific abundance) across countries (fixed, Sweden vs Finland) and sites (random factor, three levels nested in country) was performed using non-parametric multivariate analyses of variance (PERMANOVA) based on the Bray-Curtis resemblance measure calculated from 4th-root transformed data (4999 unrestricted permutations, Type III). Epifauna assemblages were examined using only two factors (Country and sites). Changes in the macrofauna abundance (individuals per m^{-2}) and the number of taxa were analysed through a 3-way (macroinfauna) and a 2-way (epifauna) PERMANOVAs (same factors as above). Distance resemblance matrices were calculated using Euclidean dissimilarity measures based on non-transformed data (4999 unrestricted permutations). For the eDNA analyses, a matrix with the relative abundance of each macroinfauna species (normalized by presence/absence) was used to examine the habitat dissimilarity of the assemblages across countries and sites (same as above) based on the Bray-Curtis resemblance measure. Changes in the number of species (log (x+1)- transformed eDNA asv reads) were analysed through a 3-way PERMANOVA (same as above) using Euclidean dissimilarity resemblance matrix. Only significant effects ($p < 0.05$) were further investigated by pairwise comparisons. PERMANOVA analyses were performed using PRIMER7 (Anderson et al., 2008). Abundance (individuals per m^2 or sequences per site replicate) and species richness were plotted using ggplot2 (Wickham, 2016) for the macroinfaunal (both habitats), epifaunal, and eDNA samples. Additionally mean abundances (per m^2) for the two conventional approach sampling methods were combined per *Fucus* site and plotted alongside bare-sediment site abundances in ggplot2 (Wickham, 2016). Shannon-Wiener diversity indices were calculated for the combined conventional approach and eDNA using the vegan package (Oksanen et al., 2022) and plotted using ggplot2 (Wickham, 2016).

3. Results

3.1. The number of taxa using different approaches

The conventional approach collected 9737 specimens (*Fucus*: macroinfauna 337, epifauna 8808; bare-sediment: macroinfauna 592). The conventional approach returned average sample macroinfauna and epifauna abundances of 3493 and 1811 organisms per m^2 respectively. For the eDNA approach, 3.9 million reads (*Fucus* 1.9 million, bare-sediment 2 million) remained after quality control and merging the paired reads. Totals of 41 and 130 separate taxa were detected through the combined conventional or the eDNA approaches respectively. Venn diagrams showed that there was variation between the ability of the approaches to capture biodiversity (Fig. 2). However, the two

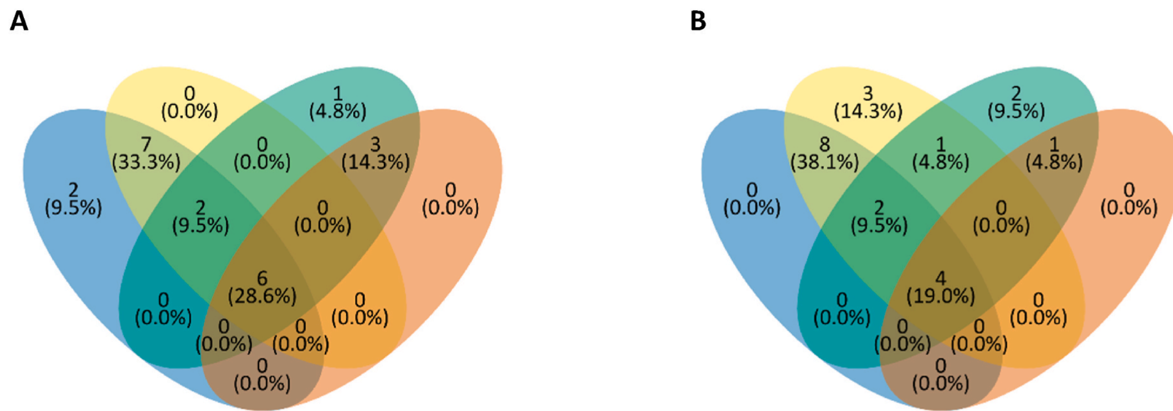

Fig. 2. Proportion of classes detected by conventional vs eDNA approaches across habitats for Askö (A) and Tvärminne (B). Colour key: blue, bare-sediment eDNA; yellow, *Fucus* eDNA; green, *Fucus* conventional; orange, bare-sediment conventional. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

approaches had considerable overlap in the classes detected (Askö 8, Tvärminne 7), although both approaches also detected exclusive taxa (eDNA: Askö 9, Tvärminne 11; conventional: Askö 3, Tvärminne 3). Overall the eDNA approach detected a larger amount of classes (Askö 17, Tvärminne 18) compared to the conventional approach (Askö 12, Tvärminne 10).

3.2. Assemblage composition

The sampling approaches determined relatively dissimilar biodiversity estimates with a greater similarity seen among samples from each approach compared to between habitat types with different approaches (Fig. 3A and B). The composition of assemblages was characterised by a high level of variability, with the conventional approach represented by three major phyla (Annelida, Arthropoda, and Mollusca) and the eDNA approach by four major phyla (Annelida, Arthropoda, Nematoda, and Platyhelminthes). Overall the conventional approach presented far simpler assemblage compositions with less variability between sites/habitat, detailing a more traditional description of community assemblages comparable to many previous studies (e.g. Rinne et al., 2022; Schagerström et al., 2014) (Fig. 3C and D). Several taxa with high detection rates in one approach were often undetected by the other (Fig. 3C, D, E, Supplementary material S2, S3). For example, *Semicytherura striata* and *Sabatieria* sp. were common within the eDNA approach but absent from the conventional approach. Of the taxa that were present within both approaches the detection rates varied considerably. For example, the Mollusca taxa (*Macoma balthica* and *Peringia/Hydrobia* spp.) and Annelida taxon (*Hediste* spp.) were frequently detected by the conventional approach, but poorly detected by the eDNA approach (Supplementary material S2, S3). In general, Mollusca were well represented by the conventional approach whilst being poorly represented or entirely absent from the majority of eDNA samples. Notability, a large proportion of the relative abundance captured by the eDNA approach was represented by taxa too small to be detected by the conventional approaches applied (e.g. Nematoda, Kinorhyncha, and Rotifera). Conversely, the approaches showed similar abilities to capture certain taxa (e.g. Arthropoda). Although relative abundances of Arthropoda in the eDNA approach were in general far larger. No taxa were clearly confined to each habitat, irrelevant of the approach used (Supplementary material S2, S3).

The assemblage composition was fairly similar in all sites in the conventional approach, although ordination was influenced by country (Fig. 4A, B, C). The eDNA approach also showed close ordination with high similarity between all sites (Fig. 4D and E). PERMANOVAs supported the trend of similarity between countries, showing no significant differences by country (macroinfauna: Pseudo-F = 2.38, P = 0.103; epifauna: Pseudo-F = 3.65, P = 0.103; eDNA: Pseudo-F = 1.36, P = 0.304; Supplementary material S4, S5). Within each country the number of exclusive taxa ranged from 17.1 to 36.9% of the observed taxa depending on the approach (Supplementary material S6, S7, S8). In the conventional dataset, the ordination of the two countries is partially influenced by groups of significant species, particularly in the case of epifauna assemblages (Fig. 4A, B, C). Both abiotic (depth, salinity) and biotic (thallus width, wet weight and height) environmental variables significantly influenced the ordination of MRPPs (Fig. 4).

Trends in biodiversity estimates were heterogenous, irrelevant of approach used, with the significance being influenced by habitat, country, and site (Figs. 4 and 5, Supplementary material S4, S5).The assemblages significantly differed by site, when nested within countries, for all approaches (epifauna: pseudo-F = 3.69, P(perm) 0.0001; macroinfauna: pseudo-F = 3.00, P(perm) = 0.0001; eDNA: pseudo-F = 3.80, P(perm) = 0.0001; Supplementary material S4). Habitat had significant effects on the assemblages and abundance of both approaches at differing scales. In the conventional approach, habitat had a significant effect on the assemblage and abundance of macroinfauna between sites when nested within country (pseudo-F = 1.66, P(perm) = 0.04; Supplementary material S4). For the eDNA approach, habitat significantly affected the assemblages and taxa between the two countries (Assemblages: pseudo-F = 2.33, P(perm) = 0.0318; Abundance: pseudo-F = 21.00, P(perm) = 0.0114; Supplementary material S4). Post hoc testing indicated that country was a significant effect on assemblages at Askö but not at Tvärminne (Askö: t = 1.85; P = 0.017; Tvärminne: t = 1.08; P = 0.399; Supplementary material S5).

Conventional and eDNA approaches provided dissimilar outlooks for the biodiversity estimates. For example, the conventional approach universally supported higher species richness, whereas the eDNA approach defined species richness by country (Fig. 5D, E, F, H). Additionally, high abundances within specific sites recorded by one approach were not reflected within the other approach (e.g. bare-sediment AS1 and AS3). Species diversities, in the form of the Shannon index, also contrasted by sampling approach (Fig. 5I). Using the conventional approach species diversity was greater in soft sediments associated with *Fucus* whilst the eDNA approach illustrated no link between species diversity and presence/absence of *Fucus*.

Unlike the eDNA approach the conventional approach combined two separate sampling methods to achieve the biodiversity estimates. Assemblages from the two methods shared taxa (e.g. *Chironomus* sp., Oligochaeta, and *M. baltica*) although exclusive taxa were also present (Fig. 3C and D). Generally, the methods provided comparable abundances for macroinfauna and epifauna assemblages, with the exception of two sites (bare-sediment AS1, TZ1) (Fig. 5A and B). Conversely, species richness was always higher in the epifauna compared to the

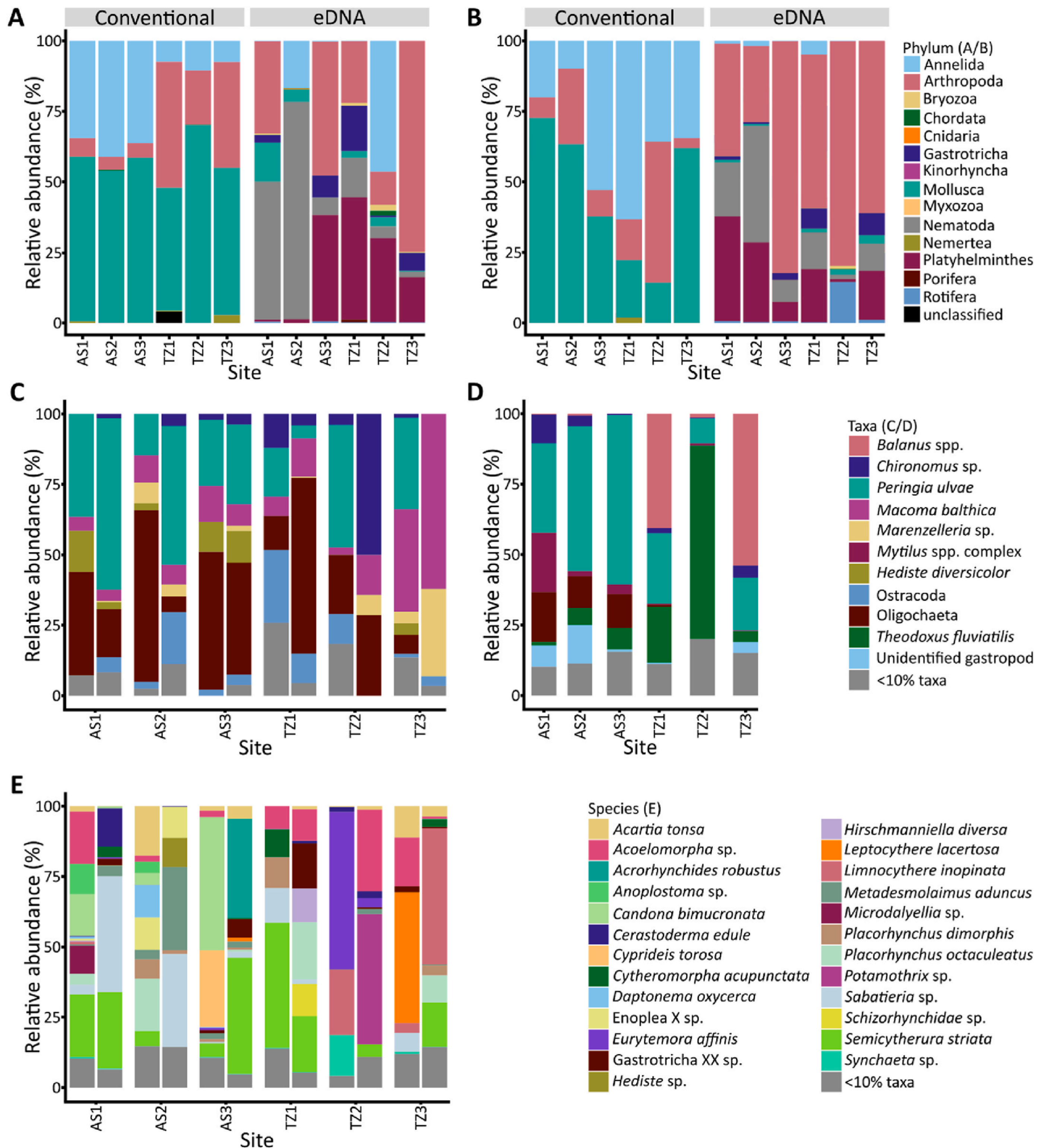

Fig. 3. Phylum-level metazoan diversity of *Fucus* (A) and bare sediment (B) habitats when using the conventional or eDNA approaches. Diversities to the lowest available taxonomic ranking of macroinfaunal (C) and epifaunal (D) assemblages when using the conventional approach and eDNA approach (E). *Fucus* (left) and bare sediment (right) habitat diversities plotted side by side (C) and only for *Fucus* habitats (D). In plots C and D taxa with a relative abundance <10 % grouped into a single category.

macroinfauna (Fig. 5D and E). Consequently, the additional epifauna assemblages within *Fucus* habitats elevated both the abundance and species richness to be higher than in the bare-sediment habitats with the exception of the abundance in the two aforementioned sites (AS1, TZ1) (Fig. 5C–F). The eDNA approach supported the trend of higher biodiversity estimates within the *Fucus* habitat at Tvärminne, but not at Askö. Overall, the two approaches provided discordant biodiversity estimates, although this appears to be partially a result of the complex and variable relationship between benthic metazoan diversity and habitat-forming algae in soft sediments.

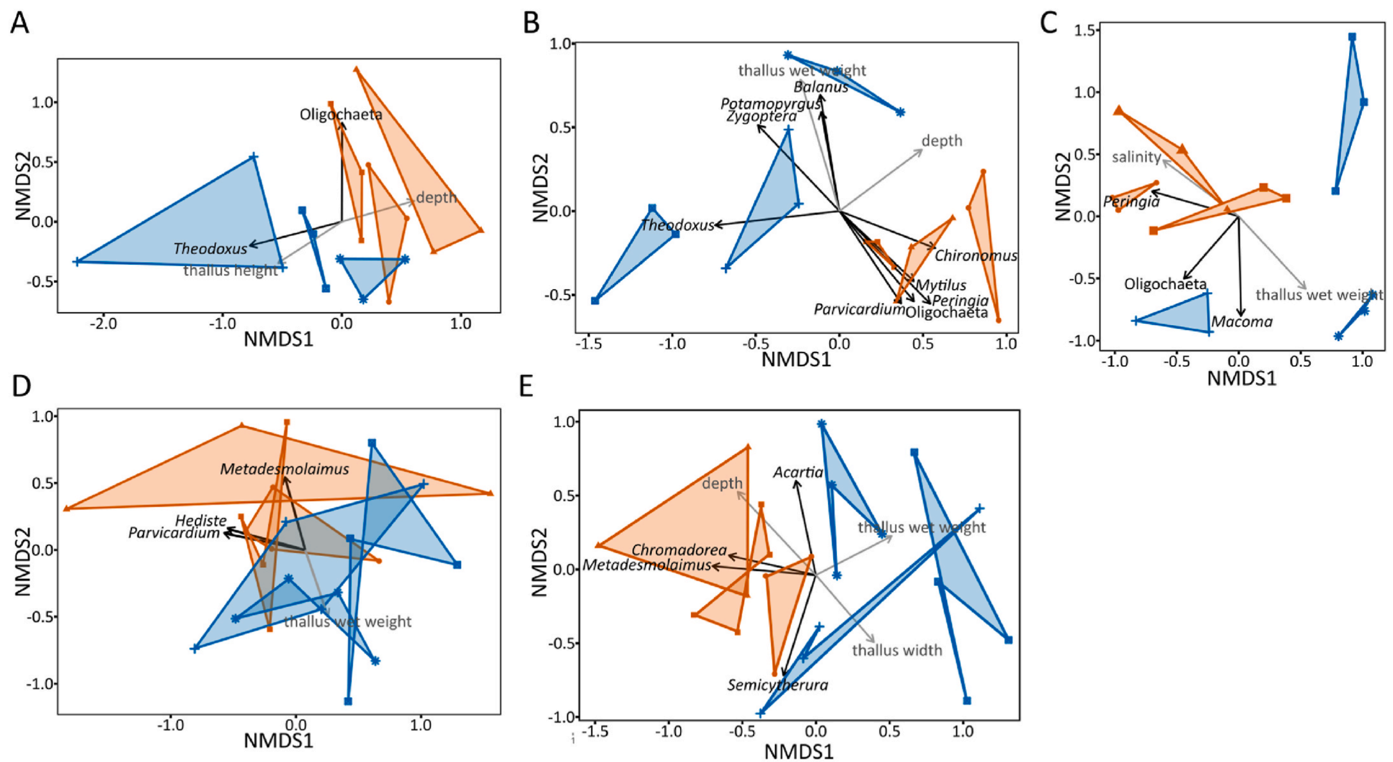

Fig. 4. Multiple Response Permutation Procedure (MRPP) ordination of taxa-specific abundance of the macroinfaunal (A, C), epifaunal (B), and eDNA (D, E) benthic assemblages of free-living *Fucus* (A, B, D) and bare sediment (C, E). Three (A, B, C) or four[1] (D, E) samples per site are shown. Coordinates are shown as Non-Metric Multidimensional Scaling (NMDS) ordination, based on the dissimilarity matrix between sites. Site abbreviation/colours: AS/orange, Askö; TZ/blue, Tvärminne. Site symbols: ●, AS1; ▲, AS2; ■, AS3; +, TZ1; ⊠, TZ2; ✳, TZ3. [1]eDNA sites with failed reactions represent 3 samples per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

This study shows that (i) *Fucus* had varying influence on the faunal benthic assemblages but appeared to increase species richness in many of the sites, and (ii) biodiversity estimates varied depending on the approach employed.

4.1. Influence of Fucus on faunal benthic diversity

The data provides new insights into the associated assemblages of free-living *Fucus*. Free-living *Fucus* supports similarly diverse macrofauna assemblages to the attached form, with abundant taxa including *Chironomus* sp., Oligochaeta, *P. ulvae*, and *T. fluviatilis* (Rinne et al., 2022; Schagerström et al., 2014). *Idotea* spp., a genus commonly associated with attached *Fucus* (Korpinen et al., 2007; Schagerström et al., 2014) were poorly detected through either approach, although *Idotea* spp. have also in cases been observed in very low abundances on attached *Fucus* (Rinne et al., 2022). Thus the absence of *Idotea* spp. may relate to stochastic changes (Engkvist et al., 2000; Kangas et al., 1982) or directly to the characteristics of the free-living form. Patterns of assemblages were highly variable, at both the small (site) and large (country) spatial scale, with few consistent trends being observed within and between sites and habitat types. Several exclusive taxa were observed for each country, as was expected due to the previously determined spatial patterns of benthic diversity (Zettler et al., 2014). Similarly to the attached form, different abiotic and biotic drivers may shape the associated assemblages (Rinne et al., 2022). Thus the high observed variability is likely due to the varying abiotic and biotic factors among the sites. For example, benthic assemblages are known to differ by depth (Orav et al., 2000), oxygen concentration (Lauringson and Kotta, 2006), eutrophication (Rinne et al., 2022), sediment type (Kotta and Orav, 2001; Mosbahi et al., 2016), thickness of algal cover (Lauringson and Kotta, 2006), algae thalli size (Schagerström et al., 2014), and algae thalli structural complexity (Cacabelos et al., 2010; Hansen et al., 2011; Kraufvelin et al., 2006). The free-living *Fucus* sites within this study differ abiotically, including in the recorded variables of salinity (5.9–6.1) and depth (max 1.9–3.4 m) but also in the morphotypes present at each site (Preston and Rodil, 2023), which may explain the lack of congruence. In several of the MRPPs depth was seen as a significant influence, with the sites with similar max depths grouping more closely (e.g. AS2/AS3 and TZ1/TZ2). Due to the often small patch size (~10–20 m^2) of free-living populations on mostly gently sloping, bottom gradients and the current poor understanding of their distribution, it is challenging to select sites to mitigate the influence of these varying abiotic conditions. Thallus morphology was also a significant factor in the MRPPs. Mitigating the influence of morphological variation is problematic because *Fucus* morphology is controlled both by the local environmental conditions and genetic characteristics (Preston and Rodil, 2023; Ruuskanen et al., 1999; Ruuskanen and Bäck, 1999). Thus the ability to select sites that represent convergent morphology is severely limited. Consequently the study highlights that the free-living sites were heterogeneous in nature.

Nevertheless when considering all sites using the conventional approach and Tvärminne using the eDNA approach, species richness was positively associated with *Fucus* as a result of additional epifaunal assemblages. Higher Shannon diversity was also observed in the *Fucus* sites of the conventional approach. Therefore it can be suggested that the presence of *Fucus* can positively influence biodiversity through creating a complex habitat which increases the number of niches and consequently supporting more species (Kostylev et al., 2005; Levin, 1992; Pianka, 2011). Similar trends have been observed within the Baltic Sea for various macrophyte communities including *Zostera marina* (Boström and Bonsdorff, 1997), *Furcellaria lumbricalis* (Kotta and Orav, 2001), and drift algae mats (Lauringson and Kotta, 2006). However, using eDNA,

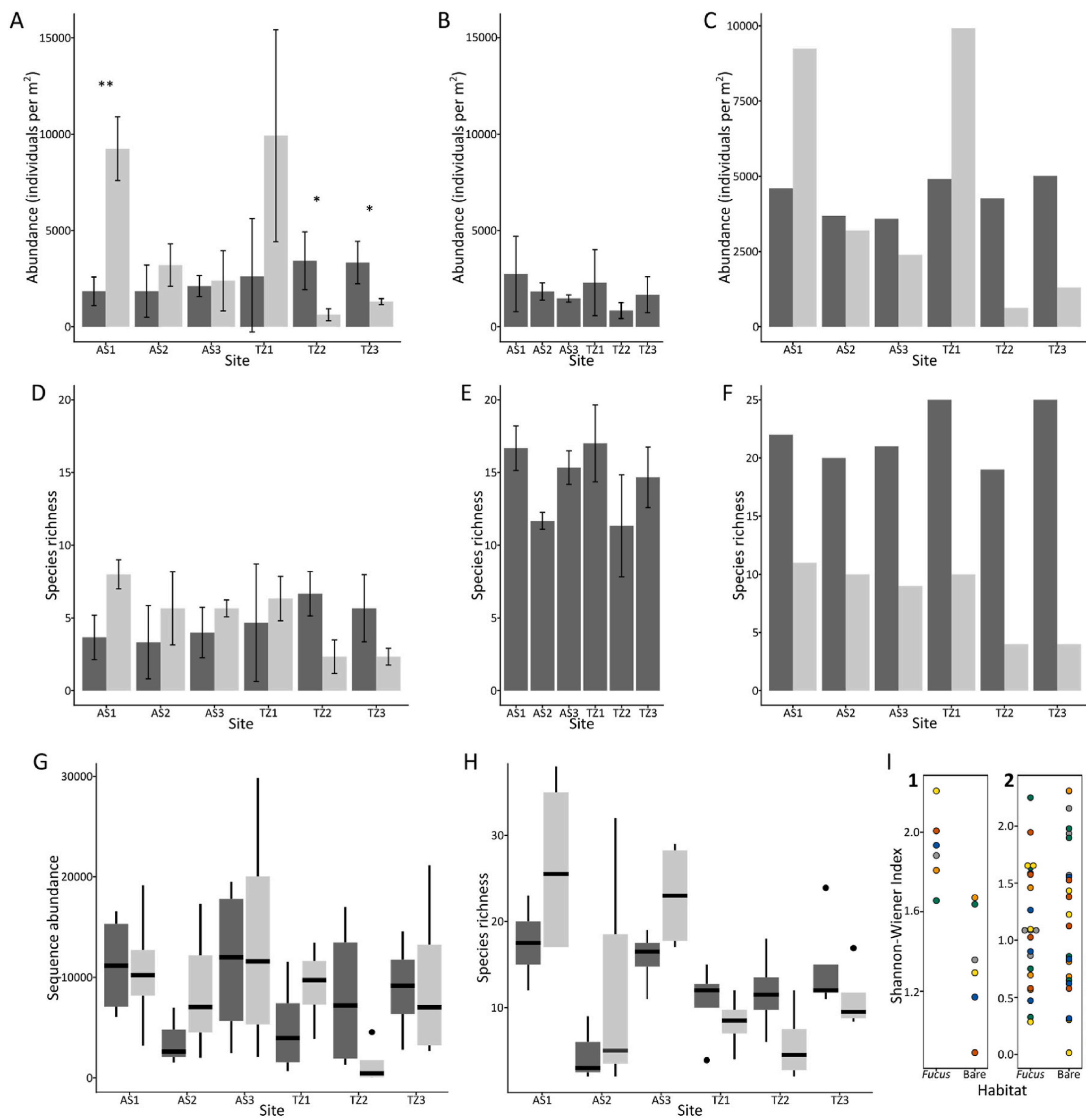

Fig. 5. Total abundance (A, B, C) and species (taxa) richness (D, E, F) of macroinfauna (A, D), epifauna (B, E), and the combined macroinfauna and epifauna communities (C, F) in samples from sites with and without free-living *Fucus* in Askö and Tvärminne using the conventional approach. Sequence abundance (G) and species richness (H) of benthic communities from sites with and without free-living *Fucus* in Askö and Tvärminne using eDNA. Shannon-Wiener diversity index (I) of habitat types by sampling approach: conventional (1), eDNA (2). Abbreviations: AS, Askö; TZ, Tvärminne. Colour codes: Dark grey, *Fucus* habitats; light grey, bare-sediment habitats. Significance levels of PERMANOVA post hoc tests between habitat types within sites.

this trend is not as apparent, with the mean species richness being higher within the three *Fucus* habitats at Tvärminne but lower within all sites at Askö compared to the bare-sediment habitats. Shannon diversities were also highly variable with no apparent trend relating to habitat type.

Fucus constrains the species richness and abundance of macroinfauna assemblages in four of the six sites. In fact, the bare-sediment habitats of these four sites showed far greater macroinfaunal abundances (e.g. a 5-fold difference at AS1) compared to the neighbouring *Fucus* habitat. A comparable macroalgae, *F. lumbricalis*, which also forms free-living populations throughout the Baltic Sea, has been found to demonstrate a similar trend of reducing macrozoobenthos (Kotta and Orav, 2001). However within attached *Fucus* canopies, abundance has been found to be higher in poor quality habitats despite the lower observed species diversity (Rinne et al., 2022). Consequently the relationship between algae habitat-formation and patterns of biodiversity is complex and partially depends on the biodiversity metric used. It appears that the presence of *Fucus* often has a filtering effect, exerting exclusionary pressure on organisms with traits suited to bare sediments. Overall, *Fucus* appears to frequently be detrimental to macroinfauna assemblages yet is able to alleviate some of the negative effects through the provision of habitat useable by epifaunal organisms. Although this trend is not universal since in two sites (TZ2, TZ3) *Fucus* greatly improves species richness and abundance of both the macroinfauna and epifauna assemblages.

4.2. Comparison of approaches

The two approaches illustrated discordant sensitivity in generating biodiversity estimates. It is an unavoidable characteristic of most biodiversity estimation approaches to produce imperfect taxa detection and consequentially introduce methodological bias (Ficetola et al., 2015; Tyre et al., 2003). Thus, the lack of congruence was expected. The conventional approach is largely dependent on detection probability, capture efficiency, and taxonomic enterprise. These often limit the

efficiency of biodiversity estimates, particularly with the current impeded taxonomic knowledge and persistent decline in taxonomic research (Hopkins and Freckleton, 2002; Kim and Byrne, 2006; Wheeler et al., 2004). Environmental DNA is a rapidly expanding field (Banerjee et al., 2021; Barnes and Turner, 2016; Pawlowski et al., 2020), but comes with its own set of challenges (Mathieu et al., 2020). The eDNA approach is contingent on detection probability, capture efficiency, extraction efficiency, sample interference, and assay sensitivity (Ficetola et al., 2015; Goldberg et al., 2016; Schultz and Lance, 2015).

The occurrence of false positives (detection of absent taxa) and false negatives (non-detection of present taxa) varies between approaches and characterises the limitations of each biodiversity estimate approach. Conventional approaches provides a real-time determination of taxa, with lower likelihood of detecting false positives (Eble et al., 2020). However false negatives can occur, especially in relation to taxa distribution and behaviour. Motile or sporadically and rarely occurring taxa can be misrepresented (Andrew and Mapstone, 1987; Eleftheriou, 2013; Majaneva et al., 2024) and the detection of small size meiobenthos, such as Nematoda and Platyhelminthes (Ojaveer et al., 2010), is also restricted by logistic and taxonomic constraints. Conversely, in the eDNA approach a positive-detection does not necessarily indicate that the taxa is currently present because the eDNA could have been transported over space or preserved over time, equally the non-detection of taxa does not automatically imply its absence (Roussel et al., 2015). In fact, false positives and negatives are common in eDNA (Beng and Corlett, 2020). Sediment-bound DNA can remain detectable for long periods of time (Turner et al., 2015), though the highly variable degradation rates of eDNA can lead to bias by certain taxa over temporal scales (Barnes et al., 2014; Beng and Corlett, 2020). Consequently, conventional approaches often provide higher sensitivity under shorter temporal scales whilst eDNA approaches are more suited to longer temporal scales. Spatial scales also differ by approach. The conventional approach represents a local (micro-)habitat, whereas eDNA has the potential to represent larger scales, particularly those habitats upstream of the direction of water movement (Deiner, et al., 2016). Environmental DNA can be transported over large spatial scales and can represent habitats outside of the sampled area (Deiner et al., 2016; Deiner and Altermatt, 2014). The presence of terrestrial species within the eDNA data suggests that signals from terrestrial habitats were recorded alongside the target habitat in this study. These approach characteristics can explain the relatively simple biodiversity estimates produced by the conventional approach, which resemble previous known assemblages of these habitats, whilst the eDNA approach produced more complex biodiversity estimates.

The conventional approach specifically targets taxa by size, whilst the eDNA approach employs taxonomically informative genetic markers specific to the target taxa. The conventional approach is therefore more limited in the taxa that it can detect (i.e. macrofauna [0.5 mm–5 cm]). This can be seen in the data with the simpler assemblage compositions detected by the conventional approach as well as with the detection of several meiofauna taxa within the eDNA data. However, the careful selection of genetic markers and molecular protocols is required to efficiently target taxa of interest in eDNA. Primer bias (preferential amplification of certain taxa), degraded DNA, PCR inhibition, PCR/sequencing induced errors, poorly defined pair merging and quality filtering, and the quality of reference databases can impede the accurately of taxa detection (Ficetola et al., 2015; Foster et al., 2022; Harper et al., 2019; Jane et al., 2015; Schrader et al., 2012; Stadhouders et al., 2010). Within the eDNA data, several signatures of these potential limitations were observed. Both *Chironomus* sp. and *Marenzelleria* sp. were abundant within the conventional data yet absent from the eDNA data. However, mismatches on the reverse primer for *Marenzelleria arctia* sequence suggested that *Marenzelleria* spp. were present yet limitations in the eDNA protocol hindered their detection. Likewise, the Baltic Sea *Chironomus* species (*Chironomus plumosus*) is known to represent a 450 bp long fragment while the sequence reads within this study were max 401 bp. Consequently *Chironomus* spp. reads were likely within the raw data but forward and reverse reads did not merge, hence the apparent absence. Similarly, the greater observed abundances of Arthropoda and the failure to detect higher abundances of Mollusca in the eDNA data both appear experimental artefacts of the eDNA process. Thus, both approaches describe a discrete portion of the entire benthic metazoan diversity, contingent on the limitations of each approach.

Overall, the discordant limitations of each approach resulted in the approaches illustrating differing biodiversity estimates, showing far greater similarity among biodiversity estimates within each approach irrelevant of habitat type, site, and country. Thus the use of either approach favours the selection of certain taxa whilst hindering others. Consequently, the two approaches are not directly comparable, yet the combined use can provide new insights into the biodiversity supported by these habitats at diverse scales. Although biodiversity estimates diverged by approach, both supported similar assemblages between the two habitats suggesting that the presence of *Fucus* does not exert extreme habitat filtering effects. Overall, this study highlights the necessity to consider the limitations of sampling approaches when generating biodiversity estimates; but also that biodiversity estimates can benefit greatly from a multi-approach design where both conventional and eDNA approaches are employed in complement.

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CRediT authorship contribution statement

Roxana Preston: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Markus Majaneva:** Writing – review & editing, Formal analysis. **Viivi Halonen:** Writing – review & editing, Investigation, Funding acquisition, Formal analysis. **Iván F. Rodil:** Writing – review & editing, Methodology, Investigation, Conceptualization.

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

Conventional and metabarcoding biodiversity data are available on Figshare: 10.6084/M9.FIGSHARE.20472768

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Appendix A. Supplementary data

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