Science of the Total Environment 901 (2023) 165991

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Using environmental DNA to better inform decision making around decommissioning alternatives for offshore oil and gas infrastructure

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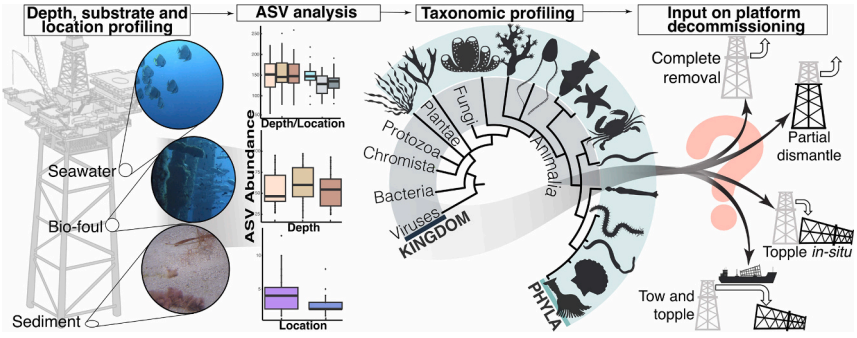
HIGHLIGHTS

- No substrate was able to holistically document the entire detected diversity on or off platforms.
- Taxonomic and taxonomy independent analyses showed similar trends.
- Database resolution was sufficient to inform on biodiversity trends.
- Highly variable results were observed between assay and substrates.
- eDNA methods successfully able to be scaled up to inform hypothetical decommissioning options.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Kyle Bibby

Keywords:
eDNA metabarcoding
Diversity detection
Marine diversity
Oil and gas platforms
Impact assessment
Gulf of Thailand

ABSTRACT

Artificial reefs are being utilised globally to aid in natural resource management, conservation, restoration or the creation of unique marine habitats. There is discussion around the optimal construction materials and designs for artificial reefs, the influences these have on biological communities, and the resulting ecological and social benefits. This discussion also includes the ecological value of repurposed marine infrastructure, such as decommissioned oil and gas platforms. Platforms often have an operational life spanning multiple decades, over which time they can develop extensive and unique community assemblages. The creation of artificial reefs by repurposing oil and gas platforms can have ecological, economic and sociological merit. However, with >12,000 platforms requiring decommissioning globally, there is the need for holistic assessment of biological communities associated with these platforms to inform the potential outcomes of different decommissioning options. We use environmental DNA metabarcoding (eDNA) of water, bio-foul and sediment samples to census broad eukaryotic diversity at eight platforms in the Gulf of Thailand (GoT) and five nearby soft sediment habitat locations. We sampled three target depths at sites (shallow, mid, deep) and detected 430 taxa at platforms, with higher diversity in shallow (near-surface) samples (313 taxa), compared to mid (30 m collection depth; 261 taxa) and

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https://doi.org/10.1016/j.scitotenv.2023.165991
Received 6 May 2023; Received in revised form 28 July 2023; Accepted 31 July 2023
Available online 2 August 2023

deep (50 m; 273 taxa). Three percent of taxa were shared among all depths at platforms with distinct assembles at each depth. Introduced species are an ongoing risk for platforms, however the eDNA detected no known introduced species. While the eDNA data provide broad taxon coverage and significant assemblage patterns, ongoing sampling innovation, assay design and local reference material still require development to obtain the maximum benefit of the technique. This study highlights the versatility and scalability of eDNA metabarcoding to holistically census marine infrastructure and inform the management and potential conservation of extant communities.

1. Introduction

Oil and gas platform jackets (hereafter termed 'platforms'), can provide habitat and refugia to a broad range of biotic diversity (Harvey et al., 2021; Kolian et al., 2017; Todd et al., 2020, 2018) and can also act as surrogate marine protected areas due to the enforcement of exclusion zones surrounding most operational structures (Alexander et al., 2022; Jagerroos and Krause, 2016). Once platform infrastructure has reached the end of operational life, it is typically decommissioned, which by accepted international guidelines and standards, has required the complete removal of all infrastructure (Techera and Chandler, 2015; Watson et al., 2023) resulting in the loss of the naturally accrued biotic diversity (Chandler et al., 2017; Fowler et al., 2014; Macreadie et al., 2011).

With an increasing number of artificial reefs being installed globally for the purposes of fisheries enhancement, conservation and habitat restoration, the repurposing of existing marine infrastructure is starting to be viewed as a viable alternative to the construction and installation of purpose-built artificial reefs (Elrick-Barr et al., 2022). The operational lifespan of marine oil and gas platforms can span decades with the infrastructure developing unique and significant biotic assemblages (Harvey et al., 2021; Rezek et al., 2018; Schutter et al., 2019; Torquato et al., 2017). Depending on the decommissioning strategy, these structures can provide ecological and socio-economic benefits either immediately, or with a reduced timeframe than purpose deployed artificial reefs (Marnane et al., 2022). Given this diversity, the removal of infrastructure from the marine environment may work against the principles of environmental management that aims to protect biological diversity (Fowler et al., 2014).

The Rigs-to-Reefs (hereafter RtR) framework explores alternatives to the complete removal of oil and gas infrastructure, in particular the conversion of platforms to artificial reef habitats. These alternatives are being considered and legislated in some jurisdictions on the condition that environmental and shipping safety concerns can be addressed (Fowler et al., 2018; Osmundsen and Tveterås, 2003; Techera and Chandler, 2015). The complete removal of infrastructure is still a viable option within the RtR framework, and can still preferred in some scenarios, such as in the presence of introduced populations. However, alternatives to this include the conversion of the infrastructure to permanent biotic refugia by toppling (laying the framework on its side), partial removal (cutting off the top section to facilitate safe shipping) of the structure in-situ in the original location, or the moving of the structure to a location where they are repurposed as an artificial reef, for example, in shallower water (Fowler et al., 2014; Macreadie et al., 2011). In some cases this can provide a socially acceptable alternative location to promote tourism (recreational fishing and SCUBA diving; Sommer et al., 2019) to natural reefs with positive economic outcomes (Elrick-Barr et al., 2022). However, literature on reefing processes and RtR conversions has focused on platforms within the Gulf of Mexico (with ~2900 active platforms; Sammarco et al., 2014) or the North Sea (Sommer et al., 2019).

Decommissioning decisions require information on the environmental and socioeconomic risks and benefits of each option. With an estimated 12,000 global offshore platforms requiring decommissioning (van Elden et al., 2019), it is unlikely that these structures would all be suitable, or required, for RtR conversion. Information on the biotic diversity, an assessment of the presence and implications of non-endemic or introduced marine species (IMS), and the presence of species of conservation significance can all inform the predicted outcomes of different decommissioning options. Biodiversity assessment methods used on oil and gas platforms have predominantly focused on identifying target groups, and have mostly focused on fish (Harvey et al., 2021; Love et al., 2020; Torquato et al., 2017) and invasive marine species (Braga et al., 2021; Page et al., 2006). Methods used have relied predominantly on morphological methods for identifying taxa, such as photographic sampling (Page et al., 2006), the analysis of video footage from remotely operated vehicle (ROV) (Harvey et al., 2021), SCUBA underwater visual counts (Consoli et al., 2013), or the morphological analysis of scraped or suctioned epibenthic fauna (Page et al., 2007).

Environmental DNA (hereafter 'eDNA'), which is defined by Taberlet et al. (2012) as any "*DNA that can be extracted from environmental samples (such as soil, water or air), without first isolating any target organisms*", shows promise as an alternative to holistically census and document platform biomes. Marine eDNA metabarcoding has been shown to be an effective tool in broad-scale diversity detection (Stat et al., 2017; Takahashi et al., 2023; West et al., 2021), censusing cryptic and low abundance taxa, including invasive marine species (Ardura et al., 2015; Bowers et al., 2021). It has been employed effectively as a stand-alone, or complementary method to traditional monitoring (Closek et al., 2019; Pearman et al., 2021). eDNA has been explored as a molecular approach for broadening survey focus, including sampling of oil and gas infrastructure (Alexander et al., 2022; Cordier et al., 2019; Laroche et al., 2017). This can be achieved by applying broad or "universal" metabarcoding assays, that amplify broadly across the taxonomic tree of life. Metabarcoding data are then cross referenced to databases to provide taxonomic resolution. Broader sampling of biota can also be attained by incorporating different sampling methods and target substrates (Alexander et al., 2023; Koziol et al., 2018) such as sediment, the water column, and epibenthic surfaces or bio-foul.

The Gulf of Thailand (GoT) is a diverse, relatively shallow (approximately 80 m maximum), tropical gulf bordering Thailand, Cambodia and Vietnam (Wattayakorn, 2006). The gulf is dominated by silt and clay substrate, but there is approximately 75 km^2 of coastal coral reef systems (Cheevaporn and Menasveta, 2003; Wattayakorn, 2012). Due to the diversity in habitat, the GoT has important ecological and fisheries value (Ahmed et al., 2007; Cheevaporn and Menasveta, 2003). However, it also has an extensive history of oil and gas extraction, with around 450 fixed installations currently in place (Thailand Department of Mineral Fuels, 2022; Tularak et al., 2007). Some of the infrastructure within the GoT is reaching the end of operational life, and will require decommissioning. Currently, there is no legal requirement for the censusing of existing communities occupying oil and gas infrastructure in Thailand (Fam et al., 2018). However, data on the presence or absence of species of interest, such as conservation significant species (such as rare, threatened or endangered), introduced species, or species of economic importance, such as fished species, can provide valuable information to predict the biodiversity outcomes of a range of decommissioning alternatives.

This study used eDNA methods to holistically assess the biotic composition of eight oil and gas platforms in the Gulf of Thailand, as well as natural benthic habitats, which provide a contrast for the diversity that might have existed prior to the installation of the platforms. Utilising a suite of assays, substrates and depth profiles, we holistically

explore the biotic diversity, with the specific aims of; (a) evaluating the ability of eDNA methods to differentiate broad and fine scale spatial changes in location and depth; (b) exploring the effectiveness of eDNA detections using the existing taxonomic frameworks, as well as independent of taxonomy, using amplicon sequence variants (ASVs) to investigate if current frameworks (both local species taxonomy and reference databases) are sufficient to inform biodiversity trends; and (c) assessing what taxa are driving community characteristics. We then explore and apply these results to determine if the level of resolution provided within this study is sufficient to inform the possible effects of different decommissioning strategies.

2. Methods

2.1. Study area

Eight platforms and five off-platform (OP) sites were sampled in March 2018. Platforms were located approximately 133 km east of Koh Samui in the Gulf of Thailand within a field of oil and gas infrastructure (see Fig. 1). OP sampling sites were located a minimum of 5 km from any subsea infrastructure. The eight platforms included seven four-legged structures and one three-legged structure. These platforms had been installed between 5 and 23 years prior to sampling and were selected as they were inactive at the time of sampling and targeted for upcoming decommissioning (supplementary Table S1). OP locations were selected and sampled as they represent the habitat composition prior to the installation of the platforms, but also theoretically, what the biotic composition will return to post decommissioning and the removal of the structures. All sample locations were between 133 and 163 km from the closest natural reef systems, and ranged between 61 m and 73 m deep.

2.2. Field sample collection

2.2.1. Water

A total of 156 water samples were collected at three depths, 0 m (surface), 30 m and 50 m below the surface, using a sterilised (10 % bleach solution) 1.7 L Niskin bottle, with three to four replicates taken at each depth (depending on the number of legs of the platform). Water samples were collected adjacent to corners of each platform and, at OP sites, to spatially mimic platform site replication. At each site the Niskin was opened, set and rinsed with surface water to remove excess bleach, and lowered to the required sampling depth. The Niskin was then closed remotely using a weighted 'messenger' before being retrieved to the vessel. On the vessel, the water sample was subsampled into bleached, pre-labelled bottles that were rinsed with reverse osmosis (RO) water to remove bleach. These samples were then refrigerated (4 °C) until filtration, which occurred on the research vessel within 4 h of collection. Filtration was completed using peristaltic Sentino pumps (Pall Life Sciences, USA) through 0.22 μm polyethersulfone membranes. All filtration equipment was sterilised in a 10 % bleach solution between filtrations for a minimum of 15 min, and rinsed with RO water, to minimise risk of cross contamination between samples. One litre control samples were collected and filtered for both the RO and bleach solution between each site to test efficacy of decontamination.

2.2.2. Bio-foul

Bio-foul is an accretion or accumulation of macro and micro-organisms on the surface of the marine infrastructure, such as platforms, which were not the primary purpose of placement of the infrastructure. Bio-foul samples were collected using a prototype aluminium scraper attached to a forward-facing manipulator arm on a work class ROV (Quasar 125 hp; SMD, UK) that was operated from the vessel. Ninety-six bio-foul samples were collected from each platform leg at a depth of 10 m, 30 m and 50 m using a medium sized. Samples of the ROV, which was scraped against the infrastructure to collect small

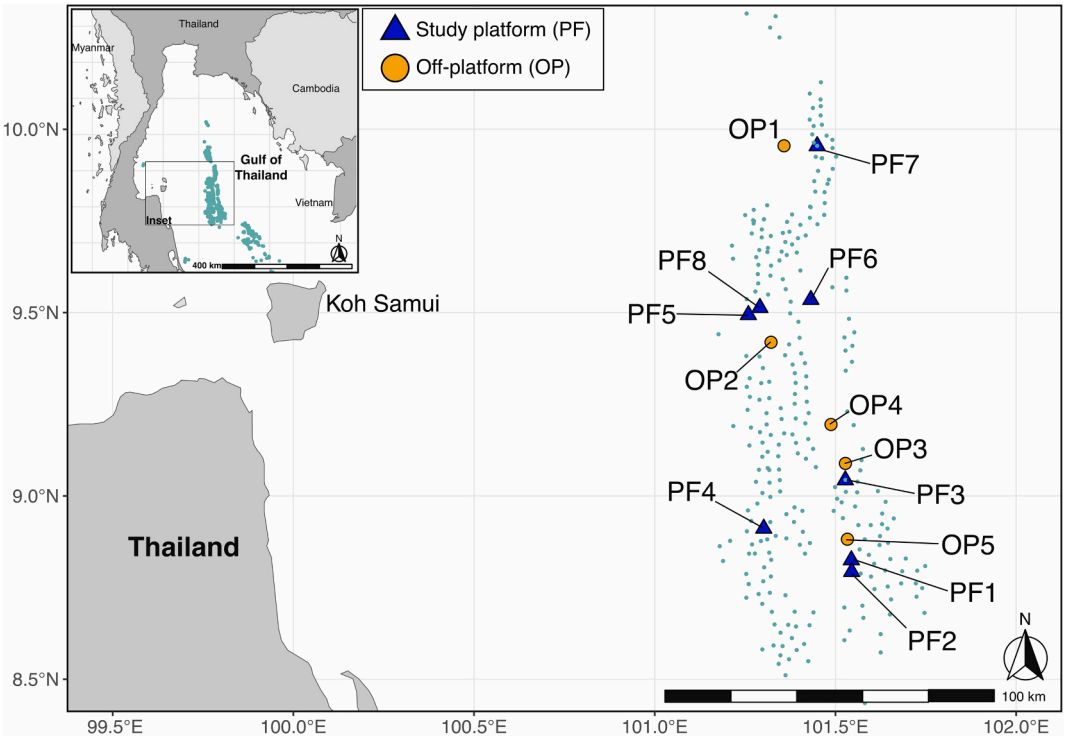


Fig. 1. Location of study area within the Gulf of Thailand (inset), as well as platforms (PF) and off-platform (OP) sites in relation to the closest land mass (Koh Samui) and the mainland Thailand coast. A combination of water, bio-foul and sediment were collected at both PF and OP sites. Teal-coloured points represent other oil and gas platforms not sampled in the current study.

fragments of the bio-foul. Each sample was transferred into individual mesh bags on a collection tray before collecting the next sample. While not logistically feasible to sterilise the prototype scraper between each sample on a platform, the scrapers and bags were sterilised prior to sampling and between ROV dives (with two dives completed at each platform) and platforms. Bio-foul samples were homogenised using an Omni TH (OMNI International, USA) and approximately 30-50 mL of the homogenised sample was placed in a 50 mL falcon tube.

2.2.3. Sediment

Sediment samples were collected using a small (3.5 L) Ekman grab within 50 m of each platform leg. The sterilised Eckman grab's jaws were opened and the grab was lowered from the vessel to the seafloor and triggered via a messenger to close. Upon retrieval to the vessel, the top doors of the Ekman grab were opened and sediment was sampled from approximately 5 locations among the top few millimetres of sediment within the Ekman grab using a sterilised and rinsed spoon, resulting in approximately 30–50 g of sediment in total at each sample. A total of 52 sediment samples were collected, which were placed in 50 mL falcon tubes. Between samples, the Ekman grab and subsampling equipment were thoroughly cleaned and sterilised using a 10 % bleach solution for a minimum of 15 min, and rinsed with RO water prior to re-deployment. OP sites were sampled to spatially mimic sampling at platforms.

After collection, all eDNA samples were stored in uniquely labelled Ziplock bags at −20 °C until transport back to the laboratory. Samples were transported on dry ice under a non-prohibited goods permit (number 0001530842).

2.3. Laboratory workflow

2.3.1. DNA extraction

Environmental DNA samples were extracted in dedicated clean laboratories. All equipment used during the digestion and extraction processes were soaked for a minimum of 15 min in a 10 % bleach solution prior to use, and irradiated for 15 min using a UV oven, and all equipment re-bleached between samples. To determine sterilisation efficacy and detect potential cross-contamination, digestion, extraction and non-template controls were taken with each batch.

DNA digestion for both water and bio-foul samples followed the DNeasy Blood and Tissue (Qiagen; Netherlands) protocol. Water filters were defrosted, dissected in half using bleached scissors in a dedicated clean laboratory, with half returned to storage in −20 °C as backup and for archiving purposes. The remaining half was further dissected and incubated overnight (minimum of 12 h) in a solution 540:60 μl ratio of ATL buffer and Proteinase K. Bio-foul samples were homogenised and tissue lysed using a TissueLyser II (Qiagen; Netherlands) in 30 s intervals for 90 to 180 s (sample dependant), on a 30 Hz setting. Following homogenisation, approximately 140 mg of sample was combined in a solution of 1260:140 μl ratio of ATL buffer and Proteinase K and digested overnight. After digestion, all water and bio-foul digests were extracted using a custom eDNA protocol on a QIACube platform (Qiagen; Netherlands). Sediment samples were homogenised with a TissueLyser II (settings: 20 Hz for 120 s in 30 s intervals) and then extracted manually, containing 250 mg of sample, using a DNeasy Powersoil extraction (Qiagen; Netherlands) protocol. All extraction resulted in approximately 100 μl of extract in AE buffer.

2.3.2. Tagged amplification and sequencing

Assays were selected from scientific literature to provide a broad coverage of biotic diversity, which could be analysed as taxonomy dependent and independent (using amplicon sequence variants, or ASVs). A broad "universal" assay was selected and applied to all samples to provide a taxonomic baseline comparison between target substrates, as drawing comparisons between assays targeting different barcode regions can be challenging. Specialised assays targeting hard coral, fish, elasmobranchs (sharks and rays), molluscs and crustaceans were applied to bio-foul and/or water samples (Table 1). These target assays were applied to samples from substrates to maximise detection, as such the targets were not applied unanimously across substrates as, for example, fish detections were perceived to be low in bio-foul and sediment samples. To mitigate paucity in reference material, assays were selected targeting varied barcode regions, including the mitochondrial CO1 region, mitochondrial 16S rRNA, and the nuclear ribosomal ITS2 region. Two of the assays were multiplexed comprising PCR with either two forward (elasmobranch assay; West et al., 2021) or reverse (hard coral, or Scleractinia) PCR primers (Table 1).

Multiplex identifier tags, consisting of 6 to 8 bp, were assigned in unique combination to each sample to allow sequences to be bioinformatically assigned back to a sample. Initially, samples were explored via (untagged) PCR using neat and 1/10 dilutions to determine the optimal DNA input to progress with identifier tags (Murray et al., 2015). Both exploratory and final PCR were completed on a StepOnePlus Instrument (Applied Biosystems) with an initial denaturation stage of 95 °C for 5 min; followed by 45 (exploratory PCR) or 50 (final tagged PCR) cycles of; 95 °C for 30 s, followed by 30 s of the assay specific annealing temperature; 72 °C for 45 s; and a final extension stage at 72 °C for 10 min. The PCR master mix comprised a total 25 μl of 2.5 mM $MgCl_2$ (Applied Biosystems; USA), 10× PCR Gold buffer (Applied Biosystems), 0.25 mM dNTPs (Astral Scientific; Australia), 0.4 mg/mL bovine serum albumin (Fisher Biotec; Australia), 0.4 μmol/L forward and reverse primers, 0.6 μL of a 1:10,000 solution of SYBR Green dye (Life Technologies; USA), and AmpliTaq Gold DNA polymerase (Applied Biosystems), with tagged PCR completed in duplicate.

The indexed duplicates were combined if the amplification curves, melt plots and ΔRn values were similar, otherwise minipools were formed with only the optimal reaction. Minipools were blended based on equi-molar ratios of the amplification ΔRn values with no more than seven samples included in each minipool. All minipools were quantified (Qubit 4.0 Fluorometer; Invitrogen) and amplicon peaks visualised (Qiaxcel; QIAgen) before being blended into a single library based on equimolar values. This library was then size selected using a Pippen Prep instrument (Sage Sciences, USA) to exclude erroneous amplicons. Sequencing was completed on a Miseq platform (Illumina, USA), with custom sequencing primers, using 500-cycle V2 (paired-end) and 300 cycle V2 (single-end) kits, using a Q-score threshold of Q30.

2.4. Bioinformatics and analysis

Raw sequence files were downloaded directly from the online Illumina Sequence Hub. Where feasible, raw sequence files from the same assay and substrate type were concatenated and processed through the bioinformatic workflow as one file to avoid the replication of ASVs within datasets. The demultiplexing and deconvolution of both paired and single-end sequence files were processed in R (v3.6.3; R Core Team, 2020) on RStudio (v1.2.5042; RStudio Team, 2020) using the package Insect (v1.4.0.9000; Wilkinson et al., 2018), and efficacy verified using the cutadapt package (v3.7; Martin, 2011). Quality filtering (maxN = 0, truncQ = 2, maxEE = 2 and a minimum amplicon length of 50 bp) was completed using the dada2 (v1.8.0; Callahan et al., 2016) pipeline in R, which was then subsequently used to merge paired-end reads, then identify and remove chimeric sequences. The resulting ASVs were queried against publicly available reference material from the National Center for Biological Information's (NCBIs) GenBank Nucleotide Database, which was accessed in May 2022. A 100 % coverage was required, as well as an e-value of 1e-3 and a 90 % minimum percent identity in order to return a maximum of 10 taxonomic assignments. Species level taxonomic assignments required a minimum 98 % identity match and were taxonomy assigned based on the lowest common ancestor (LCA) using the Python script within the eDNAFlow automated workflow (Mousavi-Derazmahalleh et al., 2021). Taxonomic assignments were manually vetted back against the initial blast results, known distributions and against publicly available databases, World Register of Marine

Table 1
PCR primers applied to water filters, bio foul and sediment eDNA collections from platform and sediment sites in the Gulf of Thailand. These assays were selected to detect broad eukaryotic diversity.

Assay name (target taxa)	PCR primers (Reference)	Target barcode	Sequence (5′-3′)	Amplicon range (bp)	Annealing temp (°C)	Substrate		
						Water	Bio-foul	Sediment
CO1 universal (broad target)	mlCOIintF[a] (Leray et al., 2013)	CO1	GGWACWGGWTGAACWGTWTAYCCYCC	313	46	Y	Y	Y
	jgHCO2198_R (Geller et al., 2013)		TANACYTCNGGRTGNCCRAARAAYCA					
Coral[b] (Scleractinia)	SCLER5.8SForw[a] (Brian et al., 2019)	ITS2	GARTCTTTGAACGCAAATGGC	220–440	55	Y	Y	
	SCLER28SRev (Brian et al., 2019)		GCTTATTAATATGCTTAAATTCAGCG					
	CoralAcro_874Rev (Alexander et al., 2019)		TCGCCGTTACTGAGGGAATC					
Fish (fish)	16SF[a] (Berry et al., 2017)	16S	GACCCTATGGAGCTTTAGAC	200	54	Y		
	16S2R-degen. (Deagle et al., 2007)		CGCTGTTATCCCTADRGTAACT					
Elasmobranch[b] (sharks and rays)	FishF1-degen[a] (West et al., 2020)	CO1	ACCAACCACAAAGANATNGGCAC	110–240	52	Y		
	FishF2-degen[a] (Fields et al., 2015)		TCNACNAATCATAAAGATATCGGCAC					
	Shark COI-MINIR-degen (West et al., 2020)		GATTATTACNAAAGCNTGGGC					
Crustacea (Crustacea)	Crust16S_F (short)[a] (Berry et al., 2017)	16S	GGGACGATAAGACCCTATA	150–170	51		Y	
	Crust16S_R (short) (Berry et al., 2017)		ATTACGCTGTTATCCCTAAAG					
Mollusca (molluscs)	Limacina_F[a] (Berry et al., 2019)	CO1	TAATTGGNGGVTTTGGRAAYTG	118	52		Y	Y[c]
	Limacina_R (Berry et al., 2019)		GTTCAHCCTRAYCCTRCNCC					

[a] Indicates forward primer.
[b] Multiplexed assay with either two forward or reverse primers.
[c] Substrate/assay combination failed to amplify in PCR and was not proceeded through sequencing.

Species (WoRMS; accessed August 2022; WoRMS Editorial Board, 2022) and FishBase (accessed September 2022; Froese and Pauly, 2022). Results from field or laboratory controls were removed manually across that assay or sequence dataset, as indicated. Finally, reads were filtered by relative abundance with a 0.1 % threshold using the R packages Phyloseq (v1.28.0; McMurdie and Holmes, 2013) and Vegan (v2.5.7; Oksanen et al., 2020), and data merged to form one dataset using the "merge_phyloseq" function in Phyloseq. All resulting samples with no reads were removed. Sampling effectiveness was explored using the BiodiversityR (Kindt and Coe, 2005) package within RStudio. This analysis was completed at the ASV level within each substrate for all assays to determine if asymptote had been reached or to extrapolate what sampling effort was required.

2.5. Data exploration and multivariate analysis

All analyses were completed on presence/absence transformed data, as read abundance is not analogous with individual abundance and can be impacted by environmental conditions such as trophic interactions, season, and water movement. The overall differences in assemblage composition were explored at the ASVs level to capture data from taxa that were missing from the GenBank reference database. This taxonomy independent approach was explored using the Primer 7 software plus PERMANOVA+ add on (Anderson et al., 2008; Clarke and Gorley, 2015) with each method, water, bio foul and sediment, analysed independently. A fixed, two-factor PERMANOVA analysis was completed on Location (platform and OP) and Depth factors (0 m, 30 m and 50 m) on water samples, with pair-wise analyses completed on both Location and Depth. Fixed design PERMANOVA analyses were also completed on Depth (10 m, 30 m and 50 m) for bio foul samples and Location for sediment samples. All PERMANOVA analyses were completed on Jaccard similarity matrices with 9999 permutations. ASV detection composition was further visualised by the above factors using non-metric MDS plots, which were bootstrapped to reduce variability within factors. Indicator species were explored for the above factors using the packages labdsv (v2.0.1; David W. Roberts, 2019) and indicspecies (v1.7.12; De Cacares and Jansen, 2016) completed on RStudio. The dataset was then collapsed at the species taxonomic level for taxonomic dependant analysis and the same PERMANOVA analysis re-run to determine if database resolution for the region impacts analysis. With focus on the diversity at platforms only, an analysis of similarity percentages was completed on combined depth profiles, shallow, mid, deep and sediment, which was completed using the SIMPER function on the Primer 7 software, on Jaccard similarity matrix. A phylogenetic tree was produced using the taxonomy from NCBI through the "phyloT" website (https://phylot.biobyte.de/).

3. Results

3.1. Sequencing results and metrics

A total of 143,569,001 raw reads were returned from sequencing across all assays and methods employed (Supplementary Section S2 and Table S3). On completion of demultiplexing, quality filtering, the merging of paired-end sequences, and the removal of chimeric sequences, mean reads per sample were 74,062 (±SE 4010), however varied by assay. The Crustacea and Mollusca assays (bio-foul samples) had the highest mean read abundance, comprising (106,026 ± 4579 and 120,985 ± 3112 respectively), with the elasmobranch assay the lowest read abundance per sample (39,124 ± 2113). Mean quality filtered reads per substrate using the CO1 universal assay, the only assay applied to samples of all three substrata, ranged from 51,997 (±1886; sediment) to 79,207 (±3466; water; Supplementary Table S3).

Contamination from field and laboratory workflows were removed

from subsequent analysis, however contamination ASVs varied by assay. ASVs that were identified as contamination and removed included non-target taxa such as bacterial and unassigned eukaryotes (from CO1 universal, Mollusca, elasmobranch and coral assays). Additionally, target ASVs that were identified from field and laboratory controls belonging to the species *Ostorhinchus semilineatus* (half-lined cardinal, phyla Chordata; 1 ASV, 19,199 reads, 0.6 % of quality filtered reads), the anchovy family Engraulidae (phyla Chordata; 26 ASVs, 101 reads, <0.1 %) were removed (fish assay), *Petroscirtes* sp. (phyla Chordata; 1 ASV, 12 reads, <0.1 %; elasmobranch assa) and *Urostyla grandis* (phyla Ciliophora; 8 ASVs, 29,994 reads, 0.8 %; coral assay). In addition, ASVs aligning to species that were used as positive controls in laboratory workflow, namely *Menippe mercenaria* (phyla Arthropoda; 2 ASVs, 109 reads; CO1 universal), *Homarus americanus* (phyla Arthropoda; 1 ASV, 2 reads; Crustacea), and *Plesiastrea versipora* (phyla Cnidaria; 1 ASV, 23 reads; coral assay) were detected in some laboratory controls. Lastly ASVs that aligned to known non-marine species were omitted, which included the species *Gallus gallus* (chicken), *Homo sapiens* (human), the genus *Ovis* (likely sheep), where DNA likely resulted from waste due to proximity to vessels and platforms.

Analysis of sampling effort by assay determined that Mollusca (bio-foul), CO1 universal and coral (water and bio-foul) were sufficient to detect 90 % of the ASV diversity (Supplementary Fig. S4), however for remaining assays additional sampling would have resulted in increased ASV diversity. Similarly, the success of each assay to assign ASVs to taxonomy was highly variable, which is reflective of the resolution of reference databases for the GoT, which is an understudied region. Combined, 25 % of ASVs were assigned to species taxonomic level and ranged from 12 % (coral) to 67 % (Crustacea; Supplementary Fig. S5). The coral assay was unable to assign 69 % of ASVs past kingdom.

3.2. Location comparison

Overall, 462 taxa were identified overall with 236 resolved to species level, 146 at genera and a further 46 at family, and the remaining 34 taxa aligning at a higher taxonomic level. A total of 431 (of which 216 were species-level) taxa were detected at platforms and 160 (87 species) at OP locations (Fig. 2). The PERMANOVA showed significance across all factors (Location, Depth and Location x Depth) in a (substrate) combined, and within bio-foul and sediment substrates (supplementary Tables S6). The factor Location X Depth within the water substrate was non-significant (Pseudo-F = 0.9, P (perm) = 0.734, Unique Perms = 9830). Pairwise analysis on combined species level data showed similarities in assemblages at both locations between shallow and mid, and mid and deep, however within the individual substrates, pairwise tests were significant (supplementary Tables S7). Taxonomic diversity varied

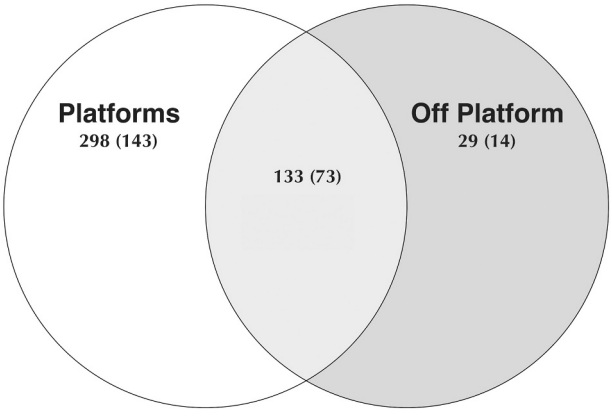


Fig. 2. Comparison of taxa detected at both platform and OP locations across all sampling methods and assays. Numbers in brackets indicate species level designations.

across substrates at each location (platform and OP). A higher mean diversity per sample was detected at platforms compared to OP locations in both water samples (platforms: 25.4 ± SE 0.6- total taxa: 214; OP: 23.4 ± 0.6, total taxa: 155), as well as sediment samples (platforms: 3.4 ± 0.4- total taxa: 33; and OP: 2.2 ± 0.2- total taxa: 14). At platforms, bio-foul had a mean of 26.4 (± 0.7- total taxa: 250) taxa per sample.

3.3. Platform diversity

Higher species diversity was detected in shallow samples at platforms (313 taxa), compared to deep (273) and mid (261; Fig. 3). Three percent of taxa were shared among all depths at platforms, whereas 36 % of species level taxa were common to shallow, mid and deep samples. Overall diversity comprised seven kingdoms and 33 phyla (Fig. 3).

Shallow bio-foul and water samples were dominated by zooplanktonic copepods (contributing 10 %), phytoplankton (Chromista and Chlorophyta contributing 22 %), and benthic species (contributing 12 %; see SIMPER analysis supplementary Table S8), with shallow samples displaying and average percent similarity of 24 %. Planktonic species increased in percentage dominance with depth (mid 36 %, deep 39 %) whereas benthic fauna dominance decreased (mid 11 %, deep 8 %). INDVAL analyses completed on depths revealed shallow samples were characterised by 28 taxa, 26 from the phyla Animalia including reef associated fish species *Atherinomorus lacunosus* (wide-banded hardyhead silverside), *Scomberoides tol* (needlescaled queenfish) and *Selar crumenophthalmus* (Bigeye scad), as well as brittle stars (phyla Echinodermata; *Ophiactis savignyi* and *Ophiactis modesta*), amphipods (Arthropoda; *Elasmopus nkjaf* and *Stenothoe nhatrangensis*). Mid samples were characterised more by benthic diversity with five of the six taxa identified, including polychaete worms (Annelida; *Phyllochaetopterus ramosus* and *Phyllochaetopterus* sp.), sponges (Porifera; *Crambe crambe* and *Tedania* sp.) and soft coral (Alcyonacea; *Dendronephthya* sp.). Twenty-one taxa characterised deeper samples, which were predominantly benthic, comprising sponges, *Ophlitaspongia papilla*, oysters (Mollusca *Hyotissa hyotis*), soft coral (*Carijoa riisei*), ascidians (Chordata; *Ascidia ahodori*) and polychaetes (Annelida; *Dipolydora armata*), however also planktonic jellyfish (phyla Cnidaria; *Nausithoe punctata*) and copepods (Arthropoda; *Clausocalanus minor*). Sediment samples were dominated by meiofauna, *Terschellingia longicaudata* and *Terschellingia* sp. (Nematoda) and protozoan, *Cunea thuwala* (Discosea).

3.4. ASV assemblage composition

By restricting eDNA data to groups that have good representation on reference databases, it is possible that patterns and insights are lost. Accordingly, taxonomy independent analysis on all samples (platform and OP sites) revealed a total of 3112 ASVs detected from all substrates, including 2261 from water samples (mean per sample 145 ± SE 3), 883 from bio-foul (56 ± 2), and 62 ASVs from sediment (4 ± 3). ASV diversity was higher at platforms in both water (151 ± 4) and sediment (4 ± 0.5) samples, in relation to OP samples which comprised 136 (±4) and 3 (±0.4) respectively. Assemblage composition at platforms differed with depth for both water and bio-foul samples, with mid depth having a higher mean ASV diversity (61 ± 4) than deep (54 ± 4) and shallow (49 ± 4), compared to depth profile in water samples which had the highest diversity in shallow samples, deep, and then mid, which was a trend mirrored in OP depth profiles (Fig. 4).

PERMANOVA analysis showed that the detected assemblages of ASVs were non-significant across the factors Location X Depth (Pseudo-F = 1.123, P (perm) = 0.105, Unique Perms = 9708; Supplementary Table S9) within the water dataset, with factors Location and Depth both significant. Fixed factor PERMANOVA for sediment (Location only) and bio-foul (Depth only) were significant (Supplementary Table S7).

The ASVs that characterised a location or depth within each substrate were characterised by indicator species analysis. Although most indicator ASVs were unassigned at the species level, some species were

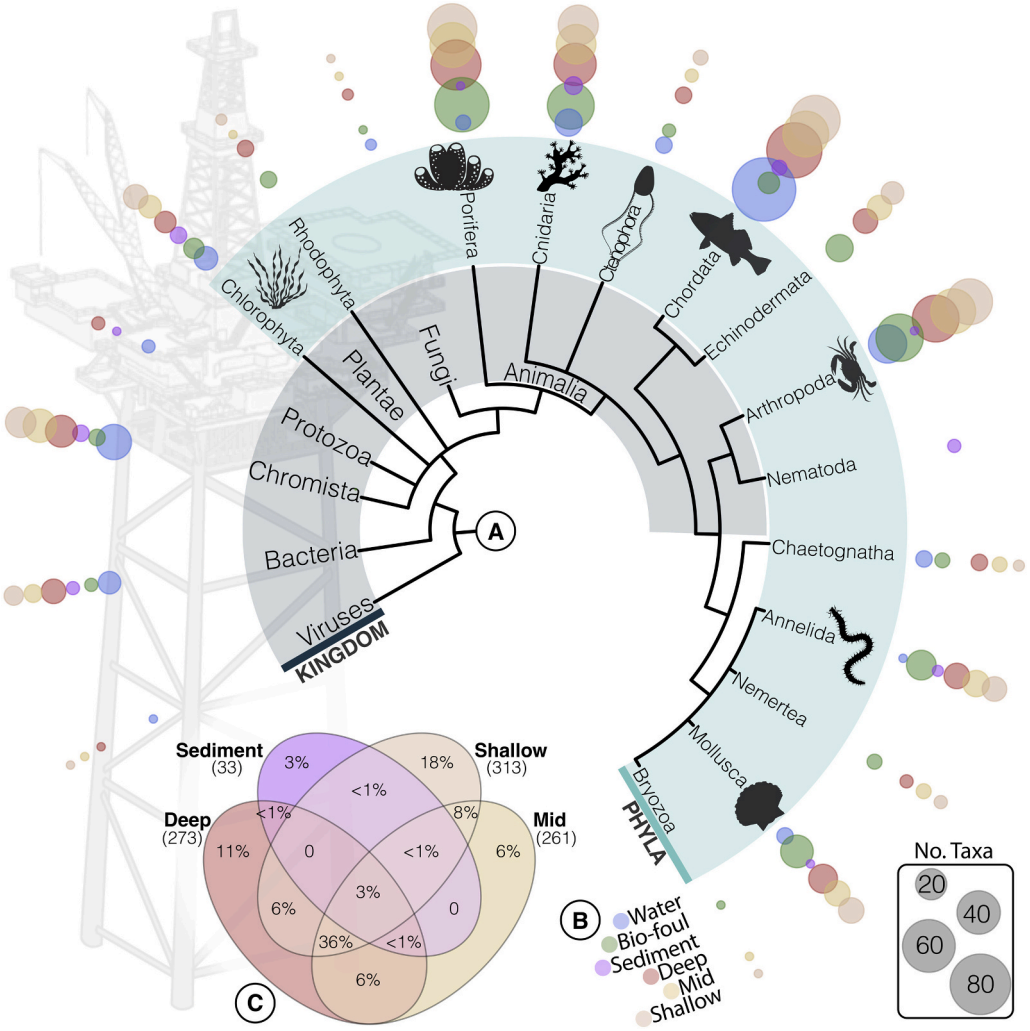


Fig. 3. Biotic kingdoms detected from eight platforms within the Gulf of Thailand, with further phyla level breakdown of detected Plantae and Animalia (A). Circles indicate the number of species level taxa detected per group from each substrate and also each depth (combined water and bio-foul) sampled (shallow, mid and deep) (B), and Venn diagram showing the percentage of taxa common to all depths at platforms utilised in this study (C).

identified driving differences in substrate detections (Table 2). Platform water samples were characterised by fish and zooplankton species (all within the genera *Calocalanus*), whereas deeper samples were characterised by phytoplanktonic groups, with 13 ASVs aligning to the algal species *Micromonas commoda*, and a further 19 ASV aligning to four species within Chromista (Table 2). Bio-foul samples were characterised by known fouling and epibenthic associated fauna such as amphipods (*Elasmopus nkjaf* and *Stenothoe nhatrangensis*), brittlestars (genus *Ophiactis*), annelids. Filter feeders, such as sponges (phyla Porifera), oyster (*Hyotissa hyotis*) and three ASVs aligning to soft coral (*Carijoa riisei*), dominated mid and deep bio-foul sample characterisation. While no indicator species, or ASVs, were identified from sediment OP sites. However, a phytoplanktonic chromist (*Pelagomonas calceolata*) characterised sediment samples associated with platforms, as well as the only meiofaunic nematode species detected, *Terschellingia longicaudata*. In OP water samples, INDVAL analysis identified fewer significant species, however a greater number of indicators unable to be aligned below kingdom or phyla at each depth. Similar to platforms, shallow and mid depths at OP sites were dominated by zooplanktonic copepods, in addition a sponge species, *Tethya seychellensis*, was also identified. Similarly, phytoplankton (Chromista and the Plantae phyla Chlorophyta) were characteristic of deeper OP samples.

4. Discussion

Using eDNA metabarcoding, we documented a broad eukaryotic diversity at oil and gas platforms in the highly diverse, Gulf of Thailand, and revealed differences in assemblages among substrates, depths and locations. We demonstrate that platforms had developed complex biotic communities associated with the vertical relief of the infrastructure, a result reflected in both taxonomic and taxonomy-independent analysis. This study also shows the taxonomic scalability of eDNA methods over conventional sampling, which often target specific taxa or assemblage components. In the complex, tropical community of the GoT, multiple assemblage components can be investigated through eDNA sampling with careful assay selection and analysis. This holistic, multi-substrate, multi-assay approach can be applied beyond oil and gas platforms to other marine infrastructure or natural habitat surveys. In particular, the ecosystem level data generated from this eDNA study can provide additional data to inform managers and regulators about the possible outcomes of different decommissioning options.

In marine eDNA research, the dominant collection method to date has comprised replicate water samples of varying volumes (Takahashi et al., 2023), which has been assumed to provide an overview of the adjacent substrates. From this research, we conclude that no single substrate was able to holistically document the entire detected diversity on or off platforms, a result corroborated by existing marine literature

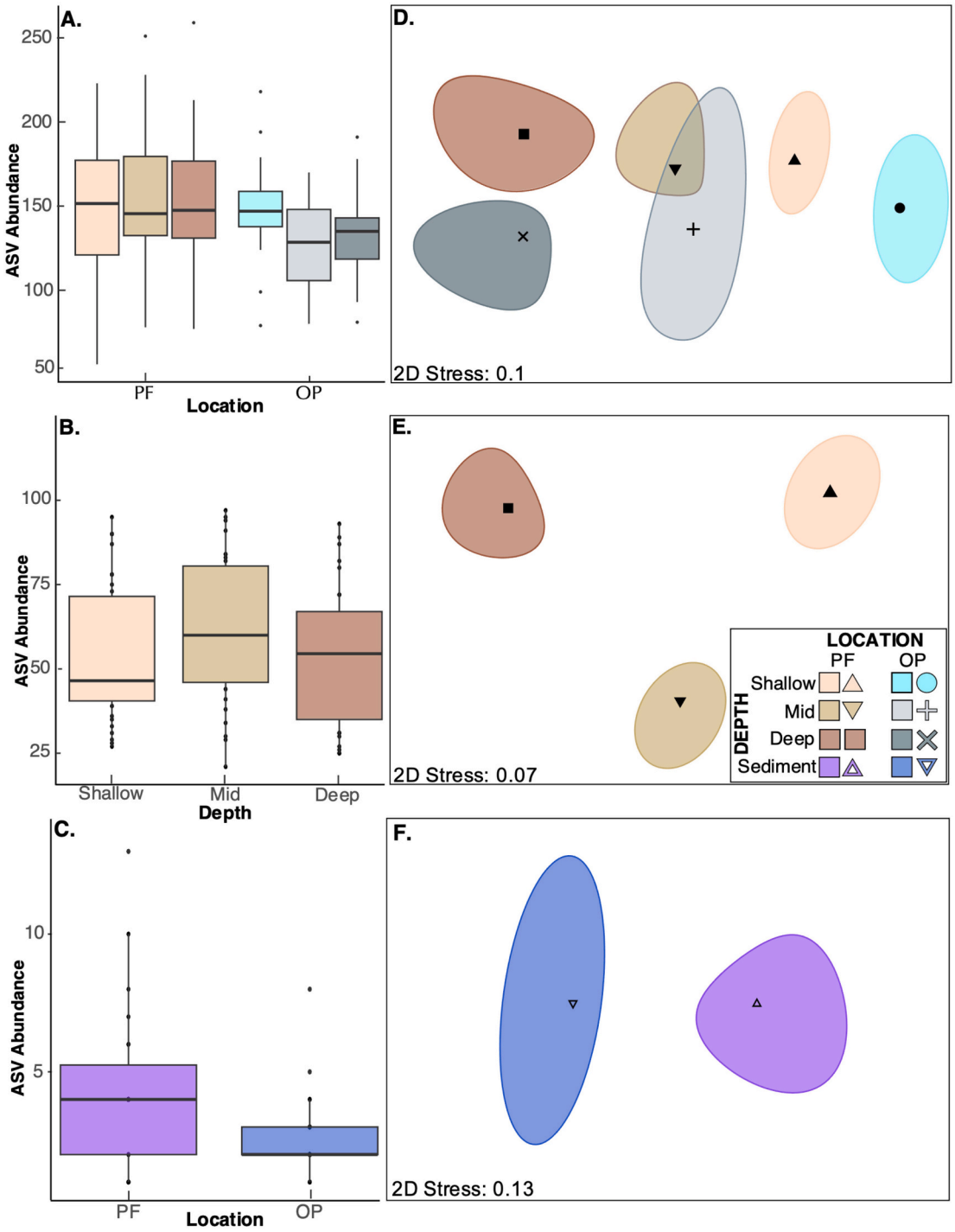


Fig. 4. Observed alpha diversity for all assays applied to the target substrates including water (A.) from different locations (platform and OP) and depths; bio-foul (B.) at platform depths; and sediment (C.) at both locations, and bootstrapped nMDS plots (D-F) for the corresponding substrates showing 95 % confidence intervals and centroids. Data based on 9999 permutations of a presence/absence transformed Jaccard resemblance matrix.

(Alexander et al., 2023; Koziol et al., 2018). The distinct assemblages associated with the various substrates have important implications for future monitoring surveys using eDNA methods at marine infrastructure, where the selection of substrate should be considered with an a priori knowledge of the primary study objectives.

Current Thailand legislation allows for a case-by-case assessment of decommissioning options using comparative assessment tools, such as the Best Practicable Environmental Option (BPEO; O'Riordan, 1989). While there are non-biological considerations involved, such as the potential for residual contaminants, physicochemical and geochemical parameters, we focus here on an ecological diversity perspective. The decommissioning of platforms typically involves the removal of all equipment that has contacted hydrocarbons (e.g. risers, valves and topsides), which is then followed by the cutting and decommissioning of the jackets (platform legs) (Bull and Kendall, 1994; Jagerroos and Krause, 2016). Platforms are then either completely removed, toppled in place, partially removed, or moved to an alternate location and repurposed as an artificial reef (Macreadie et al., 2011).

In the present study, the patterns of biotic diversity in relation to depth and the differences between biotic diversity associated with

Table 2
Indicator species identified from Platforms and OP sites in the GoT showing ASVs aligning to species, significantly characterising water (location and depth), bio-foul (depth only) and sediment (location).

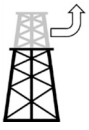
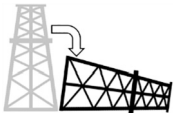
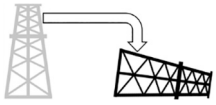
	Depth	Kingdom	Phyla	Species	No. Sig. ASVs	*P*-value (range)
Water						
PF	Shallow	Animalia	Arthropoda	*Calocalanus pavo*	1	0.01
		Animalia	Arthropoda	*Calocalanus plumulosus*	1	0.02
		Animalia	Chordata	*Atherinomorus lacunosus*	1	0.0
		Animalia	Chordata	*Oxyporhamphus micropterus*	1	0.03
		Animalia	Chordata	*Upeneus guttatus*	1	0.04
	Mid	Animalia	Chordata	*Euthynnus affinis*	1	0.01
	Deep	Animalia	Arthropoda	*Clausocalanus minor*	1	0.04
		Animalia	Chordata	*Ascidia ahodori*	2	0.01–0.03
		Animalia	Cnidaria	*Antipathes curvata*	1	0.03
		Animalia	Cnidaria	*Nausithoe punctata*	1	0.04
		Chromista	Haptophyta	*Phaeocystis globosa*	4	0.0–0.04
		Chromista	Ochrophyta	*Pelagomonas calceolata*	6	0.0–0.03
		Chromista	Ochrophyta	*Pseudo nitzschia cuspidata*	1	0.0
		Chromista	Radiozoa	*Dictyocoryne truncatum*	8	0.0–0.03
		Plantae	Chlorophyta	*Chloropicon laureae*	1	0.02
		Plantae	Chlorophyta	*Micromonas commoda*	13	0.0–0.03
OP	Shallow	Animalia	Arthropoda	*Calocalanus plumulosus*	1	0.04
		Animalia	Chordata	*Selar crumenophthalmus*	1	0.03
		Animalia	Porifera	*Tethya seychellensis*	1	0.05
	Mid	Animalia	Arthropoda	*Farranula gibbula*	1	0.02
		Chromista	Radiozoa	*Dictyocoryne truncatum*	1	0.04
	Deep	Chromista	Haptophyta	*Phaeocystis globosa*	1	0.0
		Plantae	Chlorophyta	*Chloropicon roscoffensis*	2	0.0–0.01
		Plantae	Chlorophyta	*Micromonas commoda*	1	0.0
		Plantae	Chlorophyta	*Pseudoscourfieldia marina*	1	0.01
		Bacteria	Proteobacteria	*Vibrio fluvialis*	1	0.05
Bio foul						
PF	Shallow	Animalia	Annelida	*Lumbrineris perkinsi*	1	0.02
		Animalia	Arthropoda	*Elasmopus nkjaf*	1	0.0
		Animalia	Arthropoda	*Stenothoe nhatrangensis*	1	0.0
		Animalia	Echinodermata	*Ophiactis modesta*	1	0.0
		Animalia	Echinodermata	*Ophiactis savignyi*	1	0.05
		Chromista	Ochrophyta	*Pelagomonas calceolata*	1	0.05
	Mid	Animalia	Annelida	*Lumbrineris perkinsi*	1	0.03
		Animalia	Porifera	*Crambe crambe*	1	0.0
	Deep	Animalia	Cnidaria	*Carijoa riisei*	3	0.00
		Animalia	Mollusca	*Hyotissa hyotis*	1	0.0
		Animalia	Porifera	*Chelonaplysilla erecta*	1	0.05
		Animalia	Porifera	*Ophlitaspongia papilla*	1	0.0
Sediment						
PF	–	Animalia	Nematoda	*Terschellingia longicaudata*	1	0.03
		Chromista	Ochrophyta	*Pelagomonas calceolata*	1	0.02
OP	–	–	–	–	–	–

platforms compared to OP locations can help to inform the outcomes of decommissioning options. While aspects of these impacts have been addressed at infrastructure elsewhere, such as exploring the coral or fish communities on reefed and standing infrastructure (Ajemian et al., 2015; Stunz and Coffey, 2020), or fish biomass and impacts to shell mounds under a partial dismantle scenario in California (Claisse et al., 2015), few studies have examined impacts to biotic diversity by following the fate of communities from before to after decommissioning. Consequently, there is little published information on survival rates of benthic and sedentary colonisers during the decommissioning process, particularly those taxa susceptible to photic and depth changes, such as corals and algae, which may affect subsequent colonisation of the biotic community.

The predicted outcomes for the biotic communities under each decommissioning scenario are summarised in Table 3, with specific examples for the taxa detected in the present study. Assuming that the OP sites represent a background diversity for open water and soft sediment habitats in the central GoT, the full removal of the infrastructure could see the local loss of up to 141 identified species that were only detected at platforms, or the potential loss of 36 shallow-water species (supplementary Table S10) under a partial removal scenario (top section removed from marine environment). Inversely, under the leave in-situ scenario, all taxa would be retained at all platform depths with only minimal disturbance when removing associated equipment (e.g. topside structure, valves and risers; Sommer et al., 2019). Additionally, sediment assemblages are likely impacted from nutrient filtration as a result of the biotic community inhabiting the vertical structure above (Bomkamp et al., 2004). When platforms are removed, the likely reduction in nutrient input and complete removal of physical structures is predicted to result in sediment assemblages adjacent to platforms becoming similar to those in OP sediment assemblages over time.

Under the topple or top and leave in place scenarios, it is predicted that there would be a shift in community composition from shallow water benthic colonisers, such as autotrophs, to deeper adapted taxa, such as Porifera, as indicated by distinct assemblages detected at different depths in the present study. The impact of towing structures to a reefing location on assemblages is likely to depend on tow method (wet or dry), transit time, speed of tow (Marnane et al., 2022), as well as the morphology of biota. For example, colonial and encrusting species (such as some ascidians and sponges) have documented increased survivorship at higher (vessel) transport speeds compared to softer bodied or branching benthic species (Coutts et al., 2010), which may have

Table 3
The mechanisms of decommissioning expected to impact extant biological communities under five decommissioning options, including full removal, partial removal, Topple/top in-situ, Tow and topple, and leave in-situ, and the implications for the diversity detected during this study. The grey indicates original platform position and black indicates the moved position of the platform under the decommissioning option. Figures recreated from original design in Fowler et al. (2018).

Decommissioning option	Generalised biotic impact of decommissioning alternative (from literature and present study)	Specific biotic impact of decommissioning alternative in the GoT (predicted from present study)
Full removal (of platform from marine environment) 	• Immediate local loss of diversity associated with platform • Decline in diversity of soft sediment-associated biota under and surrounding platforms due to reduced nutrient filtration (Bomkamp et al., 2004) • Potential triggered spawning of some benthic colonisers (Hewitt, 2022), with potential implications for introduced species, if present (Donelan et al., 2022), and release of organic material during cutting removal and cleaning	• Local loss or dispersal of at least 141 identified species that were detected at platforms but not OP sites • Potential decline in soft sediment inhabiting species detected adjacent to platforms, such as the polychaete, *Timarete ceciliae*, and nematode, *Terschellingia longicaudata* • Potential triggered spawning of benthic colonisers, such as ascidia (*Ascidia ahodori*) or Bryozoa (*Parasmittina* sp.) during cutting and removal
Partial removal (top section removed from the marine environment) 	• Immediate local loss of diversity associated with shallow sections of platforms • Potential change in soft sediment-associated assemblages under and surrounding platforms due to reduced nutrient filtration from top section of platform • Potential triggered spawning of some benthic colonisers, with potential implications for introduced species, if present, and release of organic material during cutting removal and cleaning	• Local loss of shallow water benthic species such as bivalves (*Barbatia trapezina*, *Isognomon legumen*, and *Pinna attenuata*), sponges (*Tethya wilhelma*, *Oscarella viridis*, *Crella cyathophora*) and algae (*Dictyota humifusa*), as well loss or dispersal of amphipods (*Podocerus jinbe*) and other arthropods (*Galathea* sp.) • Potential induced spawning of benthic colonisers, such as ascidia (*Ascidia ahodori*) or Bryozoa (*Parasmittina* sp.) during cutting and removal
Toppled or topped in-situ  or	• Gradual loss of diversity associated with shallow section of platform due to changes in depth of toppled or topped platform • Gradual loss or re-orientation of attached biota due to changes in orientation of platform (toppled platform)	• Gradual loss or dispersal of a potential 36 identified species that were only detected in shallow sections of platform due to change in depth, including species adapted to wave surge zone, such as mussels (*Barbatia trapezina*) or encrusting sponges (*Crella cyathophora*) • Increase in deeper dwelling species, such as coral *Carijoa* sp. (Cnidaria), or *Igernella* sp. (Porifera), which were detected predominantly in deep samples
Tow and topple (creation of deployed artificial reef) 	• Loss of some/all attached biota during towing, influenced by local regulations (requirement for cleaning), tow method (wet or dry), tow duration, and body morphology (Coutts et al., 2010) • Loss of fish and other motile species during towing (unless towed slowly; Marnane et al., 2022) • Gradual loss or re-orientation of attached biota due to change in orientation of toppled platform • Change in community composition associated with platform to deeper or shallower community depending on depth of toppling compared to in-situ depth • Decline in diversity of soft sediment-associated biota at site where platform was removed due to reduced nutrient filtration or loss of hard habitat • Potential triggered spawning of some benthic colonisers, with potential implications for introduced species, if present, and release of organic material during cutting removal and cleaning	• Maintenance of some reef-associated fish species if towed slowly (Marnane et al., 2022), including Moon wrasse (*Thalassoma lunare*), Goldband fusilier (*Pterocaesio chrysozona*) or Streaked spinefoot (*Siganus javus*). Likely reduction of juveniles from reef associated species unable to follow • Decline of soft sediment inhabiting species such as polychaete, *Timarete ceciliae*, and nematode, *Terschellingia longicaudata*
Leave in-situ	• No changes to existing diversity • Gradual increase in diversity of deeper dwelling species • Potential triggered spawning of some benthic colonisers, with potential implications for introduced species, if present, and release of organic material during cutting removal and cleaning • Potential change and redistribution of sediment-associated assemblages under and surrounding platforms and extended under the toppled or topped structure	• Preservation of at least 141 identified

(*continued on next page*)

Table 3 (*continued*)

Decommissioning option	Generalised biotic impact of decommissioning alternative (from literature and present study)	Specific biotic impact of decommissioning alternative in the GoT (predicted from present study)
	associated with platforms • No changes in diversity of soft sediment communities under and adjacent to platforms • No potential triggered spawning of benthic colonisers, or release of organic material	species that were associated with platforms but not OP sites, including detected conservation significant species (*Stylophora pistillata*)

implications for towing transit success. For those species surviving the towing process, once at the reefing location their survival is likely to depend on reefing depth, with distinct assemblages detected at different depths in the present study indicating the potential for demise and transition to a different suite of species over time if the platforms are reefed at depths shallower or deeper than in-situ.

The value in conducting broad assemblage censusing pre-decommissioning is, in part, to understand the presence or absence of key taxa, such as conservation significant or introduced species, both of which can be present in low abundance and biomass, which then may help inform decisions regarding decommissioning options. eDNA methods are highly sensitive and easily tailored to finding low abundance taxa (Nester et al., 2022). However, the use of single broad metabarcoding assays (such as the CO1 universal assay used in this study) may not be ideal for informing on the presence of low abundant searches (Wilcox et al., 2013; Xia et al., 2021). This can be mitigated by the use of narrow focus assays (target species or group specific), by increasing site replication, adopting a multi-assay approach, and incorporating *in-silico* analysis of target taxa.

In this study, two conservation significant species were detected, of a potential 400 occurring within the broader GoT (IUCN red list database accessed in December 2022): the smooth cauliflower coral (*Stylophora pistillata*; family Pocilloporidae; Near-threatened) and the pelagic Indo-Pacific sailfish (*Istiophorus platypterus*; family Istiophoridae; Vulnerable) species. However, the sailfish species, detected from multiple replicates at one site, is likely only loosely to be associated with platform habitats. None of the eight regionally documented introduced marine species from Thailand were detected in this study. These included sponges (*Tetilla japonica*), arthropods (*Penaeus vannamei* and *Leucothoe spinicarpa*), tunicates (*Clavelina cyclus* and *Ecteinascidia thurstoni*), and mollusc species (*Mytilopsis adamsi*, *Mytilopsis sallei* and *Mytella strigata*) (Chavanich, 2010; Sanpanich and Wells, 2019). However, reference material for these species is limited, with three of the eight species entirely unrepresented, and *in-silico* analysis (allowing for two primer mismatches) indicating that only *M. strigata* had the potential to be amplified with the primers used in this study, yet was not detected. While no congeneric taxa for this introduced species were detected, these species may be represented in higher order taxonomic assignments (family or above) and unable to be assigned to species-level. However, this list may not reflect earlier introductions and cryptogenic species, and therefore not reflect the true number of extant non-native species present (Chavanich, 2010). While the primary aim of this study was to characterise broad diversity at the platforms, this finding highlights the importance, and implications, of assay selection, in targeted taxonomy searches.

While successful in the broad characterisation diversity using both taxonomic and taxonomy independent analysis at oil and gas platforms in the GoT, efficiencies in the selection of assay and substrate were evident in our results. The coral assay, for example, was unable to assign almost 70 % of ASVs past kingdom, with only 12 % assigned to species, the lowest rate of all the assays used here. Given the estimated 292 identified scleractinian species occurring within the GoT (IUCN redlist database accessed in December 2022), the primary detection target of the coral assay (Alexander et al., 2019), this result could indicate a lack of hard corals inhabiting the structures, although four species were successfully detected (including 3 species of the genera *Tubastraea*). Alternatively, this could result from a lack of reference material from the ITS2 barcode region. Similarly, while the CO1 universal assay detected broad assemblages from water and bio-foul substrates, sediment substrate yielded few meiofauna species and significantly less ASVs by comparison. Detected sediment fauna comprised single annelid and nematode species and the remaining detections largely unassigned or green algae (genus *Chloropicon*). Informing management decisions currently relies heavily on taxonomic resolution (Kelly et al., 2014; Nielsen et al., 2023), especially for the detection of target taxa. Therefore, for future studies on platforms within the central GoT, we would not recommend the use of the coral assay, or the combination of the sediment substrate with broad CO1 universal assay, for informing on taxonomic resolution.

Given the developing field of marine eDNA, and in particular its use for censusing marine epibenthic assemblages, the optimisation of sampling and laboratory methods is ongoing, and likely dependent on location, environmental conditions and survey objectives. A number of recommendations can be drawn from this study, and existing literature, for future surveys on oil and gas platforms, which may be applicable to wider marine epibenthic sampling. During study design, the availability of assays and completeness of reference databases should be considered (through in-silico analysis; Bylemans et al., 2018) for target or local dominant taxa, which can determine potential for taxonomic analysis of results, or analysis of assemblages independent of taxonomic frameworks. Sampling substrates and methods target different biotic assemblages, and therefore careful consideration should be given to the objectives of the research study, with recent studies indicating increased diversity detections utilising methods that collect minimal bulk material (Alexander et al., 2023). Increasing vertical sampling replication may increase detected diversity and provide finer spatial nuance in informing on decommissioning, such as informing on optimal structure dissection. Finally, the application of an appropriate universal barcode assay, such as the CO1 universal assay used here, to all substrates may provide initial broad results and inform further assay direction required or on unrepresented target groups, if necessary for the study objectives.

The application of eDNA for the assessment of biotic diversity associated with marine infrastructure is relatively new, and particularly so in the Gulf of Thailand. eDNA metabarcoding can provide a very valuable, non-destructive tool for holistically censusing benthic, sedimentary and planktonic organisms, either as a complementary of stand-alone method broadly across the tree of life. While eDNA samples can be expensive to analyse, they can be efficiently collected offshore, significantly reducing vessel time that carries considerable costs and also reduces sampling risks. Importantly the ability to store the digital data and the DNA extracts provide a powerful way to revisit sites and samples to build up temporal and spatial datasets. With appropriate collection of samples, selection of assays and analysis, eDNA censusing has considerable potential to aid in determining the decommissioning course of action. For example, exploring how biota respond to reefing or towing options may help shape future decisions. Likewise, these methods can be further tailored to detected known IMS. Finally, ongoing eDNA surveys of the GoT sites could provide valuable sentinel data on how oceans are responding to a range of anthropogenic pressures.

CRediT authorship contribution statement

Conceptualisation – JBA, MJM, TSE, ESH; Investigation – MJM, ESH;

Laboratory workflow – JBA; Statistical analysis – JBA, ESH; Preparation of Manuscript – JBA, MJM, ESH; Editing of manuscript - JBA, MJM, TSE, MB, SS, PS, ESH; and Funding acquisition – ESH, MJM.

Author contributions

JBA, MJM, MB, TSE and ESH conceived and designed the project. ESH, MJM and PS undertook the data collection. JBA, TSE, ESH undertook the statistical analysis. JBA wrote the initial draft with guidance from MJM, TSE, MB and ESH. All authors were involved in contributing and editing the manuscript.

Funding

This project was funded by Chevron through a research grant to Curtin University from the Western Australian Energy Research Alliance (AES 17-P2TD-151-A1). JBA received industry scholarship funding from CSIRO.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jason B Alexander reports financial support, article publishing charges, equipment, drugs, or supplies, and writing assistance were provided by Chevron Energy Technology Co. Jason B Alexander reports financial support was provided by Commonwealth Scientific and Industrial Research Organisation. Euan S Harvey reports a relationship with Chevron Energy Technology Co that includes: funding grants. Michael Bunce reports a relationship with Chevron Energy Technology Co that includes: funding grants. Travis Elsdon reports a relationship with Chevron Energy Technology Co that includes: employment. Michael Marnane reports a relationship with Chevron Energy Technology Co that includes: employment. Paweena Sitaworawet reports a relationship with Chevron Energy Technology Co that includes: employment. Se Songploy reports a relationship with Chevron Energy Technology Co that includes: funding grants. Sarin Chaiyakul reports a relationship with Chevron Energy Technology Co that includes: employment.

Data availability

Data will be made available on request.

Acknowledgments

We gratefully acknowledge the field and logistical support provided by and the Crew of The Resolution. We are particularly grateful to Donnie Cameron and the ROV crews from Mermaid Subsea Services. We would also like to thank Tina Berry and Georgia Peverley from eDNA Frontiers for their expertise in processing samples through the eDNA workflow. This work was supported by resources provided by the Pawsey Supercomputing Research Centre, and from the CSIRO Environomics Future Science Platform through the iPHD program.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.165991.

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