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NOTES

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¹ The FISP scheme is managed by the Department for Environment, Food and Rural Affairs (Defra), and sits under the £100 million UK Seafood Fund which was set up to support the long-term future and sustainability of the UK fisheries and seafood sector. The FISP scheme aims to improve and share knowledge of fisheries and aquaculture by funding data collection and research to support sustainable fisheries management. All projects are delivered in collaboration between researchers and the fishing and seafood industry.

² The Fishmongers' Company's Fisheries Charitable Trust is a charity whose principal aim is to develop sustainable interaction with, and conservation of, the UK's marine environment, supporting both conservation and sustainable aquaculture activities in the UK.

2. Executive Summary

The 'Ecosystem Benefits of UK Oyster Aquaculture Sites' (ECM_65323) project is part of the Fisheries Industry Science Partnerships (FISP) scheme. Surveys were undertaken in 2023 to measure the abundance and variety of marine life at three Pacific/rock oyster farms and one naturalised Pacific oyster reef, compared to sites with similar environmental conditions but no live oysters (control areas). Ecosystem function testing was also undertaken at these sites to improve understanding of the ecosystem services that oyster sites provide in nitrogen cycling processes.

Recognising the importance of conservation and restoration of native oyster populations, surveys were also carried out to measure oyster abundance and associated variety of marine life (biodiversity) at an historical native oyster site, which was commercially restocked with native oysters. Surveys included collection of underwater imagery, sediment samples, oyster dredging and ecosystem function testing.

Findings from the oyster farm site surveys showed that from measurements of the sediment, including its composition and organic content, the variety of marine life and levels of disturbance and ecological status, the oyster sites had no demonstrable impact on the marine environment. Underwater imagery showed that at some locations diversity on the seabed was higher at the oyster sites than at the control areas, and other wildlife such as birds, fish and marine mammals were observed using the sites. This indicates that the oyster sites may have a positive influence on their surroundings by providing additional habitat in the marine environment and support a greater diversity of marine life than at other areas. Results from the ecosystem function testing showed that while there was some evidence that oyster sites may increase ammonia inputs to the marine environment, microbial communities were responding to this, particularly on the live oyster shells where greater numbers of microorganisms that break down ammonia and nitrogen compounds were found.

The surveys at the historical native oyster site which was restocked with young native oysters in 2021 had less demonstrable results. No consistent differences were recorded between the oyster site and the control area (having no live oysters) for sediment measurements or the diversity of seabed marine life. However, none of the young oysters were found during the survey, meaning relationships to the results could not be made. Some larger native oysters were recorded, which had been relaid as part of a previous native oyster restoration trial, suggesting that native oysters may need to be of a larger size or to be settled to shelly material (cultch) to survive in at sites such as these.

In summary, this project has highlighted that Pacific oyster farms had minimal impact on the marine environment, with some locations exhibiting higher seabed diversity than control areas. Additionally, other wildlife such as birds, fish and marine mammals were observed using the sites, indicating their role in enhancing marine habitat. Furthermore, ecosystem function testing demonstrated the vital role of oyster sites in nitrogen cycling processes, crucial for mitigating elevated nitrogen inputs in coastal environments. Although surveys at historic native oyster sites yielded less demonstrable results, they highlight the importance of ongoing restoration efforts to ensure the survival and development of native oyster populations. Overall, the research highlights the positive influence of oyster farms, not only in promoting biodiversity but also in providing essential ecosystem services for coastal ecosystems.

3. Introduction

The cultivation of oysters, with a low carbon footprint and little requirement for supplementary inputs such as feed or nutrient inputs, represents a sustainable method of producing high quality marine protein whilst providing employment and economic activity in coastal communities. Environmental concerns have created uncertainty over the future of Pacific/rock oyster (*Magallana gigas*) cultivation due to the invasive non-native status of this species in the UK, yet fisheries policy supports growth of the aquaculture sector and promotes sustainable sources of low-carbon and high-quality protein³. Legislation⁴ does not prohibit aquaculture activities for the Pacific oyster but attempts to ensure the sustainable management of the resourceⁱ. Decisions related to aquaculture restrictions are required to be fully justified and based on available data and a realistic assessment of any risk that the Pacific oyster poses to biodiversity and the environmental status of coastal areasⁱ. This study aimed to broaden the data available for such decisions, primarily by investigating the ecosystem benefits of oyster cultivation, which could help establish the role of oyster aquaculture as a Nature Based Solution or 'Other Effective Area-Based Conservation Measure' (OECM).

As part of the Fisheries Industry Science Partnerships (FISP) project 'Ecosystem Benefits of UK Oyster Aquaculture Sites', surveys were undertaken by ENVISION in 2023 to measure the biodiversity associated with three oyster farms and one naturalised Pacific oyster reef in comparison to control sites with similar environmental conditions and no live oysters, as well as ecosystem function (nitrification/denitrification activity) at these sites (oyster farm site surveys). Recognising the importance of conservation and restoration of native oyster populationsⁱⁱ, surveys were also carried out in 2022 and 2023 to measure oyster abundance and associated biodiversity at an historical native oyster site, commercially restocked with native oysters in 2021 (native oyster ranch site surveys). Survey works included recording of underwater imagery, sediment sampling, oyster dredging and ecosystem function sampling. Methods and results are described in full in separate site survey reports^{iii, iv, v, vi, vii, viii, ix}.

³The Joint Fisheries Statement (JFS), which sets out policies for achieving, or contributing to the achievement of, the Fisheries Act Objectives and forms part of the UK Fisheries Management and Support Framework (the Fisheries Framework. JFS: produced as stipulated in section 2 of the Fisheries Act 2020.

⁴ including the Water Framework Directive (2000/60/EC); Habitats Directive (92/43/EEC); Marine Strategy Framework Directive (2008/56/EC) and the Council Regulation concerning use of alien and locally absent species in aquaculture (Regulation No. 708/2007)

4. Oyster Farm Site Surveys

Oyster farm site surveys were undertaken in June/July 2023 to measure the biodiversity associated with three oyster farms (in Pembrokeshire, Lindisfarne and the River Avon, Devon) and one naturalised Pacific oyster reef (in the River Exe, Devon) in comparison to control sites, as well as ecosystem function (nitrification/denitrification activity) at these sites (Figure I).

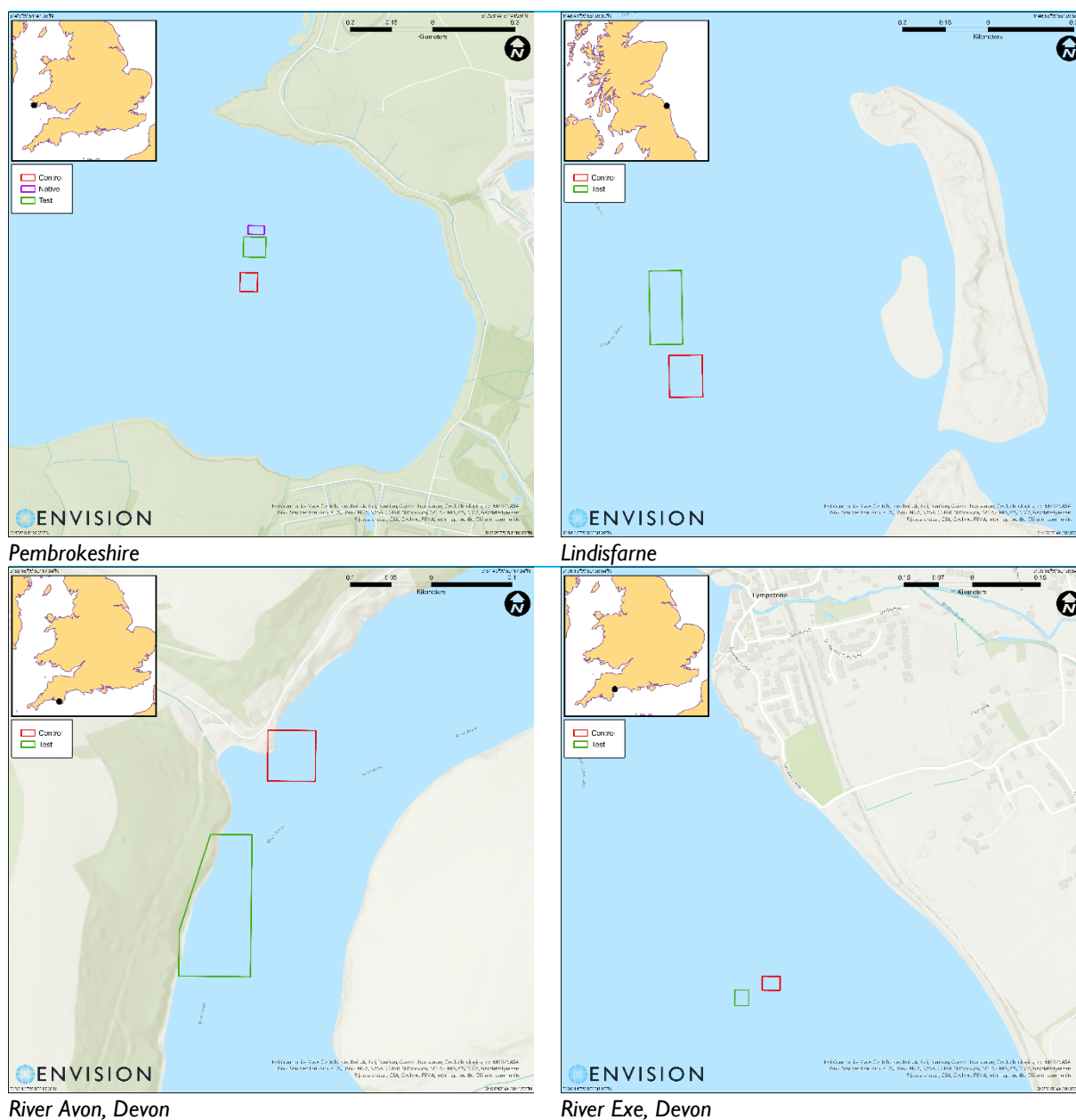


Figure I.

Test and control areas indicated at the oyster farm site surveys (and one naturalised Pacific oyster reef) in Pembrokeshire, Lindisfarne, the River Avon, Devon, and the River Exe, Devon.

The objectives of the surveys were to collect the following data at test and control areas:

- I. Two sediment samples, at five locations in each area, using a sediment corer, one for particle size analysis (PSA) and total organic carbon (TOC) analysis and a second for macrofauna analysis.

2. Underwater imagery (timelapse still imagery) using an underwater camera system, for identification and enumeration of biota around live oysters and in the control area.
3. Swab and sediment samples for ecosystem function testing, to be undertaken with genomic analysis.

4.1. Methods

4.1.1. Sample Planning

Sample locations were selected for PSA/TOC, macrofauna and ecosystem function sampling to provide a geographic spread around each site, and where it was accessible and safe to collect samples. Underwater camera systems were mounted on Pacific oyster trestle tables in the centre of the table array (and on native oyster trestle tables at the farm site in Pembrokeshire), or on frames in the naturalised oyster reef and control areas. The control areas were selected to have similar environmental conditions (depth, exposure and distance up the shore) to the oyster test area and were approximately 100 metres away from live oysters.

4.1.2. Sediment Sampling

Sediment samples were collected (five from each test area, five from each control area) and processed in accordance with national guidance^{x,xí,xii}. A 10cm diameter sediment corer was used to collect samples for PSA/TOC (500ml sediment), and samples for macrofauna analysis (approx. 2 litres sediment), which were sieved through a 1.0 mm sieve with the retained macrofauna and sediment preserved in ethyl alcohol (70% seawater buffered solution). Samples were transported to relevant laboratories for analysis.

4.1.3. Underwater Imagery Sampling

Waterproof camera systems were used to capture the biota present at the oyster sites, with camera systems comprising two or three GoPro Hero 8 cameras set up on frames at each area (Figure 2 and Figure 3).



Figure 2.
Example of oyster farm (test area) camera system.



Figure 3.
Example of control area underwater camera system.

At the Pacific oyster and native oyster farm test areas, one camera was fixed above the table, focussing on the upper surface of the oyster bag (upper), another was placed below the table focussing on the bottom of the bag (middle), and a third camera was placed under the table focussing on the underlying substrate (lower) (Figure 2). At the control area and naturalised Pacific oyster reef (no tables), two cameras were set up on frames in similar positions to the upper and lower cameras at the test area (Figure 3). Lamps were used to improve lighting over the area captured by the cameras.

Cameras were programmed using GoPro Labs Beta Firmware to begin collecting timelapse imagery approximately 30 minutes before high-water slack tide (for conditions with best visibility) around spring tides, during daylight hours. Underwater lamps were set up with batteries and timers in waterproof housing to enable lighting to be active over the same time. To maximise the duration of footage collected, timelapse still imagery was used to span a period of approx. 2-3 hours over high tide. Set up of camera systems was restricted to one hour either side of low tide as sites were located in the lower part of the intertidal shore area.

4.1.4. Ecosystem Function Sampling

Microbial-driven nitrogen (N) transformations are especially crucial in coastal systems, which often receive high anthropogenic N inputs (e.g. from river discharge, agricultural run-off etc) that can lead to eutrophication of coastal waters and sediments, and other associated ecologically damaging effects^{xiii, xiv}. Two key processes in the N-cycle are nitrification and denitrification which are tightly coupled together. Ecosystem function testing was undertaken to provide evidence of the ecosystem services of UK oyster sites in nitrogen cycling.

Sediment and swab samples were collected from across five UK sites Pembrokeshire, Lindisfarne, Avon, Exe and Maldon. Samples (denoted Test) were collected from beneath trestle tables where bags

of oysters were located (either Pacific oysters, *Magallana gigas*, or native oysters, *Ostrea edulis*), or around the naturalised Pacific oyster reef at the Exe site. In addition, control samples were collected from nearby uncultivated intertidal sediments where live oysters were absent. Replicate sediment (n=10) and swab (n=10) samples were taken from each test and control area by surveyors in 15ml plastic vials, with geospatially referenced photographs taken. Approx. 7-15ml of sediment was collected at each location, and swab samples were taken by swabbing oyster surfaces in bags or equivalent hard substrate at control area (e.g. small stones, bivalve shells (non-living)) for 60 seconds. Both sediment and swab samples were frozen for transport to the laboratory for subsequent analysis.

4.1.5. Analysis of Sediment Data

PSA results were used to determine sediment classes and attribute each sample with a Broadscale Habitat (Marine Nature Conservation Review (MNCR) Level 3 category based upon the varying percentages of gravels, sands and muds^{xv}. The sediment fractions at each sample location were presented in a map. A t-test was undertaken to test if there was a statistical difference in the values of TOC between the test and control site.

4.1.6. Analysis of Macrofauna Data

Macrofauna analysis data were rationalised by checking taxon names with the WoRMS species list and removing juveniles, taxa with damage/uncertain identification and mobile taxa such as fish. Colonial or encrusting taxa were recorded as presence/absence data and were retained within the analyses as these represent an epifaunal component of the seabed communities potentially associated with coarse habitats including shell which may be relevant to oyster grounds.

Multivariate statistical analysis was undertaken with the macrofauna data to investigate any groupings of macrofauna communities between the test and control areas. The SIMPROF routine was run in PRIMER-E (Plymouth Routines in Multivariate Ecological Research version 7 (v7)^{xvi} and resulting dendrograms and Multidimensional Scaling (MDS) plots were reviewed based on group-averaged Bray-Curtis similarities computed on standardised, square root transformed abundances, with 'slices' performed at various similarity levels to best differentiate between the main groupings. Groups with less than two samples or outlier samples with zero abundances were removed. The resulting groups were reviewed in relation to contributing taxa and sediment particle size to explore the differences in the faunal communities.

Statistical tests were conducted in 'R'^{xvii} on the macrofauna data. Species richness, evenness and diversity tests were calculated, and diversity indices from the samples were then compared using a t-test to reveal if any differences were statistically significant.

Macrofauna data were assessed using the AZTI Marine Biotic Index (AMBI⁵), a measure of the overall pollution sensitivity of a benthic assemblage. Taxa are classed into different ecological groups, which are related to the degree of sensitivity/tolerance to an environmental stress gradient (i.e. from sensitive to pollution tolerant/opportunistic species, from I, through II, III, IV to V). Results for each sample

⁵ <https://ambi.azti.es/>

included the percentage of taxa belonging to each ecological group, AMBI value, richness, diversity and the resulting disturbance classification. Factorial analysis, involving AMBI, richness and Shannon's diversity, was also undertaken, resulting in the 'Multivariate AMBI' or M-AMBI which can be used to assess the status of samples based on the WFD ranges for ecological status. Results were compared between test and control areas.

4.1.7. Analysis of Imagery Data

The imagery was reviewed, processed, and analysed in line with the principles of current guidelines^{xviii, xix} to identify and enumerate biota present. Timelapse still imagery was compiled as video footage, and reviewed to record the imagery quality, physical and biological characteristics, seabed character, habitat and biotopes present, conspicuous taxa, and life forms along with any modifiers or visible impacts present. Quantitative data were collected by recording the number of appearances of each taxon in the field of view, irrespective of whether they were unique individuals. Biota were recorded with counts for solitary/erect taxa and percent cover estimated for algae/colonial/encrusting taxa. Where appropriate, any relevant features of conservation interest or 'Habitats Directive: Annex I Habitats'^{xx} were noted, and all data were recorded in a MEDIN^{xxi} compliant proforma spreadsheet, with reference to the World Register of Marine Species (WoRMS) database for taxon nomenclature.

Abundances from the imagery data were standardised for different durations of video by using the average number of individuals observed per hour (of footage in real time) and compared between the test (native and Pacific oysters) and control areas. Hutcheson's t-tests were performed on the imagery data for the different camera positions to explore if differences in diversity between the test and control areas were significant, with tests undertaken separately for the different types of data recorded e.g. abundance data of solitary/erect taxa (count) and percentage cover (percent) for algae/colonial/encrusting taxa.

4.1.8. Ecosystem Function Analysis

Analysis was undertaken on samples to characterise and determine the abundance of the microbial communities driving nitrogen (N) transformations associated with oyster test and control areas in relation to nutrient concentrations. Anion and cation concentrations (e.g. ammonium, nitrate, nitrite), were measured on sediment (n=5) and swab (n=5) samples using a Dionex ICS-3000 (Thermo Scientific, UK) as previously described^{xxii, xxiii}. DNA was extracted from sediments using a Soil DNA Isolation Plus Kit (Norgen Bioteck Corp., Canada) following the manufacturers recommendations. Functional gene abundance was quantified by qPCR with a SensiFAST SYBR No-ROX Kit (Bioline) on a CFX96 Real-Time PCR Detection System (BioRad).

All statistical analyses were conducted in 'R' Studio^{xvii}. All data sets were not normally distributed, and some were less balanced sample sizes due to failed amplification in qPCR. Where possible a suitable Generalised Linear Model approach was taken – with appropriate distribution assumptions, e.g. GLM with a poisson, quasipoisson or negative binomial distribution. Model selection was determined by evaluations of dispersion scale parameters of the minimum models. Alternatively, a Kruskal Wallis test was used. Data from the native oyster samples from Pembrokeshire were not included (as a single site, but responses at this site were inferred).

4.2. Results

4.2.1. Sediment PSA and TOC Results

The results of the PSA/TOC analysis are summarised below in Table I, with sediment sample locations and full results presented in Table 9 in Appendix A, Section 7.1.

Table I.

PSA and TOC results, showing total carbon, sediment class and broadscale habitat (BSH) for samples from the control and test areas at the Pacific oyster farm (Pembrokeshire, Lindisfarne, Avon) and naturalised oyster reef (Exe) sites.

Sample	Total carbon (mg/g)	Sediment class	BSH
Pembrokeshire			
Control 1	31.28	muddy sand (mS)	Subtidal Sand
Control 2	28.38	muddy sand (mS)	Subtidal Sand
Control 3	56.59	gravelly sand (gS)	Subtidal Sand
Control 4	38.61	muddy sand (mS)	Subtidal Sand
Control 5	30.64	muddy sand (mS)	Subtidal Sand
Test 1	22.14	sand (S)	Subtidal Sand
Test 2	28.52	muddy sand (mS)	Subtidal Sand
Test 3	18.36	sand (S)	Subtidal Sand
Test 4	22.17	sand (S)	Subtidal Sand
Test 5	28.33	sand (S)	Subtidal Sand
Lindisfarne			
Control 1	193.55	gravelly sand (gS)	Subtidal Coarse Sediments
Control 2	23.06	gravelly sand (gS)	Subtidal Coarse Sediments
Control 3	29.35	gravelly sand (gS)	Subtidal Coarse Sediments
Control 4	43.33	sandy gravel (sG)	Subtidal Coarse Sediments
Control 5	35.65	gravelly sand (gS)	Subtidal Coarse Sediments
Test 1	48.21	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
Test 2	20.74	gravelly sand (gS)	Subtidal Coarse Sediments
Test 3	48.10	(gravelly) muddy sand ((g)mS)	Subtidal Sand
Test 4	95.86	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
Test 5	47.48	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
River Avon			
Control 1	36.63	(gravelly) sand ((g)S)	Subtidal Sand
Control 2	39.76	sand (S)	Subtidal Sand
Control 3	40.13	(gravelly) sand ((g)S)	Subtidal Sand
Control 4	55.69	gravelly muddy Sand (gmS)	Subtidal Mixed Sediments
Control 5	81.22	gravelly muddy Sand (gmS)	Subtidal Mixed Sediments
Test 1	42.59	sand (S)	Subtidal Sand
Test 2	44.18	(gravelly) sand ((g)S)	Subtidal Sand
Test 3	31.05	(gravelly) sand ((g)S)	Subtidal Sand
Test 4	50.52	gravelly sand (gS)	Subtidal Coarse Sediments
Test 5	44.57	sand (S)	Subtidal Sand

River Exe	Total carbon (mg/g)	Sediment class	BSH
Control 1	73.22	(gravelly) sandy mud ((g)sM)	Subtidal Mud
Control 2	62.83	sandy mud (sM)	Subtidal Mud
Control 3	73.62	sandy mud (sM)	Subtidal Mud
Control 4	72.35	sandy mud (sM)	Subtidal Mud
Control 5	73.71	(gravelly) sandy mud ((g)sM)	Subtidal Mud
Test 1	62.38	sandy mud (sM)	Subtidal Mud
Test 2	60.27	(gravelly) sandy mud ((g)sM)	Subtidal Mud
Test 3	59.77	(gravelly) sandy mud ((g)sM)	Subtidal Mud
Test 4	59.23	(gravelly) sandy mud ((g)sM)	Subtidal Mud
Test 5	54.91	(gravelly) sandy mud ((g)sM)	Subtidal Mud

Organic matter in sediments is an important source of nutrients for benthic fauna, however high concentration levels of total organic carbon can indicate organic enrichment, with oxygen depletion and toxic byproducts affecting species richness/abundance/biomass^{xxvii}.

Total organic carbon was compared at control and test areas at the oyster sites using a t-test, which showed a significant difference ($p < 0.05$) at two sites (Pembrokeshire and the Exe), with the control area having a higher average TOC value. There was no significant difference between TOC values ($P > 0.05$) for the sites in Lindisfarne or in the River Avon. None of the measurements at the oyster sites in this research showed high levels of total organic carbon in comparison with other studies^{xxvii}.

Comparison of the sediment classes and broadscale habitats between the test and control areas did not show any consistent trends throughout the oyster sites. At two of the sites, only one broadscale habitat was recorded throughout the entire site ('Subtidal Sand' at the Pembrokeshire oyster farm site, 'Subtidal Mud' at the naturalised oyster reef in the Exe), however sediment composition and classes showed the control area samples generally had a slightly higher silt content (except at Lindisfarne), with slightly more sand/coarse sediment at test area samples.

At the Avon oyster farm site, whilst both the test and control areas were largely comprised of sandy sediments, two locations at the control area were recorded as mixed sediments (with more silt present), and the test area sediments were all recorded as sand or coarse sediments. At the Lindisfarne oyster farm site, sediments in test area samples appeared to have a relatively higher silt content (majority (four) recorded as gravelly muddy Sand) than the control area (majority (four) recorded as gravelly Sand).

4.2.2. Sediment Macrofauna Analysis Results

Results of the identification and enumeration of macrofauna in the sediment samples from the oyster sites showed taxa to be dominated by Annelids (mostly polychaetes), which were the most diverse taxonomic group, present at almost every sample location. Molluscs (e.g. the gastropods *Littorina* species and *Peringia ulvae*) were also recorded in high numbers at the Lindisfarne and Exe sites. Bivalves were also commonly recorded in the control area at the Exe site, and throughout the Pembrokeshire site, as well as amphipods at both these sites. Less common taxa included brittle stars, urchins, barnacles, crabs, shrimps and mysids, bryozoans, pycnogonids and bryozoa and hydrozoa.

4.2.2.1. Multivariate Statistical Analysis Results

Multivariate statistical analysis results are summarised below. Full details of macrofauna data are provided in accompanying spreadsheets, with multivariate group also shown in Table 9 in Appendix A, Section 7.1.

Resulting of the multivariate analysis were reviewed in dendrograms and Multidimensional Scaling (MDS) plots based on group-averaged Bray-Curtis similarities computed on standardised, square root transformed abundances, with 'slices' performed at various similarity levels to best differentiate between the main groupings (Figure 4).

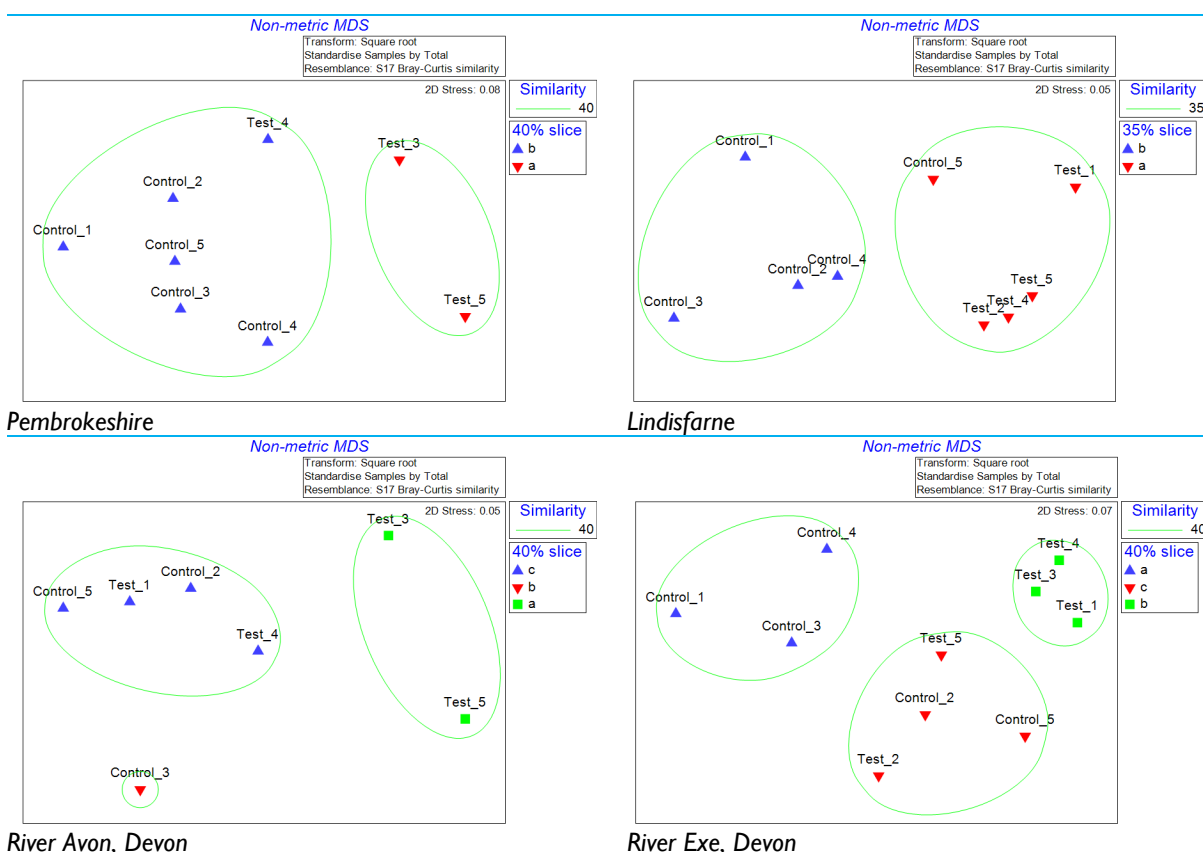


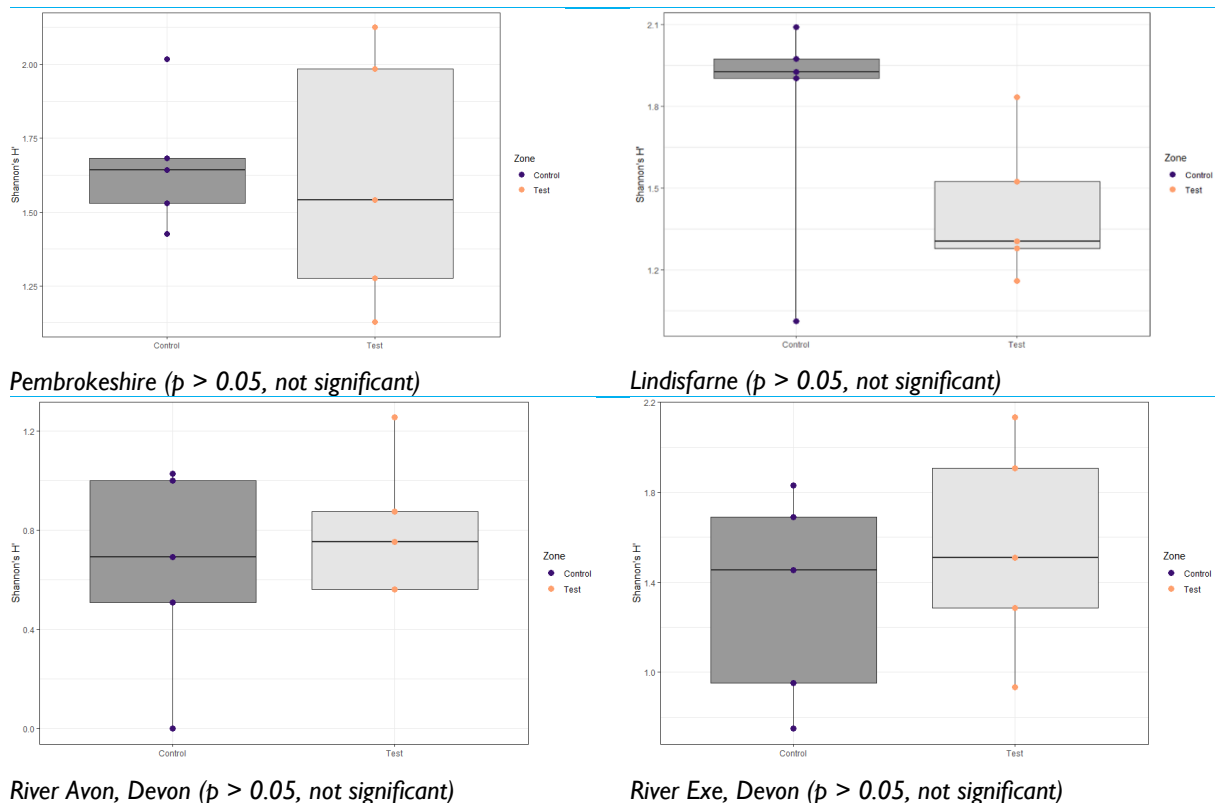
Figure 4.

Analysis of macrofauna from oyster sites in PRIMER – MSD plots from abundance data for each site.

Multivariate analysis results showed some distinction between the samples from the control and test areas at the Pembrokeshire oyster farm, with the control site samples comprised of amphipods and bivalves as well as polychaetes and having relatively higher silt content. Two groups were also differentiated at the Lindisfarne site, however both groups had polychaetes and molluscs as major contributors, but fewer taxa contributed to the group from the test area (Figure 4).

Results showed no clear distinction between the samples from the control and test areas at the Avon and Exe sites, with similar taxa found to be the major contributors to the groups, which showed some relation to sediment composition (silt or sand content) (Figure 4).

Shannon's Diversity Indices (SDI) were calculated for the samples in 'R' and compared between the test and control areas, using a t-test to determine the statistical significance (Figure 5).

**Figure 5.**

Analysis of macrofauna from oyster sites in 'R' – Shannon's Diversity for test and control area samples, with p value for t -test shown in brackets.

The results showed mean values for diversity (SDI) were higher at test areas than control areas at the Avon and Exe sites, but mean values of SDI were higher at control areas for the Pembrokeshire and Lindisfarne sites, however the differences were not statistically significant at all sites⁶.

4.2.3. Sediment Macrofauna AMBI Analysis Results

Full results for the AMBI analysis are shown in Table 10, and are summarised below in Table 2, showing the AMBI disturbance classification (Heavily Disturbed, Moderately Disturbed, Slightly Disturbed to Undisturbed), which can reflect natural as well as anthropogenic levels of disturbance, and the AMBI ecological status (Bad, Poor, Moderate, Good to High) for each site and area.

⁶ The result at the Lindisfarne site may have been influenced by one sample with lower diversity at the control area, which was seen to be grouped with the test area samples within the multivariate analysis process.

Table 2

Results from AMBI software, showing AMBI values, diversity, richness, disturbance classification, Multivariate-Ambi and assessment of ecological status from factorial analysis.

Site	AMBI Disturbance Classification		AMBI Ecological Status	
	Control (n=5)	Test (n=5)	Control (n=5)	Test (n=5)
Pembrokeshire	Moderately Disturbed (2) Slightly Disturbed (3) -	- Slightly Disturbed (2) Undisturbed (3)	Good (3) High (2)	Good (3) High (2)
Lindisfarne	Slightly Disturbed (3) Undisturbed (2)	Slightly Disturbed (5) -	Good (1) High (4)	Good (5) -
Avon	Heavily Disturbed (1) Slightly Disturbed (3) Undisturbed (1)	- Slightly Disturbed (5) -	Bad (1) Good (3) High (1)	Moderate (1) Good (3) High (1)
Exe	Slightly Disturbed (4) Undisturbed (1)	Slightly Disturbed (5) -	Moderate (2) Good (1) High (2)	- Good (4) High (1)

The results from the AMBI process showed that several of the taxa recorded in the macrofauna were not recognised (percent not assigned (%)) within the taxa lists for the software and therefore were not assigned to ecological groups (Table 10), which may decrease the relevance of the results.

Assessment of the macrofauna data from the oyster sites through the AMBI process resulted in variable levels of disturbance classification and ecological status, but no trends were evident to suggest less disturbance or better ecological status at either test or control areas consistently between sites. Disturbance classification at test areas ranged from 'Slightly Disturbed' (majority) to 'Undisturbed', whereas the majority of control area samples were also classed as 'Slightly Disturbed' but ranged from 'Heavily Disturbed' to 'Undisturbed'. The ecological status of the majority of test area samples was classed as 'Good', ranging from 'Moderate' to 'High', whereas at the control areas, samples were most frequently classed as 'Good' or 'High', but ranged from 'Bad' to 'High'.

These results are likely to reflect natural levels of disturbance at the oyster sites and show that macrofauna communities are not adversely affected by the presence of the oyster sites.

4.2.4. Imagery Analysis Results

The imagery quality from the oyster sites were assessed using the NMBAQC image quality categories (Table 3), with 14 videos assessed as 'Excellent', 17 videos assessed as 'Good', 11 as 'Poor' and two as 'Very Poor'. Imagery quality was affected by issues such as lighting at the middle camera position, which was located directly under the oyster bags, and at dusk (subsequent imagery was taken in full daylight hours), varying field of view where camera angles altered, macroalgae which obscured the field of view on occasion, and high levels of turbidity at the Exe.

Example imagery from each of the oyster sites is presented in Table 18, Table 19, Table 20 and Table 21 in Appendix B, Section 7.2.

Table 3.

Summary of NMBAQC image quality categories (Turner et al., 2016^{xix}).

Quality Category	Proportion of Tow Negatively Affected	Organism Enumeration	Biotope/Habitat
Excellent	<5%	Quantitative	Level 5
Good	5-20%	Quantitative	Level 5
Poor	20-50%	Qualitative	Level 3
Very Poor	50-80%	Not recommended	Level 2/3
Zero	>80%	Data not usable	Data not usable

Results of imagery analysis showed biota to be dominated by different taxa at different sites. At the Pembrokeshire site molluscs (e.g. periwinkles (*Littorina*) and whelks (Buccinidae)), fish and polychaete worms were recorded most frequently, with more gobies and shore crabs seen at the control, whereas at the oyster test area barnacles were also common, and fish (e.g. sea bass (*Dicentrarchus labrax*)) were seen ‘holding station’ under the tables. Molluscs (e.g. *Littorina*) and gobies were also the most abundant taxa at the naturalised Pacific oyster reef in the Exe, recorded along with fewer shrimps, crabs, bivalves and fish.

At the Avon site, biota were recorded in relatively lower numbers, with the control area dominated by fish e.g. mullets (Mugilidae) and some bass (*Dicentrarchus labrax*) moving quickly in shoals up and down the central channel of the river, and gobies (Gobiidae) on the riverbed. At the test area, crabs and gobies, shrimps and gobies were most common, with fish (e.g. *Dicentrarchus labrax*) again observed to be ‘holding station’ under the table.

At the Lindisfarne site, taxa at the control area to be dominated by shrimps (e.g. *Crangon crangon*) and crustaceans (e.g. *Brachyura*, *Carcinus maenas*, Paguridae). At the test area the most abundant taxa were crustaceans, with shrimps, polychaetes, fish and gastropods also seen here. Seals (e.g. *Halichoerus grypus*) were also observed resting on the oyster trestle table and moving adjacent to the tables.

Other taxa recorded included shrimp, hermit crabs, flatfish, jellyfish, brittle stars, terebellid worms, bivalves, crabs, macroalgae, as well as hydrozoa, bacterial mats and other faunal crusts growing on the oyster table structures

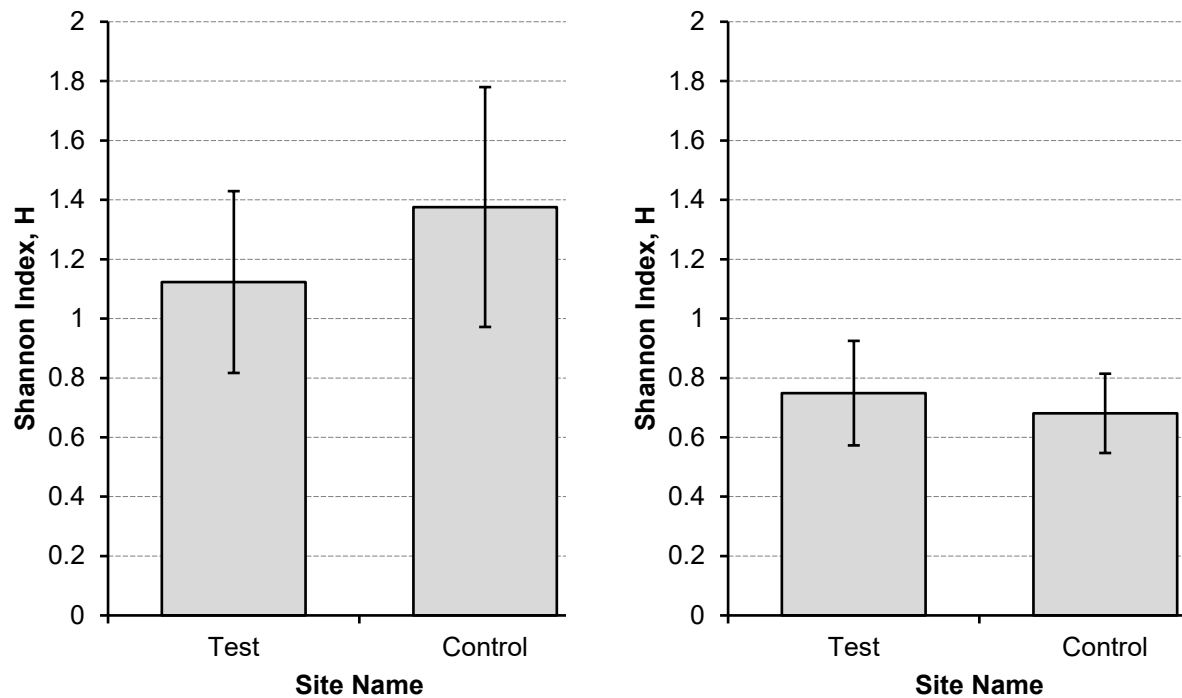
No Pacific oysters were recorded on the substrate at the farm sites around the trestle tables or at the control sites.

4.2.4.1. Results of Statistical Tests on Imagery Data

Shannon’s Diversity Indices were determined for the biota abundances recorded from the imagery data, and statistical tests (Hutcheson’s t-test) were undertaken to determine if differences in diversity between the test and control areas were significant. Tests were undertaken separately for the different types of data recorded e.g. abundance data of solitary/erect taxa (count) and percentage cover (percent) for algae/colonial/encrusting taxa.

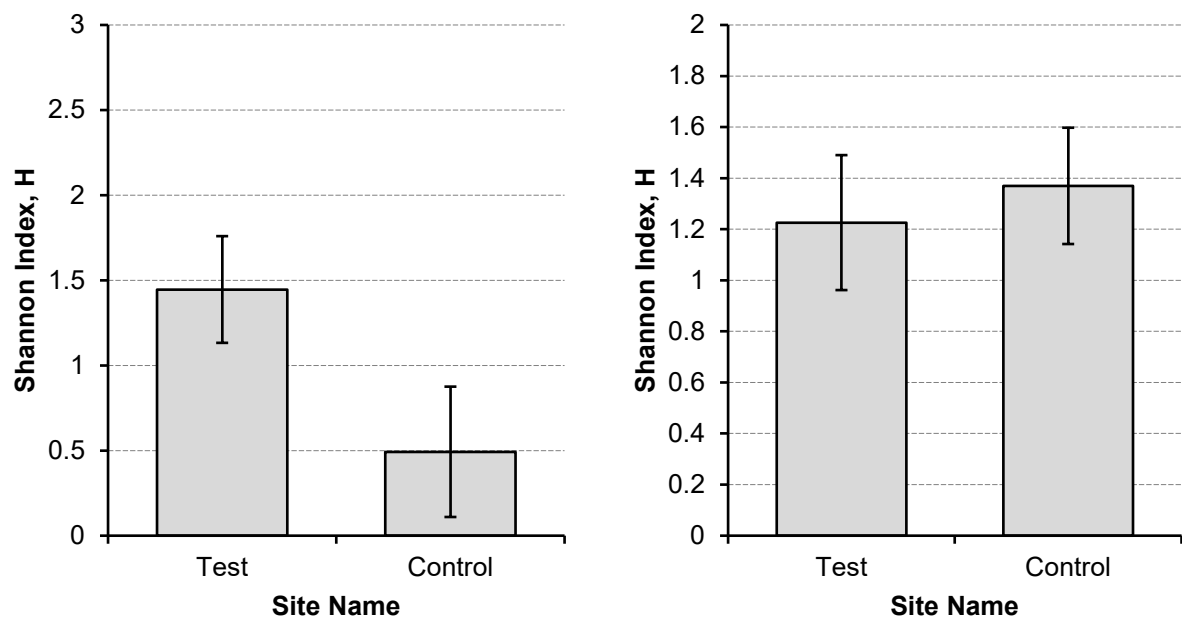
Results are shown for the solitary/erect biota (count data) recorded at the lower camera position (focusing on the underlying substrate), where most biota was recorded in the imagery data (Figure 6).

Again, no consistent trends were seen between sites, with diversity indices being higher at the control than the test area at the Pembrokeshire and Exe sites, but higher at the test area than the control at the Lindisfarne and Avon sites. However, differences in diversity indices at this camera position were not found to be significant ($p > 0.05$), with the exception of the Avon where twice the number of taxa were recorded at the oyster farm test area, which a significantly higher diversity index than the control area ($p < 0.05$).



Pembrokeshire ($p > 0.05$, not significant)

Lindisfarne ($p > 0.05$, not significant)



River Avon, Devon ($p > 0.05$, not significant)

River Exe, Devon ($p > 0.05$, not significant)

Figure 6.

Comparison of diversity of biota (count data) recorded from lower camera position at oyster test and control areas – Shannon's Diversity.

At the upper/middle camera positions, there were no significant differences in diversity of solitary erect taxa (count data) at the majority of sites, with the exception of the Avon and Lindisfarne sites, where the diversity indices were significantly higher at the test area ($p < 0.05$). Diversities were likely to have been higher at these upper camera positions due to the oyster tables providing structure for taxa to inhabit, and also diversities at the Avon control area were lowered by high numbers of one

type of taxa (fish) moving in shoals up and down the river. Diversity indices and p values from Hutcheson's t-tests are summarised for all sites in Table 4.

Table 4

Hutcheson's t-test values for comparison of Shannon's Diversity Index (SDI) between test and control areas (different camera positions, for solitary/erect (count) taxa), for imagery data from the oyster farm sites.

Biota (Count)	SDI Test	SDI Control	Higher SDI	P Value	Significant
Pembrokeshire Biota					
Lower	1.12	1.38	Control	0.3214	No
Middle	0.00	0.40	Control	0.3901	No
Upper	0.07	0.40	Control	0.4841	No
Native Lower	1.14	1.38	Control	1.1400	No
Native Upper	0.00	0.40	Control	0.3901	No
Lindisfarne					
Lower	0.75	0.68	Test	0.5380	No
Middle	1.01	0	Test	0.1378	No
Upper	1.23	0	Test	0.0033	Yes
Avon Biota					
Lower	1.45	0.49	Test	0.0003	Yes
Middle	0.69	0.52	Test	0.2980	No
Upper	0.69	0.52	Test	0.0008	Yes
Exe Biota					
Lower	1.23	1.37	Control	0.410	No
Upper	1.1	0.9	Test	0.8500	No

For the percent cover taxa (Table 5), only one taxon or no taxa were recorded at the majority of locations, showing no diversity or results which were not applicable (N/A) for statistical comparison. At the locations where diversities could be compared, diversity indices were always higher at the oyster test areas than the control area, but were only significantly higher at the Exe site, where several taxa were recorded overgrowing the naturalised Pacific oysters in the substrate.

For the middle and upper camera positions, diversity in the test areas included the taxa present on the oyster table structures, including macroalgae, turf and encrusting taxa. No percent cover taxa were recorded at the control area as this was effectively mid-water, however, with no structure present to grow over, this could not be statistically compared. This demonstrates the oyster tables provide a surface for colonisation by marine organisms, thereby introducing additional habitat to the estuarine ecosystem.

Table 5

Hutcheson's *t*-test values for comparison of Shannon's Diversity Index (SDI) between test and control areas (different camera positions, for percent cover (percent) taxa), for imagery data from the oyster farm sites.

Biota (Percent)	SDI Test	SDI Control	Higher SDI	P Value	Significant
Pembrokeshire Biota					
Lower - Percent	/	/	/	/	N/A
Middle - Percent	/	/	/	/	N/A
Upper - Percent	/	/	/	/	N/A
Native Lower	1.50	/	Native	0.40	No
Native Upper	/	/	/	/	N/A
Lindisfarne					
Lower - Percent	1.07	0.64	Test	0.34	No
Middle - Percent	1.21	/	Test	/	N/A
Upper - Percent	1.29	/	Test	/	N/A
Avon Biota					
Lower - Percent	0.00	0.00	/	/	No
Middle - Percent	1.10	/	Test	/	N/A
Upper - Percent	0.33	/	Test	/	N/A
Exe Biota					
Lower - Percent	1.26	0.00	Test	<0.001	Yes
Upper - Percent	/	/	/	/	N/A

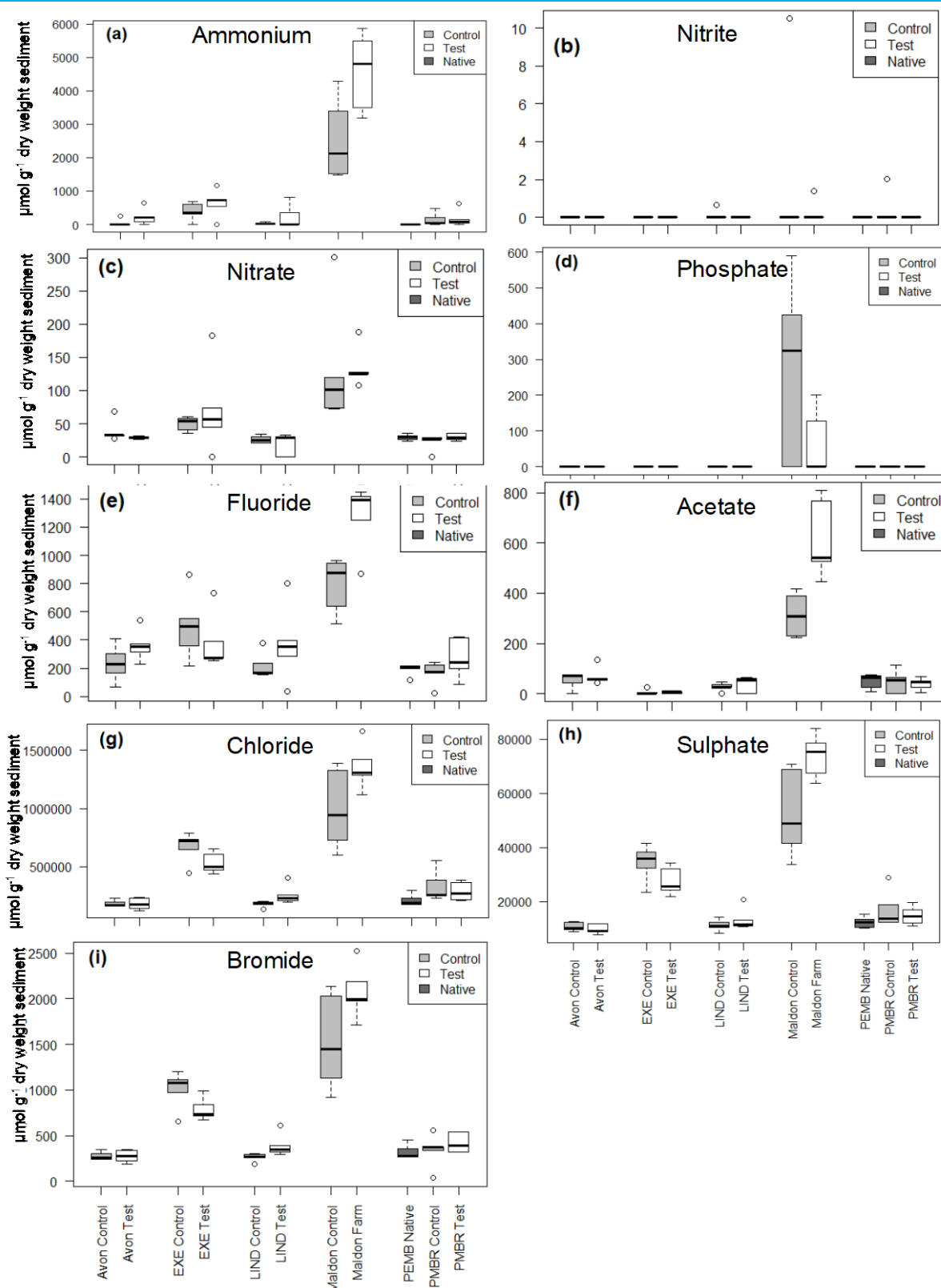
4.2.5. Ecosystem Function Analysis Results

4.2.5.1. Nutrient Levels

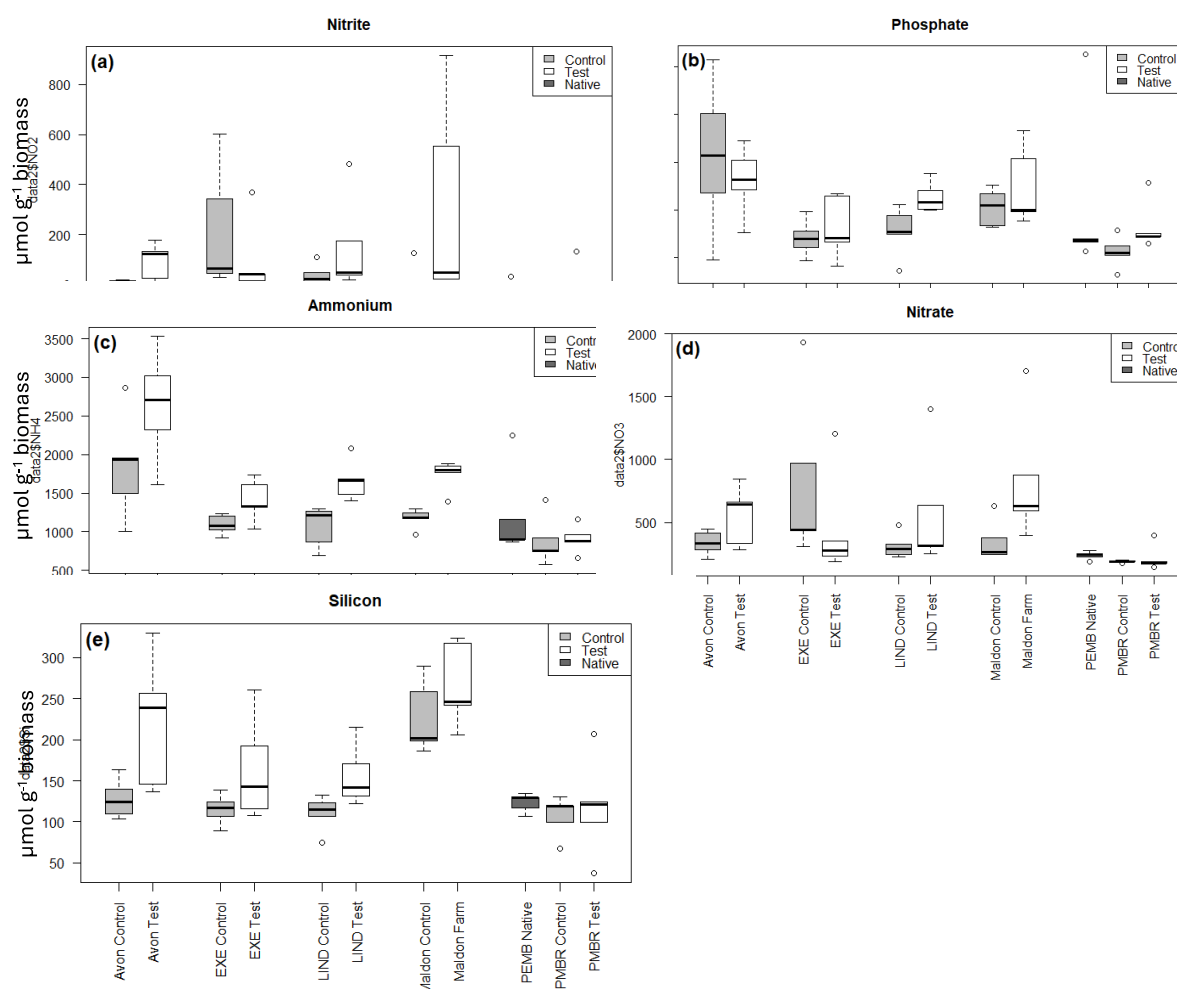
Results showed a significant difference in the ammonium levels from different sites (Figure 11.; GLM_{QUASIPois}, $F_{4,45} = 61.8$, $P < 0.001$). This was especially the case in the sediments within the oyster farm at Maldon which showed the highest ammonium concentrations (medians of 4814.38 $\mu\text{mol g}^{-1}$ dry weight sediment) compared to much lower values at Avon (206.99 $\mu\text{mol g}^{-1}$ dry weight sediment), Exe (736.95 $\mu\text{mol g}^{-1}$ dry weight sediment), Lindisfarne (2.16 $\mu\text{mol g}^{-1}$ dry weight sediment) and Pembrokeshire (85.46 $\mu\text{mol g}^{-1}$ dry weight sediment). While the largest effect size is driven by differences at Maldon, ammonium was consistently higher within oyster test sediments than in control sediments (Figure 7; GLM_{QUASIPois}, $F_{1,44} = 13.04$, $P < 0.001$). With the exception of the Pembrokeshire farm, all other oyster test sites also showed higher ammonium concentrations on the live oyster swab samples compared to the controls (Figure 8; mean difference of ammonium: control 1881 $\mu\text{mol g}^{-1}$ biomass vs farm shell surface 2591 $\mu\text{mol g}^{-1}$ biomass; GLM_{QUASIPois}, $F_{1,44} = 22.15$, $P < 0.001$).

Across all sites for both sediments and swab samples (both controls and within oyster test areas) nitrite concentrations were low, suggesting a rapid turnover of nitrite to nitrate by nitrification (Figure 7 and Figure 8). There was no significant difference in the nitrite, nitrate, and phosphate levels between oyster test and control sediment samples, but there were statistically significant differences between sites (e.g. between estuaries: sediment nitrate: GLM_{QUASIPois}, $F_{4,45} = 19.27$, $P < 0.001$). Swab samples taken from oyster shells had significantly higher nitrate levels compared to controls (Shell swab nitrate GLM_{NEGBIN}, resid deviance_{1,44} = 52.12, $P < 0.05$), where this was particularly driven by differences observed in shell swab nitrate at Maldon, Avon and Lindisfarne farms (Fig 3d). This may be related to

the increased ammonium levels at these locations which is indicative of greater nitrification at these sites/ shell samples (Figure 7 and Figure 8).

**Figure 7.**

Ecosystem Function Testing: Sediment nutrient concentrations (medians). Box and whisker plots show 25:75 quantiles and range.

**Figure 8.**

Ecosystem Function Testing: Swab nutrient concentrations (medians). Box and whisker plots show 25:75 quantiles and range.

4.2.5.2. Abundance of N cycling genes

Analysis of the functional genes involved in nitrification are shown in Figure 9. The abundance of AOA ammonia oxidisers was on average higher within oyster test area sediment samples compared to the controls (Figure 9b; sediment AOA: GLM_{QUASIPOLS}, $F_{1,74}=4.34$, $P<0.05$), but see the potential exception to this at Maldon (Fig 4b). For AOB ammonia oxidisers they were higher within oyster test area sediments only in Pembrokeshire and Lindisfarne (Figure 9d; sediment AOB Farm*treatment interaction - GLM_{QUASIPOLS}, $F_{4,70}=4.92$, $P<0.002$), with some marginal support for the same effect in the Exe. While many samples were below detection limits, the abundance of AOA and AOB genes on swabbed live oyster surface samples differed between sites and between live Pacific oyster shells and controls. Specifically, there were significant interactions such that AOA gene copies were higher within oyster test areas than controls in all sites other than Pembrokeshire where they were significantly lower (Figure 9a; GLM_{QUASIPOLS} $F_{4,70}=3.00$, $P<0.03$), and AOB gene copies were only significantly higher in Maldon and Lindisfarne (Figure 9c; GLM_{QUASIPOLS} $F_{4,70}=7.42$, $P<0.001$).

Interestingly, there was up to 10-fold greater abundance of AOA compared with AOB in both swab and sediment samples across most sites (with the exception of Pembrokeshire and Avon, where there was still an increase of AOA over AOB), suggesting it is likely that AOA driven nitrification was occurring at these coastal shellfish farms.

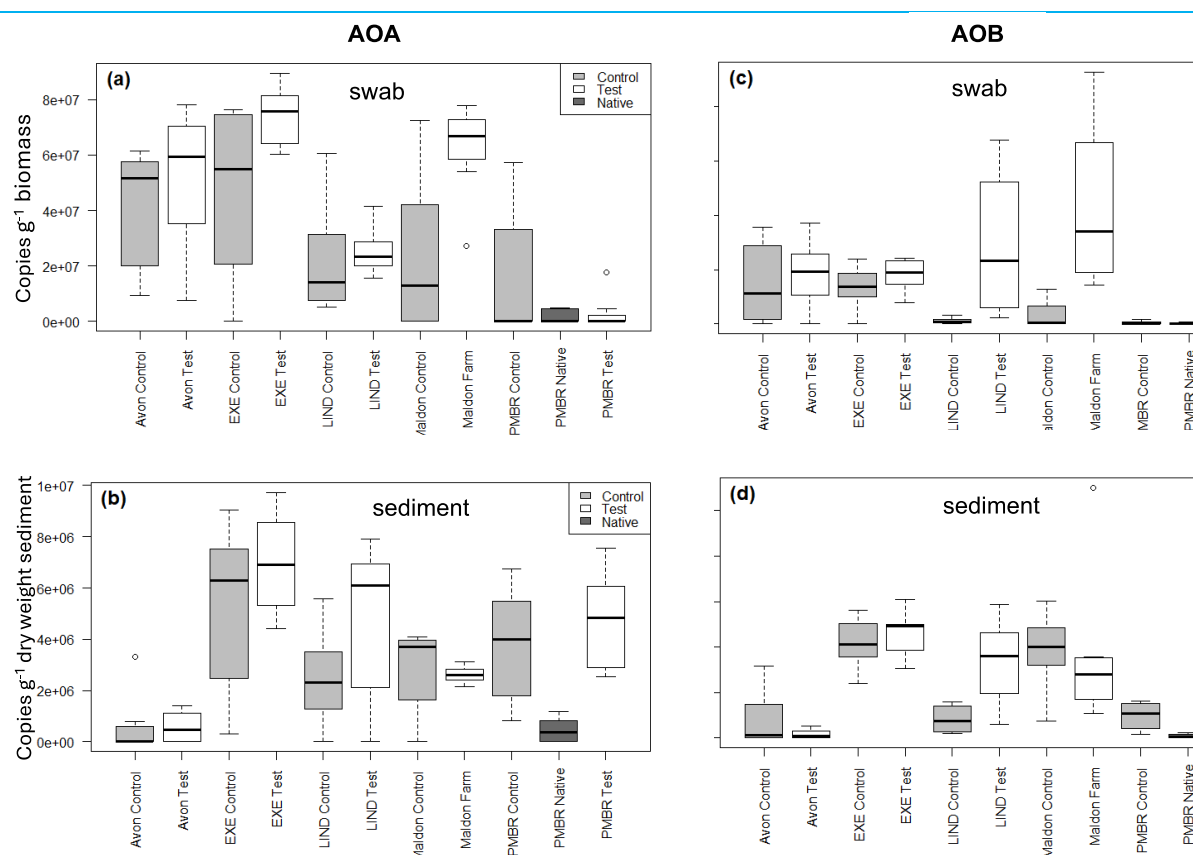
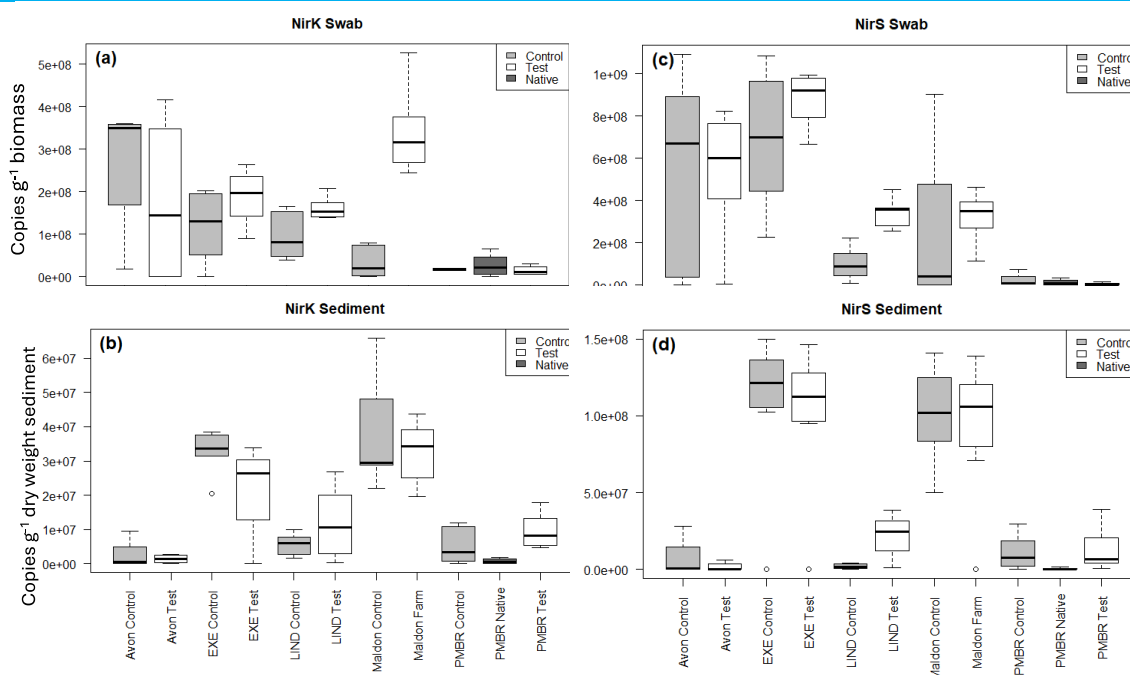


Figure 9.

*Ecosystem Function Testing: Abundance (median) of ammonia oxidising archaea (AOA) and bacteria (AOB) *amoA* gene copies from swabs (a, c) and sediments (b, d). Box and whisker plots show 25:75 quantiles and range.*

Analysis of the functional nitrite reductase genes (*nirS/nirK*) involved in denitrification are shown in Figure 10. With some exceptions (e.g. Pembrokeshire, where both background ammonia and nitrate levels were low), in general, there was a high abundance of *nirS/nirK* genes across sites (in both swabs and sediment samples). Interestingly, across most sites there was a 10-fold increase in *nirK* gene abundance on the swabs compared to the sediments. In general, there was also a greater abundance of *nirS* genes found in the swabs compared to the sediments.

Specifically, there was a greater abundance of *nirK* gene copies g⁻¹ biomass in the oyster test area swabs at the Exe, Lindisfarne and Maldon compared to controls (Figure 10a). Similarly, there was also a higher abundance of *nirS* gene copies g⁻¹ biomass in the oyster shell swab at the Exe, Lindisfarne and Maldon compared to controls (Figure 10c), suggesting a greater abundance of denitrifiers on the live oyster shell compared to controls at these locations (Figure 10).

**Figure 10.**

Ecosystem Function Testing: Abundance (median) of ammonia oxidising archaea (AOA) and bacteria (AOB) amoA gene copies from swabs (a, c) and sediments (b, d). Box and whisker plots show 25:75 quantiles and range.

4.3. Summary and Conclusions – Oyster Farm Site Surveys

Data were collected to compare the diversity of biota associated with three oyster farms and one naturalised Pacific oyster reef with control areas with no oysters nearby in June/July 2023, including underwater imagery, sediment sampling and ecosystem function sampling.

Comparison of the sediment classes and broadscale habitats between the test and control areas at the oyster sites did not show any consistent trends throughout the oyster sites. The sediment composition data showed similar sediment composition at the test and control areas at each site, with control areas generally having relatively higher silt contents at the majority of sites, except for one (Lindisfarne).

Total organic carbon measurements showed control areas to have significantly higher TOC levels ($p < 0.05$) at two sites (Pembrokeshire and the Exe), but there was no significant difference between TOC values ($P > 0.05$) for the sites in Lindisfarne or in the River Avon.

Multivariate analysis of the macrofauna data from the sediment showed some distinction between the samples from the control and test areas at the Pembrokeshire and Lindisfarne sites, but no clear distinction between the samples from the control and test areas at the Avon and Exe sites. Shannon's Diversity Indices (SDI) were calculated for the samples in 'R' and compared between the test and control areas. Results showed that diversities were higher at test areas than control areas at the Avon and Exe sites but lower at test areas for the Pembrokeshire and Lindisfarne sites, however none of the differences were statistically significant.

Assessment of the macrofauna data from the oyster sites through the AMBI process resulted in variable levels of disturbance classification and ecological status, but no trends were evident to suggest less disturbance or better ecological status at either test or control areas consistently between sites. The results were likely to reflect natural levels of disturbance at the oyster sites and show that macrofauna communities are not adversely affected by the presence of the oyster sites.

Underwater imagery data were standardised by using the average abundances of taxa observed per hour (of footage in real time) and compared between the test and control areas.

For the solitary/erect taxa (count data) there were no significant differences in diversity between the test and control areas at the majority of camera positions, with the exception of the lower camera position at the Avon site, and the upper camera position at the Avon and Lindisfarne sites, where the diversity indices were significantly higher at the test area.

For the percent cover taxa, only one taxon or no taxa were recorded at the majority of locations, showing no diversity. At the locations where diversities could be compared, diversity indices were always higher at the oyster test areas than the control area but were only significantly higher at the Exe site. For the middle and upper camera positions, no structure was present to grow over so this cannot be statistically compared, but this demonstrates the oyster tables provide a surface for colonisation by marine organisms, thereby introducing additional habitat to the estuarine ecosystem.

No Pacific oysters were recorded on the substrate at the farm sites around the trestle tables or at the control site.

Ecosystem function tests showed that, in general, higher ammonium levels were found within the oyster test areas (both on live oyster shells and in sediments), compared to controls with no oysters. There was also approx. 10-fold greater abundance of AOA than AOB (in both swabs and sediments) across most sites, suggesting that AOA were the likely drivers of nitrification at these locations. In addition, there was a greater abundance of denitrifiers found on the oyster shells, especially at the Exe, Lindisfarne and Maldon, compared to controls. Specifically, across most sites there was a greater abundance of the genes involved in denitrification (*nirS/nirK*) on the swabs compared to the sediments, suggesting that live oyster substrates are facilitating the proliferation of denitrifiers. These findings provide an advance of our understanding of the ecosystem services that oyster farms provide to N cycling processes (particularly denitrification and the removal of N) in coastal environments.

These results show that, in terms of sediment composition and organic content, diversity of sediment macrofauna and biota recorded from imagery data and disturbance and ecological status of soft-bottomed communities, the oyster farm sites had no demonstrable impact on the marine environment. Underwater imagery data show that at some sites diversity indices were higher at the oyster site test areas. These data further establish that the oyster sites have no demonstrable impact on their surroundings and may have a positive influence on the marine environment by supporting greater biodiversity than outside the farm. Furthermore, ecosystem function test analyses provided evidence of the ecosystem services of UK oyster sites in nitrogen cycling, and in particular their importance in contributing to the removal of elevated N inputs that are likely to occur in coastal environments.

5. Native Oyster Ranched Site Surveys

Surveys were undertaken in December 2022 and June 2023 to measure diversity associated with a ranched native oyster site (test) located in Mumbles, Swansea Bay (Figure 11) compared to a control site nearby, as well as the progress of restocked oysters.

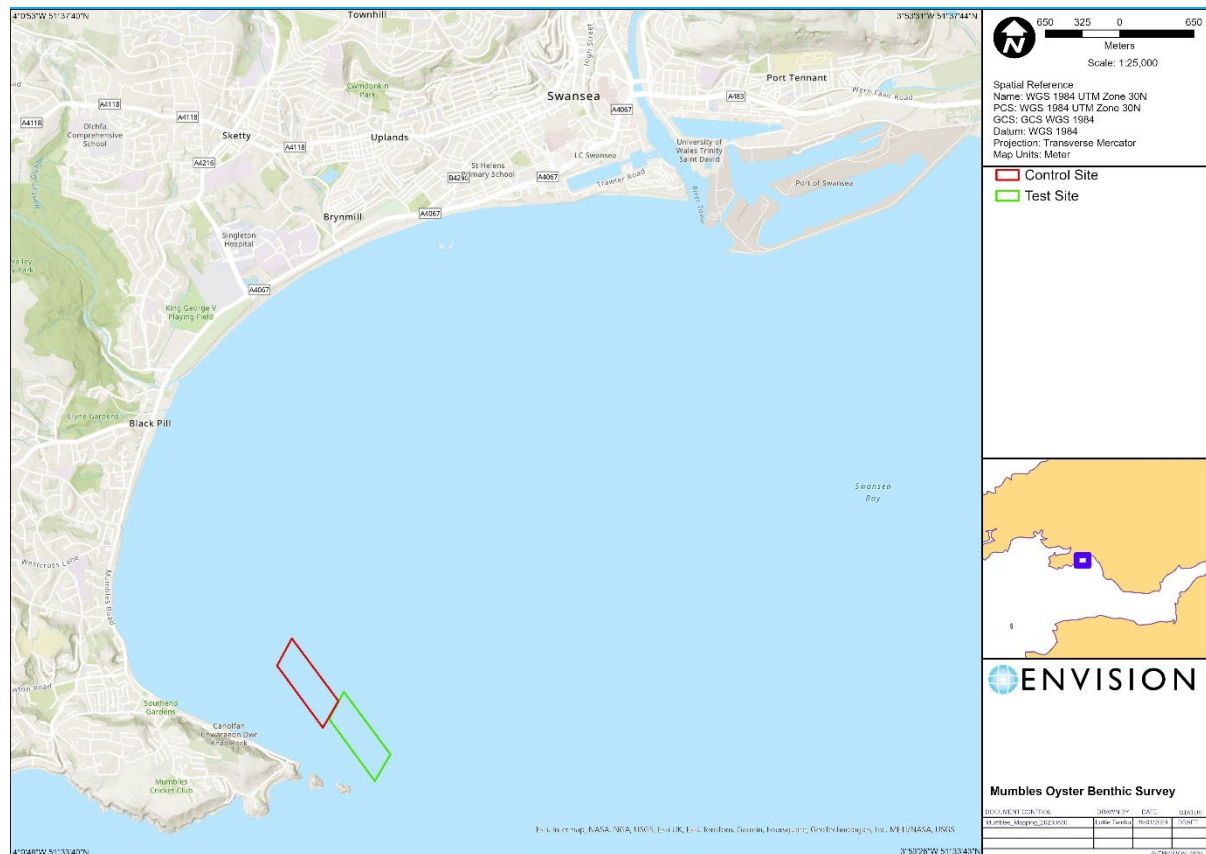


Figure 11.
Test and control sites, Mumbles, Swansea Bay.

The objectives of the survey were to collect the following data at test and control areas:

1. Sediment samples for PSA and TOC (500ml sediment) and for macrofauna analysis, to be sieved over a 1mm mesh sieve and the retained material stored and preserved for analysis.
2. Underwater imagery using a Remotely Operated Vehicle (ROV), collected to determine the extent and distribution of the subtidal habitats, identify and enumerate the biota occurring at each station, and to record any modifications or observations of anthropogenic influence.
3. Data on oyster abundance and other biota using a micro-oyster dredge undertaken in transects throughout both the control and test sites in the 2023 Mumbles survey.

5.1. Methods

5.1.1. Sample Planning

Sample stations were planned for the 2022 Mumbles survey by randomly selecting locations from a grid superimposed over the test and control sites to ensure sufficient geographical spread throughout the areas, and assigning priority levels, to allow collection of as many samples as possible within the time available and conditions encountered. Sampling design for the 2023 Mumbles survey was adapted to collect data at the same stations as in 2022 (priority 1), and some additional (priority 2 and 3) stations were selected with the aim of sampling ten stations within both the control and test sites (Figure 12). The additional (priority 2 and 3 selections) could be sampled as an alternative where sediment samples were unsuccessful at some locations (e.g. due to hard ground).

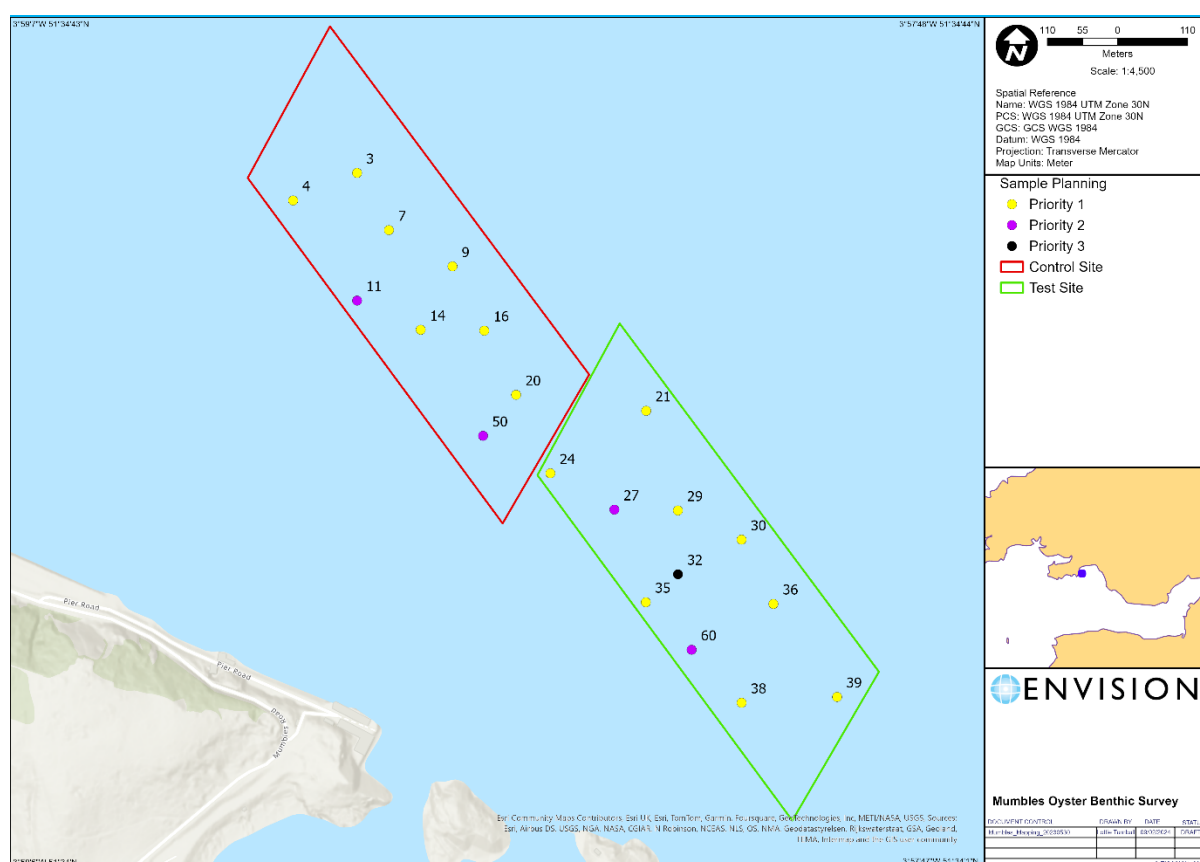


Figure 12.
Location of proposed sample stations in Mumbles oyster test and control sites, Swansea Bay.

5.1.2. Sediment Sampling

A 0.045m² Van Veen grab sampler was deployed to collect sediment samples from the seabed, and processed in accordance with national guidance^{x, xi, xii}. Samples were collected for PSA/TOC (500ml) and for macrofauna analysis, which were sieved through a 1.0 mm sieve with the retained macrofauna and sediment preserved in ethyl alcohol (70% seawater buffered solution). Samples were transported

to relevant laboratories for analysis. Any malfunction with the grab resulted in a failed sample, and the station was considered unsuitable for sampling if no sample was retained after five attempts.

5.1.3. Underwater Imagery Sampling

An observation class ROV was deployed to collect drop-down imagery information on the biology of the seabed and to verify the physical and biological nature of the seabed in compliance with national guidance^{xxiv, xxv, xxvi}. A remote controller was used to operate the ROV, which was lowered and raised by thrusters to limit disturbance to the seabed and maintain underwater visibility. The ROV, equipped with lights and lasers (approx. 10cm apart) for scale, recorded video footage at 4K resolution and was viewed in real time at the surface, with approximately two - three minutes of video footage collected at each station. At each station the vessel approached the target sample location and positioned itself so that wind and tide would cause the vessel to drift over the station coordinates, and away from the camera system whilst deployed. Positions were recorded at the start and end of each deployment using a differentially corrected GPS (dGPS) system, and a continuous log of vessel position was recorded whilst the camera was deployed, along with a survey log including position and time at the start and end of each deployment, station number, depth and brief survey notes.

5.1.4. Oyster Dredging Sampling

An oyster dredge (fitted with a finer mesh) was deployed during the 2023 survey at several locations throughout the control and test sites, after other sampling methods were completed to assess the abundance of oysters. The oyster dredge tows were evenly distributed across the test and control survey areas, with relatively short deployments used to minimise impact on the site and ensure more precise spatial recording. The contents of the dredge were released on to the deck for sorting, identification and an estimation of abundance.

5.1.5. Analysis of Sediment Data

PSA results were used to determine sediment classes and attribute each sample with a Broadscale Habitat (Marine Nature Conservation Review (MNCR) Level 3 category based upon the varying percentages of gravels, sands and muds^{xv}. The sediment fractions and classes at each sample location were presented in a map. A t-test was undertaken to test if there was a statistical difference in the values of TOC between the test and control site.

5.1.6. Analysis of Macrofauna Data

Macrofauna analysis data were rationalised by checking taxon names with the WoRMS species list and removing juveniles, taxa with damage/uncertain identification and mobile taxa such as fish. Colonial or encrusting taxa were recorded as presence/absence data and were retained within the analyses as these represent an epifaunal component of the seabed communities potentially associated with coarse habitats including shell which may be relevant to oyster grounds.

Multivariate statistical analysis was undertaken with the macrofauna data to investigate any groupings of macrofauna communities between the test and control areas. The SIMPROF routine was run in

PRIMER-E^{xvi}) and resulting dendrograms and Multidimensional Scaling (MDS) plots were reviewed based on group-averaged Bray-Curtis similarities computed on standardised, square root transformed abundances, with 'slices' performed at various similarity levels to best differentiate between the main groupings. Groups with less than two samples or outlier samples with zero abundances were removed. The resulting groups were reviewed in relation to contributing taxa and sediment particle size to explore the differences in the faunal communities.

Statistical tests were conducted in 'R'^{xvii} on all the macrofauna data. Species richness, evenness and diversity tests were calculated, and diversity indices from the samples were then compared using a t-test to reveal if any differences were statistically significant.

5.1.7. Analysis of Imagery Data

The imagery was reviewed, processed, and analysed in accordance with current guidelines^{xviii, xix} to identify and enumerate biota present, and record the broadscale habitats and biotopes in the imagery, noting where one substrate type changed to another (over 5m). The results of analyses were reported and provided in spreadsheet proformas, along with image reference collections for each taxon, habitat and biotope recorded.

Imagery was viewed at normal or slower than normal speed, noting the imagery quality, physical and biological characteristics, such as substrate type and composition (in line with current guidelines), seabed character, conspicuous taxa, and life forms along with any modifiers or visible impacts present. Taxa were identified to the most detailed taxonomic level possible and recorded with abundance counts for erect species and percent cover estimated for colonial/encrusting/macroalgae species. Where appropriate, any relevant features of conservation interest or 'Habitats Directive: Annex I Habitats^{xx}' were noted at each sample location and all data were recorded in a MEDIN^{xxi} compliant proforma spreadsheet, with reference to the World Register of Marine Species (WoRMS) database for taxon nomenclature.

Multivariate statistical analysis was undertaken with the underwater imagery data to investigate any groupings of biota communities between the test and control area. The SIMPROF routine was run in PRIMER-E using the procedure as described for macrofauna data in Section 5.1.6.

Statistical tests were also conducted in 'R' on the imagery data. Species richness, evenness and diversity tests were calculated, and diversity indices from the samples were then compared using a t-test, or Welch's unequal variances t-test, to reveal if any differences were statistically significant.

5.1.8. Analysis of Oyster Dredge Data

Multivariate statistical analysis were also undertaken with the data from the oyster dredge tows from the 2023 survey, using the SIMPROF routine in PRIMER-E as well as statistical tests in 'R' to calculate the species richness, evenness and diversity indices, which were compared using a Welch's unequal variances t-test.

5.2. Results

During the 2022 Mumbles survey (29th November to 1st December 2022), underwater imagery collection was attempted, however, due to high levels of turbidity preventing a view of the seabed, video sampling was considered unsuitable and survey effort was concentrated on grab sampling. Grab samples were collected at seven stations within the control site and seven stations within the test site, including one sample for PSA/TOC and five samples for infaunal analysis, totalling 14 PSA/TOC and 70 infaunal samples.

During the 2023 Mumbles survey (11-13th June 2023), data were collected at 10 stations in the test area and 10 stations in the control area, totalling 20 PSA/TOC and 20 macrofauna samples and underwater imagery from 20 stations, as well as oyster dredging at nine locations (five in the test area and four in the control).

5.2.1. Sediment PSA and TOC Results

Results of the PSA and TOC analysis are presented below in Table 6 for the 2022 Mumbles survey, and in Table 7 for the 2023 Mumbles survey, and in Table 12 and Table 13 in Appendix A, Section 7.1.

Table 6

PSA and TOC results, showing total carbon, sediment class and broadscale habitat for each station from the 2022 Mumbles survey.

Control / Test	Station	Total carbon (mg/g)	Sediment Class	BSH
CONTROL	STN03	122.05	muddy sand (mS)	Subtidal Sand
	STN04	97.84	sandy mud (sM)	Subtidal Mud
	STN07	136.59	muddy sand (mS)	Subtidal Mud
	STN09	80.57	muddy sand (mS)	Subtidal Mud
	STN14	82.26	muddy sand (mS)	Subtidal Sand
	STN16	114.87	muddy sand (mS)	Subtidal Sand
	STN20	123.69	muddy sand (mS)	Subtidal Sand
SITE	STN21	62.41	sand (S)	Subtidal Sand
	STN24	102.98	muddy sand (mS)	Subtidal Mud
	STN29	58.26	sand (S)	Subtidal Sand
	STN30	52.71	sand (S)	Subtidal Sand
	STN35	132.39	muddy sand (mS)	Subtidal Mud
	STN36	56.64	sandy gravel (sG)	Subtidal Coarse Sediments
	STN39	61.58	sand (S)	Subtidal Sand

Over half of the stations at the test site were sand, one station was sandy gravel (sG) and two stations were muddy sand (mS). The majority of stations at the control site were found to be muddy sand (mS), with one station of sandy mud (sM), reflecting the relatively higher silt content at the control area.

In the 2023 Mumbles survey, again results reflected a slightly higher silt content at the control area (more stations of 'Subtidal Mud') at the control site (Table 7), with an overall trend of increasing silt closer to the shore.

Table 7

Sediment analysis results, showing Total Carbon, sediment classes and broadscale habitats for each station from the Mumbles 2023 survey.

Control / Test	Station	Total carbon (mg/g)	Sediment Class	BSH
CONTROL	STN03	84.38	(gravelly) muddy sand ((g)mS)	Subtidal Mud
	STN04	76.41	(gravelly) muddy sand ((g)mS)	Subtidal Mud
	STN07	82.51	(gravelly) muddy sand ((g)mS)	Subtidal Sand
	STN08	105.57	(gravelly) muddy sand ((g)mS)	Subtidal Mud
	STN09	115.33	(gravelly) muddy sand ((g)mS)	Subtidal Sand
	STN011	102.27	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
	STN014	76.99	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
	STN016	50.63	(gravelly) sand ((g)S)	Subtidal Sand
	STN020	77.78	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
	STN050	92.09	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
TEST	STN021	53.21	sand (S)	Subtidal Sand
	STN024	75.17	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
	STN027	129.12	(gravelly) muddy sand ((g)mS)	Subtidal Mud
	STN029	88.87	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
	STN030	44.22	sand (S)	Subtidal Sand
	STN032	103.17	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
	STN035	88.70	gravelly muddy sand (gmS)	Subtidal Mixed Sediments
	STN036	49.56	gravelly sand (gS)	Subtidal Coarse Sediments
	STN039	50.38	(gravelly) sand ((g)S)	Subtidal Sand
	STN060	65.91	muddy sandy gravel (msG)	Subtidal Mixed Sediments

While organic matter in sediments can be an important source of food for benthic fauna, high concentration of total organic carbon can indicate organic enrichment, and possible oxygen depletion and toxic byproducts affecting species richness/abundance/biomass^{xxvii}. A t-test showed that TOC was significantly higher in the control area than the test area in the 2022 Mumbles survey ($p < 0.05$), but there was no significant difference between TOC values at the test and control sites in the 2023 Mumbles survey ($p > 0.05$).

5.2.2. Sediment Macrofauna Analysis Results

Results of the identification and enumeration of the macrofauna samples showed Annelids to be the most dominant and diverse taxonomic group, with high numbers of polychaete worms recorded as well as lower abundances of bivalves, gastropods, brittle stars, crabs and anemones, along with hydrozoa and bryozoa. No native oysters were recorded in the sediment samples from either the test or control area.

Multivariate statistical analysis results are summarised below (Figure 13). Full details of macrofauna data are provided in accompanying spreadsheets, with multivariate group also shown in Table 12 and Table 14 in Appendix A, Section 7.1.

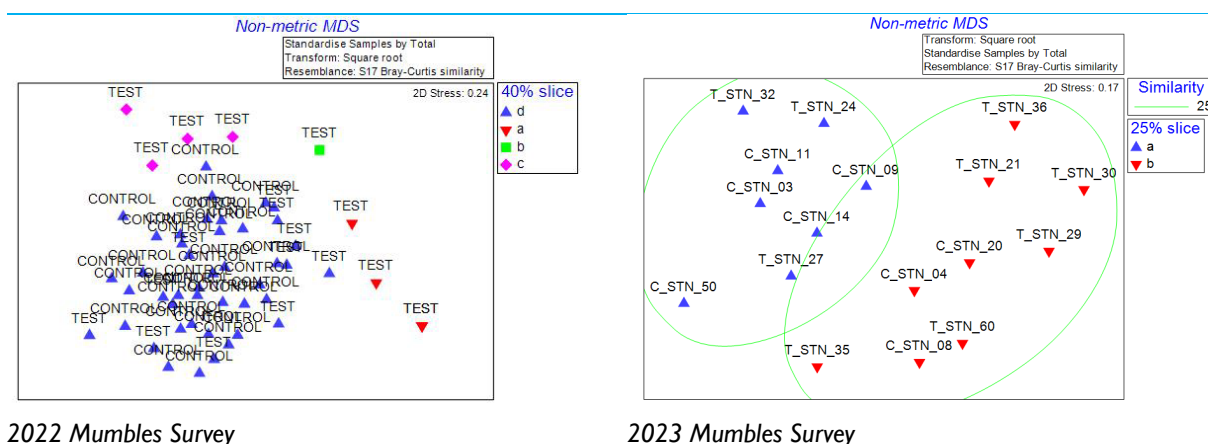
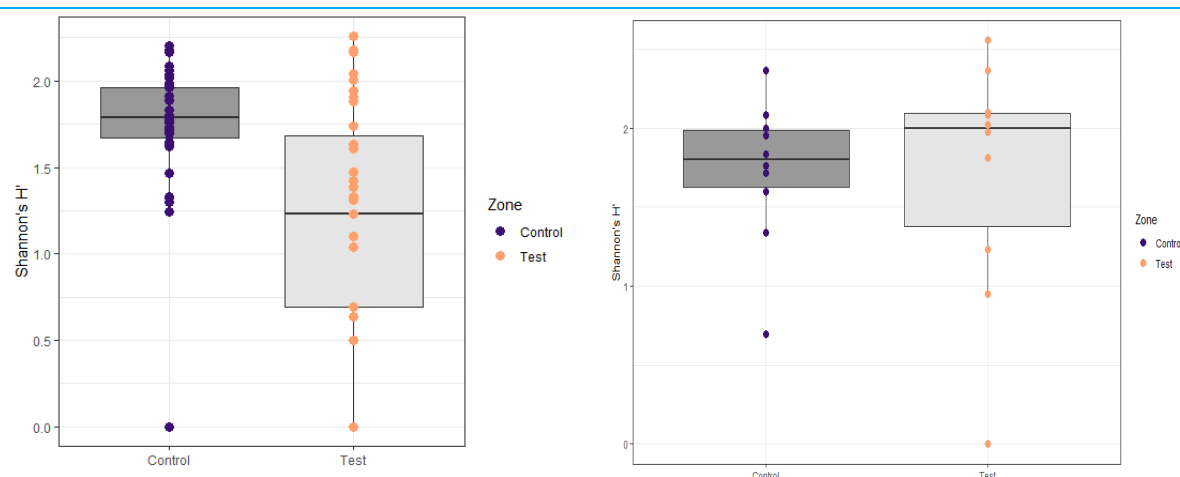


Figure 13.

Analysis of macrofauna from 2022 and 2023 surveys at Mumbles ranched native oyster site in PRIMER – MSD plots from abundance data for each site.

Initial analysis of the macrofauna data from the 2022 Mumbles survey showed the majority of samples to cluster into one group ('c'), which had the largest number of contributing taxa and comprised the samples located closest inshore and with relatively higher silt content. Multivariate analysis was then undertaken again on group 'c' (Figure 13), however there was still no clear distinction between test and control area samples or relationship to sediment composition.

Multivariate analysis on the macrofauna data from the 2023 Mumbles survey again showed no clear distinction between test (with a prefix T_) and control area (with a prefix C_) samples (Figure 13), and no clear relationship between the groupings and sediment fractions.



2022 Mumbles Survey (group 'c' samples)

2023 Mumbles Survey

Figure 14.

Analysis of macrofauna from 2022 and 2023 surveys at Mumbles ranched native oyster site in R – Shannon's Diversity for test and control area samples, with *p* value for t-test shown in brackets.

Shannon's Diversity Indices (SDI) for the macrofauna data from the 2022 and 2023 Mumbles surveys are shown in Figure 14, with significantly higher diversity indices at the control area than the test area in the 2022 data ($p < 0.05$). In the 2023 data, samples had a higher mean diversity index at the test area, however a t-tests showed there to be no statistical difference ($p > 0.05$) between the test and control areas.

5.2.3. Imagery Analysis Results

Imagery was assessed using the NMBAQC image quality categories (Table 3) and the footage collected during the 2022 Mumbles survey was unanalysable, due to previous poor weather and turbidity of the sea water. Imagery from the 2023 Mumbles survey was assessed as 'Excellent' at 12 stations and 'Good' at eight stations.

The results from analysis of the 2023 Mumbles survey imagery data (Table 15 and Table 16 in Appendix A, Section 7.1) showed that the seabed at the majority of stations within both sites was comprised of coarse sediment with a notable mud and sand component, with no clear distinction obvious between the control and test site.

A total of six habitats/biotopes were recorded from the video imagery from the Mumbles survey area (Table 8). Whilst a wide variety of biota was recorded throughout the survey area, most of the stations showed no dominance of specific taxa that allowed more detailed biotopes to be allocated, with the exception of high numbers of *Sabella pavonina* at two stations, where the biotope 'Sabella pavonina with sponges and anemones on infralittoral mixed sediment' (SS.SMx.IMx.SpavSpAn) was recorded.

Table 8

Imagery analysis results from 2023 Mumbles survey, showing broadscale habitat and MNCR code for each sample station.

Site	Station	Broadscale Habitat	MNCR Code
CONTROL	STN03	Subtidal Sand	SS.SSa.IMuSa
	STN04	Subtidal Sand	SS.SSa.IMuSa
	STN07	Subtidal Coarse Sediment	SS.SCS.ICS
	STN08	Moderate Energy Infralittoral Rock	IR.MIR
	STN09	Subtidal Mixed Sediment	SS.SMx.IMx
	STN011	Subtidal Coarse Sediment	SS.SCS.ICS
	STN014	Subtidal Mixed Sediment	SS.SMx.IMx
	STN016	Subtidal Mixed Sediment	SS.SMx.IMx
	STN020	Subtidal Mixed Sediment	SS.SMx.IMx.SpavSpAn
	STN050	Subtidal Mixed Sediment	SS.SMx.IMx
TEST	STN021	Subtidal Sand	SS.SSa.IMuSa
	STN024	Subtidal Mixed Sediment	SS.SMx.IMx.SpavSpAn
	STN027	Subtidal Mixed Sediment	SS.SMx.IMx
	STN029	Subtidal Mixed Sediment	SS.SMx.IMx
	STN030	Subtidal Coarse Sediment	SS.SCS.ICS
	STN032	Subtidal Mixed Sediment	SS.SMx.IMx
	STN035	Subtidal Coarse Sediment	SS.SCS.ICS
	STN036	Moderate Energy Circalittoral Rock	CR.MIR
	STN039	Subtidal Mixed Sediment	SS.SMx.IMx
	STN060	Moderate Energy Circalittoral Rock	CR.MIR

The biota recorded in the highest abundances throughout the Mumbles survey area included polychaete worms (e.g. *Sabella pavanina*), brittle stars, hermit crabs, urchins, starfish, anemones and bivalve siphons. No native oysters were recorded in the imagery analysis, potentially due to difficulties identifying from the underwater imagery due to faunal and algal overgrowth obscuring the substrate.

The reference collection included images 66 taxa/faunal groups and bioturbation. There were 17 instances of litter as defined in Annex 5.1 of the Joint Research Centres Guidance on Monitoring of Marine Litter in European Seas⁷, found at five stations, all within the test site, including broken ceramic pieces, glass bottles, food packaging and plastic items. No other instances of anthropogenic impacts were observed.

Multivariate analysis on the imagery data from the 2023 Mumbles survey showed no clear distinction between test (with a prefix T_) and control area (with a prefix C_) samples (Figure 15) at 60% similarity, which resulted in only one larger group and two single samples. At 65% similarity (Figure 15), three groups differentiated with more than one sample, however all groups had the same major contributing taxa, and again there was no clear distinction between the control and test areas.

⁷ <https://mcc.jrc.ec.europa.eu/documents/201702074014.pdf>

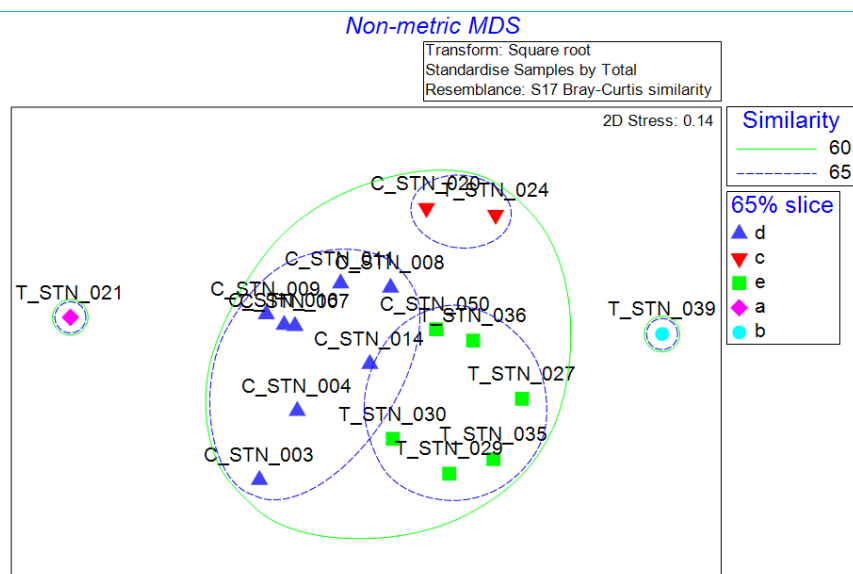


Figure 15.
Analysis of biota from Mumbles 2023 imagery survey in PRIMER - MDS plot from abundance data (60 + 65% slices).

Statistical tests conducted in R on the imagery data from all sample stations showed a greater mean diversity index at the test areas, however the difference between diversities from the control and test area samples was not significant ($p > 0.05$) (Figure 16).

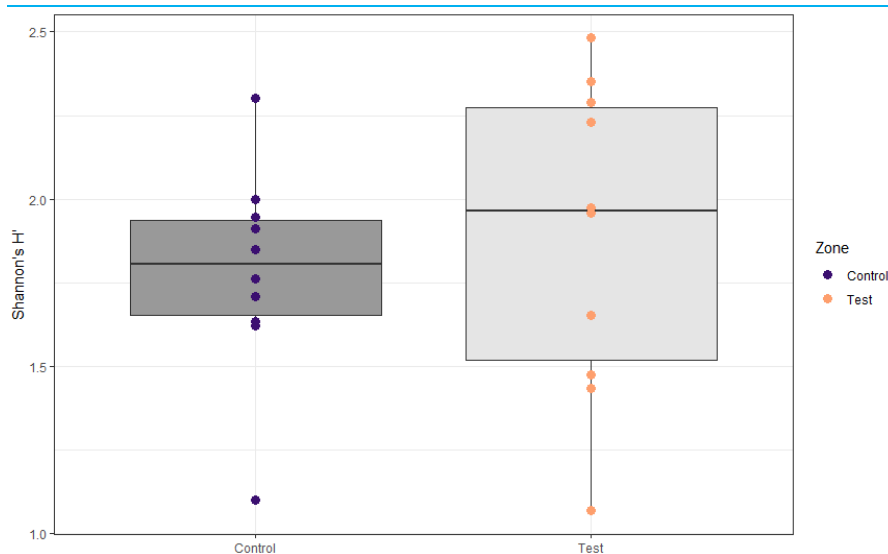


Figure 16.
Analysis of macrofauna from the Mumbles 2023 imagery survey in R – Shannon's Diversity.

5.2.4. Oyster Dredge Analysis Results

The biota collected using the oyster dredge are presented in Table 17 in Appendix A, Section 7.1. Similar taxa were recorded in the oyster dredges as that recorded in the imagery analysis, along with notable quantities of large thick shell material (old, abraded). The fauna recorded in the highest abundances were urchins (*Psammechinus miliaris*), hermit crabs, molluscs including the invasive slipper limpet *Crepidula fornicata*, starfish, brittle stars, small crabs and polychaete worms.

The oyster dredges were deployed as a method for verification of oyster densities in the control and test sites, as oysters were not found in the grab samples, and hard to identify from the underwater

imagery. Eight larger native oysters were found in the test site, as opposed to one collected from the control site, with average numbers per tow being 1.6 in the test site and 0.25 in the control site. None of the smaller native oysters used for restocking the site were sampled.

Multivariate statistical analysis was undertaken with the data from the oyster dredge tows, which showed two groups to differentiate at 50% similarity (Figure 17), with fewer samples in 'group a', all from the test site. These three dredge tows were located at the south of the test site, close to where a deeper area of hard ground 'circalittoral rock' (CR.MCR) was recorded from the imagery data and included taxa typical of hard substrate communities such as keel worms, bryozoa and hydrozoa.

Statistical tests were conducted in 'R' on the dredge data, and a Welch's unequal variances t-test showed there to be no statistical difference ($p > 0.05$) between the diversities of samples from the test and control site.

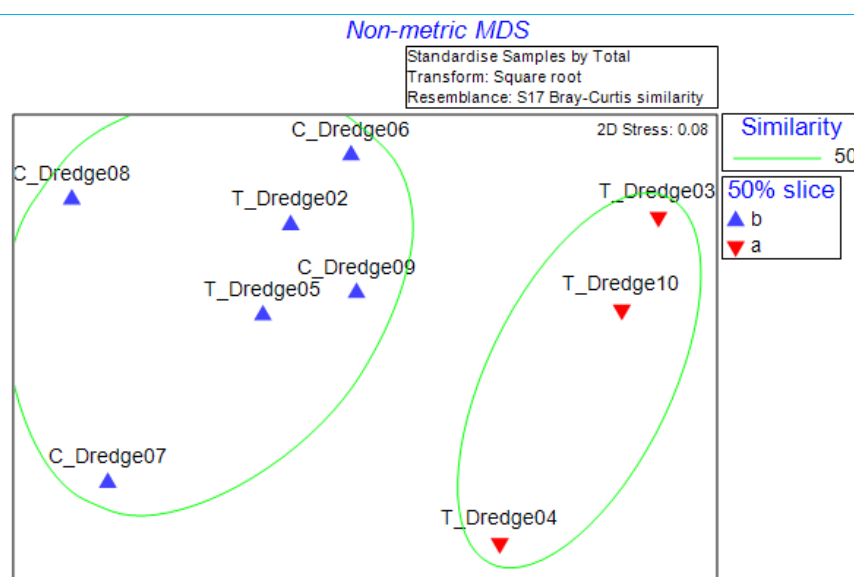


Figure 17.
Analysis of Mumbles 2023
dredge data in PRIMER -
MDS plot from abundance
data (50% slice).

5.2.5. Invasive Non-Native Invasive Species

Two invasive non-native species (INNS) were recorded during the survey which were the leathery tunicate *Styela clava*, and the slipper limpet *Crepidula fornicata*. Both have been recorded at many locations around the UK since first recorded in the middle of the last century. These taxa were recorded in low abundance in the current study.

5.3. Summary and Conclusions – Native Oyster Ranching Site Surveys

Data were collected to measure diversity associated with a ranching oyster site located in Mumbles, Swansea Bay in December 2022 and June 2023 and at a control site nearby, as well as the progress of restocked oysters.

The analyses of the sediment data from the 2022 Mumbles survey showed levels of total organic carbon to be significantly higher in the control area, where samples had a slightly higher silt content, and where diversity indices were also seen to be significantly higher than in the test area. However, multivariate analysis did not show clear grouping of the macrofauna communities between the test and control areas. Conversely, data from the 2023 Mumbles survey showed no statistical difference in total organic carbon between the test and control site, although samples again also showed a higher silt content at the control area. Multivariate analysis of the macrofauna data from 2023 also showed no clear groupings in the benthic communities between the test and control sites, and no clear relationship to sediment particle size composition, and a t-test showed there to be no statistical difference between the diversities of samples from the test and control site. No clear trends are evident from the two years' data.

Imagery data from 2023 also showed no clear distinction between the test and control sites, with the only clear groupings in communities arising where stations were located on harder ground or where the polychaete *Sabella pavonina* was present in high abundances. There was no significant difference in diversity indices between the test and control site from the imagery data. The data from the dredge tows did show some grouping of biota communities from the south of the test site in areas in the vicinity of hard ground, but many of the main contributing taxa were similar for both groups and diversity indices were not significantly different between the test and control site.

The smaller native oysters used to restock the site were not recorded in the current survey, which could potentially have been due to survey methodology or possibly due to displacement by currents or tidal movements, or potential smothering by mobile sediments. Native oysters were difficult to identify from underwater imagery due to faunal and algal overgrowth obscuring the substrate. Larger native oysters, which were relaid in 2013/14 as part of a restoration trial, were recorded using an oyster dredge tow that targeted the 2013/14 site, with eight native oysters recorded in the test site. Wild native oysters are present around the sites but only one was recorded from the control site.

Overall, the results showed no consistent differences between the test and control areas, which could be expected as the restocked smaller native oysters were unrecorded and, if absent, could not have an influence on the diversity of the test site. However, larger native oysters were recorded in greater abundance at the test site with the oyster dredge than in the control site, albeit at relatively low densities, which suggests that native oysters may need to be of a larger size to survive in environments with potentially mobile sediments and turbid waters, or that dredge equipment may need to be targeted towards smaller individuals.

6. Methodology Development & Recommendations

An experimental approach was taken to collect data at the oyster farms and naturalised oyster site during this work and was developed and refined through trials over several months. As data collection was restricted to two days survey at each oyster site, with underwater imagery collected at a single location in the test and control area, the results provide a “snapshot” of the ecological environment experienced at the oyster farm sites and the naturalised oyster site.

This current project was tailored to capture specific aspects of the interaction of oyster sites with the immediate marine environment, however observations were made during site visits indicating that these areas were utilised by other wildlife such as birds, fish and marine mammals. Further research could be designed to assess any additional benefits oyster farms might have upon wider biodiversity.

Should further research be undertaken, experimental design could be developed. Technical improvements in camera deployment would improve data collection, taking into consideration onsite environmental conditions such as currents affecting camera angles and scale. Further research would provide additional evidence to support the findings, with multiple camera system locations, replication of samples and a longer-term data collection strategy enabling more robust datasets and analysis to inform management decisions.

Further research into nitrogen recycling and the microbial activity involved would give further insight into the role of UK oyster sites in these ecosystem services, including characterisation of the diversity of total microbial communities, characterisation of the diversity of ammonia oxidiser and denitrifier communities by gene sequencing and analysis of the effects of cultivated sites on nitrification and denitrification rate measurements from the sediments. Further qPCR analysis of other functional genes involved in denitrification (e.g. *nosZ*) would establish whether complete or partial denitrification is occurring, and incorporation of more growing methods such as on-bed mariculture and also nutrient cycling measurements of other examples of naturalised reefs of Pacific oysters to enable understanding of their contributions to ecosystem function and natural capital.

7. Appendices

7.1. Appendix A: Data Tables

Table 9.

Date, location, TOC and PSA, and multivariate analysis results for sediment samples from the Pacific oyster farm (Pembrokeshire, Lindisfarne, Avon) and naturalised oyster reef (Exe) site surveys, June/July 2023.

Site	Date	Latitude	Longitude	Total carbon (mg/g)	% Gravel	% Sand	% Mud	Sediment Class	BSH	Multivariate Analysis Group
Pembrokeshire										
Control 1	05/06/2023	51.68246	-5.05444	31.28	0.31	85.15	14.54	muddy sand (mS)	Subtidal Sand	b
Control 2	05/06/2023	51.68238	-5.05446	28.38	0.19	87.25	12.55	muddy sand (mS)	Subtidal Sand	b
Control 3	05/06/2023	51.6822	-5.05451	56.59	1.07	90.70	8.23	(gravelly) sand ((g)S)	Subtidal Sand	b
Control 4	06/06/2023	51.6822	-5.05451	38.61	0.11	85.57	14.32	muddy sand (mS)	Subtidal Sand	b
Control 5	06/06/2023	51.68238	-5.05422	30.64	0.13	87.18	12.69	muddy sand (mS)	Subtidal Sand	b
Test 1	06/06/2023	51.683681	-5.054039	22.14	0.52	91.83	7.65	sand (S)	Subtidal Sand	N/A (outlier)
Test 2	06/06/2023	N/A	N/A	28.52	0.79	84.08	15.13	muddy sand (mS)	Subtidal Sand	N/A (outlier)
Test 3	06/06/2023	51.683681	-5.054039	18.36	0.50	90.20	9.30	sand (S)	Subtidal Sand	a
Test 4	06/06/2023	51.68358	-5.05405	22.17	0.23	92.12	7.64	sand (S)	Subtidal Sand	b
Test 5	06/06/2023	51.68362	-5.0542	28.33	0.46	91.89	7.65	sand (S)	Subtidal Sand	a
Lindisfarne										
Control 1	19/06/2023	55.6506	-1.8052	193.55	17.72	77.63	4.65	gravelly sand (gS)	Subtidal Coarse Sediments	b
Control 2	19/06/2023	55.6505	-1.8051	23.06	13.21	80.89	5.91	gravelly sand (gS)	Subtidal Coarse Sediments	b
Control 3	19/06/2023	55.6512	-1.8051	29.35	22.65	72.97	4.38	gravelly sand (gS)	Subtidal Coarse Sediments	b
Control 4	19/06/2023	55.6515	-1.8057	43.33	31.51	63.81	4.68	sandy gravel (sG)	Subtidal Coarse Sediments	b

Site	Date	Latitude	Longitude	Total carbon (mg/g)	% Gravel	% Sand	% Mud	Sediment Class	BSH	Multivariate Analysis Group
Control 5	19/06/2023	55.6515	-1.8056	35.65	19.61	74.98	5.41	gravelly sand (gS)	Subtidal Coarse Sediments	a
Test 1	19/06/2023	55.6495	-1.8045	48.21	13.82	69.79	16.39	gravelly muddy sand (gmS)	Subtidal Mixed Sediments	a
Test 2	19/06/2023	55.6495	-1.8045	20.74	9.52	85.47	5.01	gravelly sand (gS)	Subtidal Coarse Sediments	N/A (outlier)
Test 3	19/06/2023	55.6495	-1.8045	48.10	4.34	82.27	13.39	(gravelly) muddy sand ((g)mS)	Subtidal Sand	a
Test 4	19/06/2023	55.6495	-1.8045	95.86	8.80	80.80	10.39	gravelly muddy sand (gmS)	Subtidal Mixed Sediments	a
Test 5	19/06/2023	55.6495	-1.8045	47.48	6.22	79.46	14.32	gravelly muddy sand (gmS)	Subtidal Mixed Sediments	a
River Avon										
Control 1	06/07/2023	50.28800	-3.86629	36.63	1.16	97.17	1.67	(gravelly) sand ((g)S)	Subtidal Sand	N/A (outlier)
Control 2	06/07/2023	50.28796	-3.86576	39.76	0.17	97.97	1.87	sand (S)	Subtidal Sand	c
Control 3	06/07/2023	50.28792	-3.86611	40.13	1.19	96.92	1.89	(gravelly) sand ((g)S)	Subtidal Sand	b (one sample)
Control 4	06/07/2023	50.28800	-3.86604	55.69	24.45	66.78	8.76	gravelly muddy sand (gmS)	Subtidal Mixed Sediments	N/A (outlier)
Control 5	06/07/2023	50.28.760	-3.86648	81.22	11.24	67.45	21.31	gravelly muddy sand (gmS)	Subtidal Mixed Sediments	c
Test 1	05/07/2023	50.28608	-3.86726	42.59	0.69	91.16	8.15	sand (S)	Subtidal Sand	c
Test 2	05/07/2023	50.28620	-3.86731	44.18	1.15	94.74	4.11	(gravelly) sand ((g)S)	Subtidal Sand	N/A (outlier)
Test 3	05/07/2023	50.28559	-3.86756	31.05	3.64	94.47	1.89	(gravelly) sand ((g)S))	Subtidal Sand	a
Test 4	05/07/2023	50.28541	-3.86772	50.52	15.92	77.54	6.54	gravelly sand (gS)	Subtidal Coarse Sediments	c
Test 5	05/07/2023	N/A (closest to slipway)		44.57222	44.57	94.73	4.58	sand (S)	sand (S)	a
River Exe										

Site	Date	Latitude	Longitude	Total carbon (mg/g)	% Gravel	% Sand	% Mud	Sediment Class	BSH	Multivariate Analysis Group
Control 1	07/07/2023	50.64006	-3.43099	73.22	1.05	15.46	83.50	(gravelly) sandy mud ((g)sM)	Subtidal Mud	b
Control 2	07/07/2023	50.64084	-3.43069	62.83	0.25	23.47	76.28	sandy mud (sM)	Subtidal Mud	a
Control 3	07/07/2023	50.64012	-3.43098	73.62	0.03	10.85	89.12	sandy mud (sM)	Subtidal Mud	b
Control 4	07/07/2023	50.64000	-3.43056	72.35	0.87	10.72	88.42	sandy mud (sM)	Subtidal Mud	b
Control 5	07/07/2023	50.64016	-3.43087	73.71	1.26	12.20	86.54	(gravelly) sandy mud ((g)sM)	Subtidal Mud	b
Test 1	07/07/2023	50.66495	-3.46680	62.38	0.02	20.71	79.28	sandy mud (sM)	Subtidal Mud	a
Test 2	07/07/2023	50.63979	-3.43178	60.27	1.61	29.79	68.60	(gravelly) sandy mud ((g)sM)	Subtidal Mud	a
Test 3	07/07/2023	50.63980	-3.43180	59.77	1.90	28.08	70.02	(gravelly) sandy mud ((g)sM)	Subtidal Mud	a
Test 4	07/07/2023	50.63985	-3.43176	59.23	3.60	26.56	69.84	(gravelly) sandy mud ((g)sM)	Subtidal Mud	a
Test 5	07/07/2023	50.63982	-3.43187	54.91	4.63	34.30	61.06	(gravelly) sandy mud ((g)sM)	Subtidal Mud	a

Table 10

Results from AMBI software, showing sample composition by ecological group percent (I-V(%)), percent not assigned, AMBI values, disturbance classification, diversity, richness, , Multivariate AMBI (M-AMBI) and assessment of ecological status from factorial analysis of macrofauna samples from the Pacific oyster farm (Pembrokeshire, Lindisfarne, Avon) and naturalised oyster reef (Exe) site surveys, June/July 2023.

Area	Sample	I(%)	II(%)	III(%)	IV(%)	V(%)	% Not Assigned	AMBI	Disturbance Classification	Diversity	Richness	M-AMBI	Status
Pembrokeshire													
Control	1	43.75	31.25	25.00	0.00	0.00	0.00	1.22	Heavily disturbed	3.16	10	0.8950	High
	2	9.09	0.00	59.09	0.00	31.82	42.10	3.68	Slightly disturbed	2.06	7	0.5525	Good
	3	12.12	21.21	48.49	0.00	18.18	37.70	2.86	Slightly disturbed	2.91	13	0.8509	High

Area	Sample	I(%)	II(%)	III(%)	IV(%)	V(%)	% Not Assigned	AMBI	Disturbance Classification	Diversity	Richness	M-AMBI	Status
	4	33.33	22.22	33.33	0.00	11.11	62.50	2.00	Undisturbed	2.43	9	0.7382	Good
	5	5.88	5.88	23.53	0.00	64.71	50.00	4.68	Slightly disturbed	2.2	9	0.5663	Good
Test	6	14.29	14.29	28.57	42.86	0.00	0.00	3.00	Slightly disturbed	2.13	5	0.5482	Good
	7	23.53	29.41	47.06	0.00	0.00	39.30	1.85	Slightly disturbed	3.14	13	0.9332	High
	8	66.67	0.00	22.22	11.11	0.00	18.20	1.17	Slightly disturbed	2.22	6	0.6830	Good
	9	68.75	18.75	12.50	0.00	0.00	0.00	0.66	Slightly disturbed	1.84	5	0.6385	Good
	10	67.74	9.68	6.45	16.13	0.00	22.50	1.07	Slightly disturbed	2.86	12	0.9166	High
Lower Limit	Bad							6		0	0	8.79E-17	Bad
Upper Limit	High							0.66		3.16	13	1	High
Lindisfarne													
Control	1	15.10	3.80	67.90	1.90	11.30	13.10	2.86	Slightly disturbed	2.75	14	0.8433	High
	2	56.70	13.30	16.70	0.00	13.30	25.00	1.50	Slightly disturbed	2.78	11	0.8803	High
	3	50.00	21.90	25.00	0.00	3.10	0.00	1.27	Slightly disturbed	3.04	14	0.9900	High
	4	56.50	8.70	34.80	0.00	0.00	32.40	1.17	Undisturbed	2.85	11	0.9111	High
	5	50.00	25.00	25.00	0.00	0.00	81.80	1.13	Undisturbed	1.46	6	0.6428	Good
Test	6	33.30	33.30	33.30	0.00	0.00	53.80	1.50	Slightly disturbed	1.88	5	0.6467	Good
	7	22.20	44.40	33.30	0.00	0.00	30.80	1.67	Slightly disturbed	2.20	5	0.6735	Good
	8	40.00	10.00	40.00	0.00	10.00	0.00	1.95	Slightly disturbed	2.65	7	0.7493	Good
	9	0.00	50.00	50.00	0.00	0.00	40.00	2.25	Slightly disturbed	1.85	4	0.5692	Good
	10	25.00	25.00	25.00	25.00	0.00	63.60	2.25	Slightly disturbed	1.67	5	0.5690	Good
Lower Limit	Bad							6		0	0	0	Bad
Upper Limit	High							1.13		3.04	14	1	High
River Avon													

Area	Sample	I(%)	II(%)	III(%)	IV(%)	V(%)	% Not Assigned	AMBI	Disturbance Classification	Diversity	Richness	M-AMBI	Status
Control	1	0.00	0.00	0.00	0.00	100.00	0.00	6.00	Heavily disturbed	0.00	1	0.0600	Bad
	2	0.00	81.48	0.00	0.00	18.52	6.90	2.33	Slightly disturbed	1.44	6	0.8407	High
	3	0.00	60.00	40.00	0.00	0.00	50.00	2.10	Slightly disturbed	1.49	3	0.6818	Good
	4	50.00	50.00	0.00	0.00	0.00	0.00	0.75	Undisturbed	1.00	2	0.6085	Good
	5	0.00	92.86	7.14	0.00	0.00	0.00	1.61	Slightly disturbed	0.73	3	0.5696	Good
Test	6	0.00	100.00	0.00	0.00	0.00	25.00	1.50	Slightly disturbed	0.81	2	0.5301	Good
	7	25.00	0.00	75.00	0.00	0.00	0.00	2.25	Slightly disturbed	0.81	2	0.4871	Moderate
	8	42.86	42.86	0.00	0.00	14.29	12.50	1.50	Slightly disturbed	1.81	4	0.8372	High
	9	0.00	57.14	7.14	0.00	35.71	0.00	3.21	Slightly disturbed	1.26	3	0.5763	Good
	10	15.79	10.53	73.68	0.00	0.00	0.00	2.37	Slightly disturbed	1.09	3	0.5918	Good
Lower Limit	Bad							6		0	0	0	Bad
Upper Limit	High							0.75		1.81	6	1	High
River Exe													
Control	1	6.30	43.80	25.00	0.00	25.00	11.10	2.91	Slightly disturbed	2.64	8	0.7352	Good
	2	0.00	0.00	100.00	0.00	0.00	12.50	3.00	Slightly disturbed	1.37	4	0.4772	Moderate
	3	57.90	10.50	31.60	0.00	0.00	9.50	1.11	Undisturbed	2.26	8	0.8259	High
	4	6.30	43.80	50.00	0.00	0.00	15.80	2.16	Slightly disturbed	2.80	8	0.8053	High
	5	0.00	3.30	93.30	0.00	3.30	0.00	3.05	Slightly disturbed	1.08	5	0.4740	Moderate
Test	6	0.00	5.30	89.50	0.00	5.30	5.00	3.08	Slightly disturbed	2.18	6	0.6148	Good
	7	0.00	28.60	71.40	0.00	0.00	36.40	2.57	Slightly disturbed	1.68	4	0.5393	Good
	8	6.10	21.20	54.50	3.00	15.20	2.90	3.00	Slightly disturbed	3.08	11	0.8638	High
	9	0.00	33.30	38.90	22.20	5.60	0.00	3.00	Slightly disturbed	2.75	8	0.7399	Good
	10	0.00	18.20	45.50	36.40	0.00	8.30	3.27	Slightly disturbed	2.08	5	0.5610	Good
Lower Limit	Bad							6		0	0	0	Bad

Area	Sample	I(%)	II(%)	III(%)	IV(%)	V(%)	% Not Assigned	AMBI	Disturbance Classification	Diversity	Richness	M-AMBI	Status
Upper Limit	High							1.11		3.08	11	1	High

Table 11.

Imagery Analysis – Hutcheson's t-test values for comparison of Shannon's Diversity Index (SDI) between test and control areas (different camera positions, for both solitary/erect taxa (count) and percent cover taxa (%)) from the Pacific oyster farm (Pembrokeshire, Lindisfarne, Avon) and naturalised oyster reef (Exe) site surveys, June/July 2023.

Biota	SDI Test	SDI Control	Higher SDI	T Value	Critical Value	P Value	Significant	Comments
Pembrokeshire Biota								
Lower- Count	1.12	1.38	Control	1.00	1.99	0.3214	No	
Lower- Percent	/	/	/	/	/	/	N/A	One taxon recorded in test, 0 in control
Middle- Count	0.00	0.40	Control	0.94	2.57	0.3901	No	One taxon recorded in test
Middle- Percent	/	/	/	/	/	/	N/A	One taxon recorded in test, 0 in control
Upper- Count	0.07	0.40	Control	0.76	2.57	0.4841	No	
Upper- Percent	/	/	/	/	/	/	N/A	One taxon recorded in test, 0 in control
Native Lower- Count	1.14	1.38	Control	0.84	1.99	0.4028	No	
Native Lower- Percent	1.50	/	Native	/	/	/	N/A	No taxa recorded in control
Native Upper- Count	0.00	0.40	Control	0.94	2.57	0.3901	No	One taxon recorded in native
Native Upper- Percent	/	/	/	/	/	/	N/A	No taxa recorded in native or control
Lindisfarne Biota								
Lower - Count	0.75	0.68	Test	0.62	1.97	0.5380	No	
Lower - Percent	1.07	0.64	Test	1.00	2.26	0.3428	No	
Middle - Count	1.01	0	Test	2.41	4.30	0.1378	No	Only 1 taxon recorded in control
Middle - Percent	1.21	/	Test	/	/	/	N/A	No taxa recorded in control

Biota	SDI Test	SDI Control	Higher SDI	T Value	Critical Value	P Value	Significant	Comments
Upper - Count	1.23	0	Test	3.74	2.20	0.0033	Yes	Only 1 taxon recorded in control
Upper - Percent	1.29	/	Test	/	/	/	N/A	No taxa recorded in control
Avon Biota								
Lower - Count	1.45	0.49	Test	3.85	2.00	0.0003	Yes	
Lower - Percent	0.00	0.00	/	/	/	/	No	1 taxon recorded in test and in control
Middle - Count	0.69	0.52	Test	1.14	2.45	0.2980	No	
Middle - Percent	1.10	/	Test	/	/	/	N/A	No taxa recorded in control
Upper - Count	0.69	0.52	Test	3.43	1.98	0.0008	Yes	
Upper - Percent	0.33	/	Test	/	/	/	N/A	No taxa recorded in control
Exe Biota								
Lower - Count	1.23	0.37	Control	0.82	1.97	0.41	No	
Lower - Percent	1.27	0.00	Test	7.75	2.18	0.00001	Yes	Only 1 taxa recorded in control
Upper - Count	1.10	0.90	Test	0.25	12.71	0.85	No	
Upper - Percent	/	/	/	/	/	/	N/A	No taxa recorded

Table 12.

Grab sample information including coordinates, depth, Total Organic Carbon, Folk sediment class and multivariate analysis groups for each station from Mumbles Oyster Site survey 2022.

Date	Station	Sample Type	Latitude	Longitude	Depth (m)	Total Carbon (mg/g)	Sediment Class	Initial Analysis	Further Analysis
01/12/2022	STN03	INF1	51.57651	-3.97748	6.9	N/A	N/A	c	d
01/12/2022	STN03	INF2	51.57659	-3.97756	6.9	N/A	N/A	c	d
01/12/2022	STN03	INF3	51.57646	-3.97776	6.9	N/A	N/A	c	d
01/12/2022	STN03	INF4	51.57658	-3.97771	6.9	N/A	N/A	c	d
01/12/2022	STN03	INF5	51.57631	-3.97757	6.9	N/A	N/A	c	d
01/12/2022	STN03	PSA	51.57647	-3.97762	6.9	122.05	muddy sand	N/A	N/A

Date	Station	Sample Type	Latitude	Longitude	Depth (m)	Total Carbon (mg/g)	Sediment Class	Initial Analysis	Further Analysis
29/11/2022	STN04	INF1	51.57624	-3.97913	5	N/A	N/A	c	d
29/11/2022	STN04	INF2	51.5762	-3.97891	5	N/A	N/A	c	d
29/11/2022	STN04	INF4	51.57594	-3.97891	5	N/A	N/A	c	d
29/11/2022	STN04	INF5	51.57617	-3.97873	5	N/A	N/A	c	d
29/11/2022	STN04	INF3	51.57601	-3.97903	5	N/A	N/A	b	N/A
29/11/2022	STN04	PSA	51.57621	-3.9792	5	97.84	sandy mud	N/A	N/A
30/11/2022	STN07	INF1	51.57583	-3.97687	10	N/A	N/A	c	d
30/11/2022	STN07	INF2	51.57586	-3.97698	10	N/A	N/A	c	d
30/11/2022	STN07	INF3	51.57573	-3.97615	10	N/A	N/A	c	d
30/11/2022	STN07	INF4	51.5758	-3.97634	10	N/A	N/A	c	d
30/11/2022	STN07	INF5	51.57574	-3.97704	10	N/A	N/A	c	d
30/11/2022	STN07	PSA	51.57586	-3.9766	10	136.59	muddy sand	N/A	N/A
01/12/2022	STN09	INF1	51.57537	-3.97522	N/A	N/A	N/A	c	d
01/12/2022	STN09	INF2	51.57517	-3.97522	N/A	N/A	N/A	c	d
01/12/2022	STN09	INF3	51.57523	-3.97516	N/A	N/A	N/A	c	d
01/12/2022	STN09	INF4	51.57523	-3.97516	N/A	N/A	N/A	c	d
01/12/2022	STN09	INF5	51.57523	-3.97518	N/A	N/A	N/A	c	d
01/12/2022	STN09	PSA	51.57527	-3.97501	N/A	80.57	muddy sand	N/A	N/A
30/11/2022	STN14	INF1	51.57443	-3.97607	10	N/A	N/A	c	d
30/11/2022	STN14	INF2	51.57441	-3.97609	10	N/A	N/A	c	d
30/11/2022	STN14	INF3	51.5743	-3.97635	10	N/A	N/A	c	d
30/11/2022	STN14	INF4	51.5745	-3.97559	10	N/A	N/A	c	d
30/11/2022	STN14	INF5	51.57445	-3.97572	10	N/A	N/A	c	d
30/11/2022	STN14	PSA	51.5743	-3.97565	10	82.26	muddy sand	N/A	N/A
29/11/2022	STN16	INF1	51.57443	-3.97441	5	N/A	N/A	c	d

Date	Station	Sample Type	Latitude	Longitude	Depth (m)	Total Carbon (mg/g)	Sediment Class	Initial Analysis	Further Analysis
29/11/2022	STN16	INF2	51.57424	-3.97445	5	N/A	N/A	c	d
29/11/2022	STN16	INF4	51.57437	-3.97422	5	N/A	N/A	c	d
29/11/2022	STN16	INF5	51.57426	-3.9742	5	N/A	N/A	c	d
29/11/2022	STN16	INF3	51.57434	-3.97444	5	N/A	N/A	d	N/A
29/11/2022	STN16	PSA	51.57457	-3.97454	5	114.87	muddy sand	N/A	N/A
01/12/2022	STN20	INF1	51.57352	-3.97335	8.5	N/A	N/A	c	d
01/12/2022	STN20	INF2	51.5736	-3.9738	8.5	N/A	N/A	c	d
01/12/2022	STN20	INF3	51.57372	-3.97371	8.5	N/A	N/A	c	d
01/12/2022	STN20	INF4	51.57346	-3.97396	8.5	N/A	N/A	c	d
01/12/2022	STN20	INF5	51.57345	-3.97384	8.5	N/A	N/A	c	d
01/12/2022	STN20	PSA	51.57349	-3.97361	8.5	123.69	muddy sand	N/A	N/A
29/11/2022	STN21	INF5	51.57357	-3.97036	10	N/A	N/A	c	a
29/11/2022	STN21	INF4	51.57372	-3.97051	10	N/A	N/A	c	b
29/11/2022	STN21	INF2	51.57313	-3.97065	10	N/A	N/A	c	d
29/11/2022	STN21	INF3	51.57283	-3.9704	10	N/A	N/A	c	d
29/11/2022	STN21	INF1	51.57334	-3.97073	10	N/A	N/A	d	N/A
29/11/2022	STN21	PSA	51.57329	-3.97079	10	62.41	sand	N/A	N/A
01/12/2022	STN24	INF1	51.5724	-3.97279	9	N/A	N/A	c	d
01/12/2022	STN24	INF3	51.57229	-3.97278	9	N/A	N/A	c	d
01/12/2022	STN24	INF4	51.57235	-3.97294	9	N/A	N/A	c	d
01/12/2022	STN24	INF5	51.57225	-3.97288	9	N/A	N/A	c	d
01/12/2022	STN24	INF2	51.57236	-3.97284	9	N/A	N/A	d	N/A
01/12/2022	STN24	PSA	51.57235	-3.9728	9	102.98	muddy sand	N/A	N/A
29/11/2022	STN29	INF1	51.57137	-3.97001	9.2	N/A	N/A	c	a
29/11/2022	STN29	INF2	51.57145	-3.96991	9.2	N/A	N/A	c	d

Date	Station	Sample Type	Latitude	Longitude	Depth (m)	Total Carbon (mg/g)	Sediment Class	Initial Analysis	Further Analysis
29/11/2022	STN29	INF4	51.57214	-3.97022	9.2	N/A	N/A	d	N/A
01/12/2022	STN29	PSA	51.57183	-3.97011	9.2	58.26	sand	N/A	N/A
29/11/2022	STN29	INF3	51.57185	-3.96979	9.2	N/A	N/A	N/A	N/A
29/11/2022	STN29	INF5	51.5717	-3.9701	9.2	N/A	N/A	N/A	N/A
01/12/2022	STN30	INF5	51.5715	-3.9686	8.7	N/A	N/A	c	a
01/12/2022	STN30	INF4	51.57121	-3.96871	8.7	N/A	N/A	c	d
01/12/2022	STN30	INF1	51.57135	-3.96874	8.7	N/A	N/A	a	N/A
01/12/2022	STN30	INF2	51.57152	-3.96848	8.7	N/A	N/A	d	N/A
01/12/2022	STN30	PSA	51.57154	-3.96866	8.7	52.71	sand	N/A	N/A
01/12/2022	STN30	INF3	51.57124	-3.96847	8.7	N/A	N/A	N/A	N/A
01/12/2022	STN35	INF1	51.5707	-3.97055	9.5	N/A	N/A	c	c
01/12/2022	STN35	INF2	51.57075	-3.97044	9.5	N/A	N/A	c	c
01/12/2022	STN35	INF4	51.5706	-3.97076	9.5	N/A	N/A	c	c
01/12/2022	STN35	INF5	51.57066	-3.97056	9.5	N/A	N/A	c	c
01/12/2022	STN35	INF3	51.57057	-3.97087	9.5	N/A	N/A	c	d
01/12/2022	STN35	PSA	51.57064	-3.97082	9.5	132.39	muddy sand	N/A	N/A
29/11/2022	STN36	INF3	51.57059	-3.96789	5	N/A	N/A	c	a
29/11/2022	STN36	INF5	51.57076	-3.96787	5	N/A	N/A	c	d
29/11/2022	STN36	INF1	51.57079	-3.968	5	N/A	N/A	d	N/A
29/11/2022	STN36	INF2	51.5704	-3.96749	5	N/A	N/A	d	N/A
29/11/2022	STN36	INF4	51.57076	-3.96792	5	N/A	N/A	d	N/A
29/11/2022	STN36	PSA	51.57038	-3.96743	5	56.64	sandy gravel	N/A	N/A
30/11/2022	STN38	N/A	51.56921	-3.96878	10	N/A	N/A	N/A	N/A
30/11/2022	STN38	N/A	51.56904	-3.96869	10	N/A	N/A	N/A	N/A
30/11/2022	STN38	N/A	51.56879	-3.96862	10	N/A	N/A	N/A	N/A

Date	Station	Sample Type	Latitude	Longitude	Depth (m)	Total Carbon (mg/g)	Sediment Class	Initial Analysis	Further Analysis
01/12/2022	STN38	N/A	51.56933	-3.96862	10	N/A	N/A	N/A	N/A
01/12/2022	STN38	N/A	51.56932	-3.96867	10	N/A	N/A	N/A	N/A
01/12/2022	STN39	INF5	51.56942	-3.96651	10	N/A	N/A	c	d
01/12/2022	STN39	INF1	51.56954	-3.9662	10	N/A	N/A	a	N/A
01/12/2022	STN39	INF2	51.56928	-3.96651	10	N/A	N/A	d	N/A
01/12/2022	STN39	INF3	51.56935	-3.96659	10	N/A	N/A	d	N/A
01/12/2022	STN39	INF4	51.56934	-3.96653	10	N/A	N/A	d	N/A
01/12/2022	STN39	PSA	51.56912	-3.96648	10	61.58	sand	N/A	N/A

Table 13.

Sediment sample (PSA and TOC) locations, depths and results for each sample station (STN), Mumbles 2023 survey, June 2023.

	STN	DATE	TIME	LAT	LONG	DEPTH (m)	TOC (mg/g)	% Gravel	% Sand	% Mud	Folk Class	Broadscale Habitat (BSH)
CONTROL	STN03	12/06/2023	08:15:16	51.57680	-3.97730	2.0	84.37908	4.96	67.92	27.13	muddy sand	Subtidal Mud
	STN04	12/06/2023	08:08:40	51.57610	-3.97884	1.6	76.41308	2.47	73.83	23.69	muddy sand	Subtidal Mud
	STN07	12/06/2023	08:30:04	51.57590	-3.97672	2.2	82.51162	3.00	81.22	15.78	muddy sand	Subtidal Sand
	STN08	12/06/2023	07:59:45	51.57530	-3.97800	1.8	105.57409	4.92	64.14	30.94	muddy sand	Subtidal Mud
	STN09	12/06/2023	07:44:28	51.57510	-3.97531	2.5	115.33187	2.44	83.26	14.31	muddy sand	Subtidal Sand
	STN11	12/06/2023	07:50:44	51.57490	-3.97729	2.0	102.27341	5.50	70.38	24.11	gravelly muddy sand	Subtidal Mixed Sediments
	STN14	11/06/2023	16:23:25	51.57430	-3.97610	N/A	76.98991	13.75	75.62	10.64	gravelly muddy sand	Subtidal Mixed Sediments
	STN16	11/06/2023	10:11:52	51.57450	-3.97438	5.5	50.62762	3.14	94.23	2.63	sand	Subtidal Sand
	STN20	11/06/2023	10:06:00	51.57360	-3.97386	5.1	77.77814	9.51	69.38	21.11	gravelly muddy sand	Subtidal Mixed Sediments

	STN	DATE	TIME	LAT	LONG	DEPTH (m)	TOC (mg/g)	% Gravel	% Sand	% Mud	Folk Class	Broadscale Habitat (BSH)
	STN50	12/06/2023	08:40:20	51.57280	-3.97448	3.0	92.09331	7.71	54.07	38.22	gravelly muddy sand	Subtidal Mixed Sediments
TEST	STN21	11/06/2023	09:35:47	51.57330	-3.97093	5.5	53.21185	0.18	99.75	0.07	sand	Subtidal Sand
	STN24	11/06/2023	09:58:42	51.57260	-3.97294	6.5	75.17186	8.49	72.29	19.22	gravelly muddy sand	Subtidal Mixed Sediments
	STN27	11/06/2023	09:48:38	51.57190	-3.97141	6.3	129.12463	4.49	65.05	30.46	muddy sand	Subtidal Mud
	STN29	12/06/2023	09:47:32	51.57180	-3.96999	5.1	88.86787	20.34	69.64	10.02	gravelly muddy sand	Subtidal Mixed Sediments
	STN30	11/06/2023	10:26:05	51.57170	-3.96849	7.6	44.2224	0.95	98.52	0.53	sand	Subtidal Sand
	STN32	12/06/2023	09:35:50	51.57090	-3.96991	5.1	103.17398	9.67	66.32	24.00	gravelly muddy sand	Subtidal Mixed Sediments
	STN35	11/06/2023	11:48:20	51.57070	-3.97083	10.2	88.70342	17.00	57.28	25.73	gravelly muddy sand	Subtidal Mixed Sediments
	STN36	12/06/2023	10:09:15	51.57060	-3.96799	6.1	49.56395	12.86	83.28	3.86	gravelly sand	Subtidal Coarse Sediments
	STN39	11/06/2023	11:10:27	51.56950	-3.96542	11.1	50.37842	3.61	95.76	0.64	sand	Subtidal Sand
	STN60	12/06/2023	09:57:20	51.56990	-3.96962	7.0	65.9066	31.83	49.46	18.70	muddy sandy gravel	Subtidal Mixed Sediments

Table 14.

Information from sediment samples (macrofauna), including locations and depths for each sample station, Mumbles 2023 survey, June 2023.

DATE	TIME	STATION	SITE	LATITUDE	LONGITUDE	DEPTH (m)	Multivariate Analysis Group
12/06/2023	08:21:52	STN03	CONTROL	51.57660	-3.97742	2.0	a
12/06/2023	08:10:56	STN04	CONTROL	51.57610	-3.97912	1.6	b
12/06/2023	08:30:53	STN07	CONTROL	51.57590	-3.97672	2.2	N/A (outlier)
12/06/2023	08:00:22	STN08	CONTROL	51.57550	-3.97794	1.8	b
12/06/2023	07:45:05	STN09	CONTROL	51.57510	-3.97531	2.5	a
12/06/2023	07:52:48	STN11	CONTROL	51.57490	-3.97729	2.0	a

DATE	TIME	STATION	SITE	LATITUDE	LONGITUDE	DEPTH (m)	Multivariate Analysis Group
11/06/2023	16:20:28	STN14	CONTROL	51.57470	-3.97554	N/A	a
11/06/2023	10:12:52	STN16	CONTROL	51.57450	-3.97438	5.5	NA (outlier)
11/06/2023	10:06:47	STN20	CONTROL	51.57360	-3.97386	5.1	b
12/06/2023	08:41:27	STN50	CONTROL	51.57280	-3.97448	3.0	a
11/06/2023	09:42:08	STN21	TEST	51.57380	-3.97086	5.5	b
11/06/2023	09:57:32	STN24	TEST	51.57240	-3.97306	6.5	a
11/06/2023	09:49:49	STN27	TEST	51.57190	-3.97141	6.3	a
12/06/2023	09:50:00	STN29	TEST	51.57200	-3.97007	5.1	b
11/06/2023	10:27:45	STN30	TEST	51.57190	-3.96829	7.6	b
12/06/2023	09:36:44	STN32	TEST	51.57090	-3.96991	5.1	a
11/06/2023	11:58:35	STN35	TEST	51.57040	-3.97021	10.2	b
12/06/2023	10:11:33	STN36	TEST	51.57060	-3.96799	6.1	b
11/06/2023	11:16:58	STN39	TEST	51.56930	-3.96584	11.1	NA (no macrofauna present)
12/06/2023	09:59:09	STN60	TEST	51.57000	-3.96936	7.0	b

Table 15.

Information for video samples, including locations and depths for each station, Mumbles 2023 underwater imagery analysis survey, June 2023.

DATE	TIME	VIDEO NO	STATION	SITE	LATITUDE START	LONGITUDE START	LATITUDE END	LONGITUDE END	DEPTH (m)
12/06/2023	14:29:25	TAKE017	STN003	CONTROL	51.57670	-3.97772	51.57660	-3.97714	7.3
12/06/2023	14:40:50	TAKE018	STN004	CONTROL	51.57610	-3.97891	51.57590	-3.97817	6.9
12/06/2023	14:18:04	TAKE016	STN007	CONTROL	51.57590	-3.97705	51.57580	-3.97646	7.4
12/06/2023	14:50:59	TAKE019	STN008	CONTROL	51.57540	-3.97871	51.57540	-3.97795	7
12/06/2023	14:08:51	TAKE015	STN009	CONTROL	51.57530	-3.97569	51.57520	-3.97544	7.8
12/06/2023	15:05:30	TAKE020	STN011	CONTROL	51.57490	-3.97769	51.57480	-3.97719	7
12/06/2023	13:59:49	TAKE014	STN014	CONTROL	51.57440	-3.97604	51.57430	-3.97540	7.6
12/06/2023	13:49:22	TAKE013	STN016	CONTROL	51.57450	-3.97501	51.57440	-3.97443	7.9
12/06/2023	13:36:57	TAKE012	STN020	CONTROL	51.57350	-3.97397	51.57330	-3.97311	7.8

11/06/2023	14:34:37	TAKE011	STN050	CONTROL	51.57320	-3.97491	51.57280	-3.97353	7.4
11/06/2023	14:01:25	TAKE009	STN021	TEST	51.57370	-3.97150	51.57330	-3.97014	8.7
11/06/2023	14:15:03	TAKE010	STN024	TEST	51.57240	-3.97307	51.57210	-3.97161	8.6
11/06/2023	13:50:26	TAKE008	STN027	TEST	51.57200	-3.97185	51.57170	-3.97026	9.4
11/06/2023	13:05:35	TAKE004	STN029	TEST	51.57200	-3.97050	51.57180	-3.96962	9.7
11/06/2023	13:11:49	TAKE005	STN030	TEST	51.57170	-3.96905	51.57140	-3.96831	9.9
11/06/2023	12:52:50	TAKE003	STN035	TEST	51.57070	-3.97092	51.57040	-3.96960	11
11/06/2023	13:22:21	TAKE006	STN036	TEST	51.57070	-3.96855	51.57030	-3.96757	10.3
13/06/2023	14:45:30	TAKE022	STN038	TEST	51.56900	-3.96914	51.56870	-3.96728	14.2
11/06/2023	12:43:08	TAKE002	STN039	TEST	51.56920	-3.96691	51.56890	-3.96620	11.6
11/06/2023	13:32:52	TAKE007	STN060	TEST	51.56990	-3.97041	51.56980	-3.96899	11.9

Table 16.

Sample station information, broadscale habitat and MNCR code for Mumbles 2023 underwater imagery analysis survey, June 2023.

STN	TAKE	Site	Imagery Broadscale Habitat	Biotope/Habitat (MNCR Code)	Image Quality
STN003	TAKE017	CONTROL	Subtidal Sand	SS.SSa.IMuSa	Good
STN004	TAKE018	CONTROL	Subtidal Sand	SS.SSa.IMuSa	Good
STN007	TAKE016	CONTROL	Subtidal Coarse Sediment	SS.SCS.ICS	Excellent
STN008	TAKE019	CONTROL	Moderate Energy Infralittoral Rock	IR.MIR	Excellent
STN009	TAKE015	CONTROL	Subtidal Mixed Sediment	SS.SMx.IMx	Excellent
STN011	TAKE020	CONTROL	Subtidal Coarse Sediment	SS.SCS.ICS	Excellent
STN014	TAKE014	CONTROL	Subtidal Mixed Sediment	SS.SMx.IMx	Excellent
STN016	TAKE013	CONTROL	Subtidal Mixed Sediment	SS.SMx.IMx	Excellent
STN020	TAKE012	CONTROL	Subtidal Mixed Sediment	SS.SMx.IMx.SpavSpAn	Excellent
STN050	TAKE011	CONTROL	Subtidal Mixed Sediment	SS.SMx.IMx	Excellent
STN021	TAKE009	TEST	Subtidal Sand	SS.SSa.IMuSa	Excellent
STN024	TAKE010	TEST	Subtidal Mixed Sediment	SS.SMx.IMx.SpavSpAn	Good
STN027	TAKE008	TEST	Subtidal Mixed Sediment	SS.SMx.IMx	Good

STN	TAKE	Site	Imagery Broadscale Habitat	Biotope/Habitat (MNCR Code)	Image Quality
STN029	TAKE004	TEST	Subtidal Mixed Sediment	SS.SMx.IMx	Good
STN030	TAKE005	TEST	Subtidal Coarse Sediment	SS.SCS.ICS	Excellent
STN035	TAKE003	TEST	Subtidal Mixed Sediment	SS.SMx.IMx	Good
STN036	TAKE006	TEST	Subtidal Coarse Sediment	SS.SCS.ICS	Excellent
STN038	TAKE022	TEST	Moderate Energy Circalittoral Rock	CR.MIR	Excellent
STN039	TAKE002	TEST	Subtidal Mixed Sediment	SS.SMx.IMx	Good
STN060	TAKE007	TEST	Moderate Energy Circalittoral Rock	CR.MIR	Good

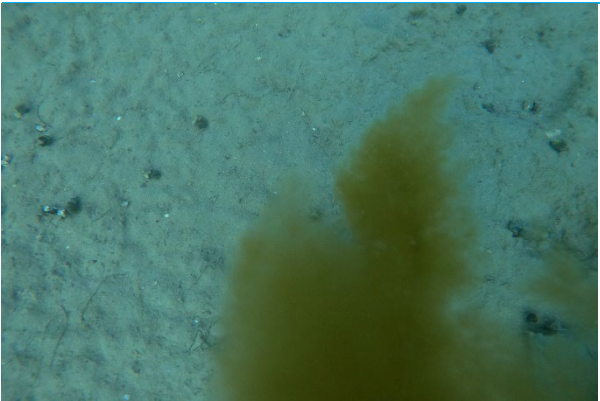
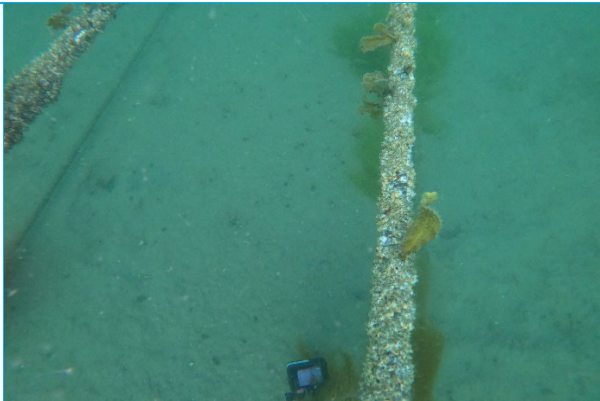
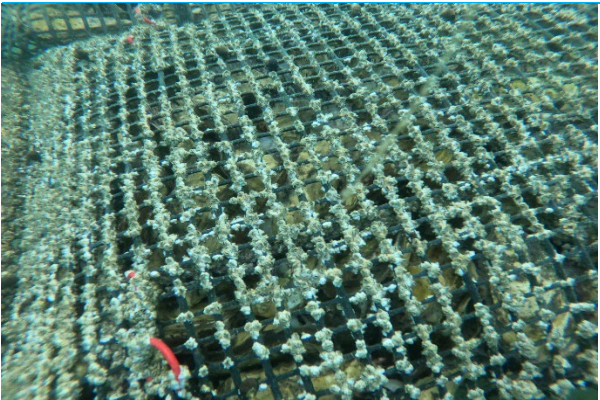
Table 17

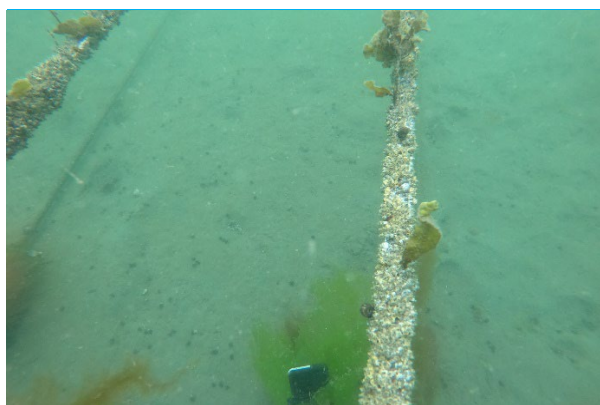
Biota recorded from dredge tows undertaken during the Mumbles 2023 survey, June 2023.

Dredge Tow	02	03	04	05	06	07	08	09	10
Actiniaria			1						
Alcyonidium	1		1	1					
Asterias rubens	18	2	1	7	6	2	11	10	1
Brachyura	10	1		1	1			8	4
Bryozoa	1	1	1	1	1	1	1	1	1
Buccinidae	5	1		1		1		1	
Caridea									1
Chlorophyta	1	1		1	1	1	1	1	1
Cirripedia							1		
Copepoda	3			1	1			2	
Crepidula fornicata	9		1		17			5	6
Flustra foliacea				1	1				
Halichondria			2	1					
Hydrozoa	1	1	1	1	1	1	1	1	1
Lanice	2				1				
Macropodia	1							1	
Mollusca	6	1	3	1	1				2
Ophiura albida	17			15			4	4	
Ophiuroidea	2		2	13	2	1			
Ostreida			2					1	6
Paguroidea	47		5	23	8	3	9	32	2
Phaeophyceae	1	1			1		1		1
Pleuronectiformes								1	
Polychaeta	1		3	1				1	1
Psammechinus miliaris	19	71	16	15	12	1	3	32	40
Rhodophyta	1	1		1	1	1	1	1	1
Sabellida	3			2	6		2	2	
Scyphozoa									
Spirobranchus triqueter	1	1	1			1		1	1
Styela clava			2	2					1
Syngnathiformes								1	
Tellinidae	8			1			1		
Trochidae					1			1	1
Tunicata	3				1	1			

7.2. Appendix B: Underwater Imagery

Table 18.
Sample imagery from each camera set up during Pembrokeshire oyster farm survey, June 2023.

	
PEMBROKESHIRE DAY 1 CONTROL LOWER	PEMBROKESHIRE DAY 1 CONTROL UPPER
	
PEMBROKESHIRE DAY 1 TEST LOWER	PEMBROKESHIRE DAY 1 TEST MIDDLE
	
PEMBROKESHIRE DAY 1 TEST UPPER	PEMBROKESHIRE DAY 2 CONTROL LOWER



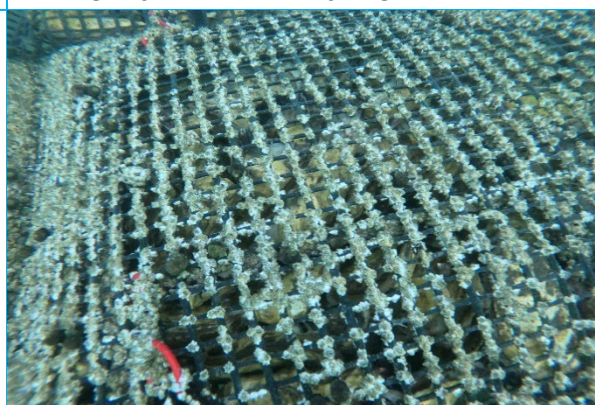
PEMBROKESHIRE DAY 2 CONTROL UPPER



PEMBROKESHIRE DAY 2 TEST LOWER



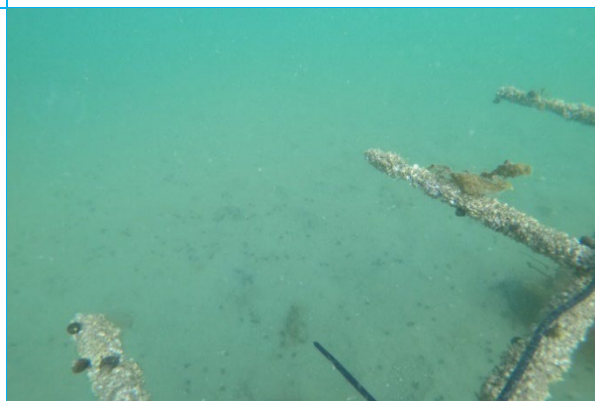
PEMBROKESHIRE DAY 2 TEST MIDDLE



PEMBROKESHIRE DAY 2 TEST UPPER



PEMBROKESHIRE DAY 3 CONTROL LOWER



PEMBROKESHIRE DAY 3 CONTROL UPPER



PEMBROKESHIRE DAY 3 TEST LOWER



PEMBROKESHIRE DAY 3 TEST UPPER



PEMBROKESHIRE DAY 3 NATIVE LOWER



PEMBROKESHIRE DAY 3 NATIVE UPPER

Table 19.

Sample imagery from each camera set up during Lindisfarne oyster farm survey, June 2023.



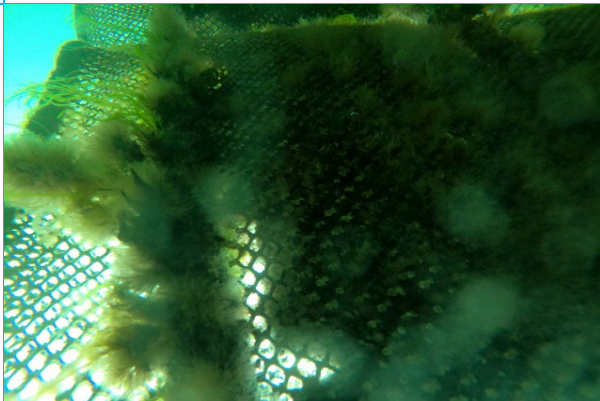
LINDISFARNE DAY 1 CONTROL LOWER



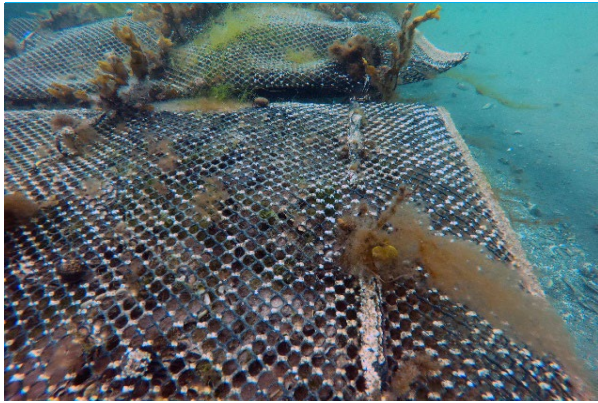
LINDISFARNE DAY 1 CONTROL UPPER



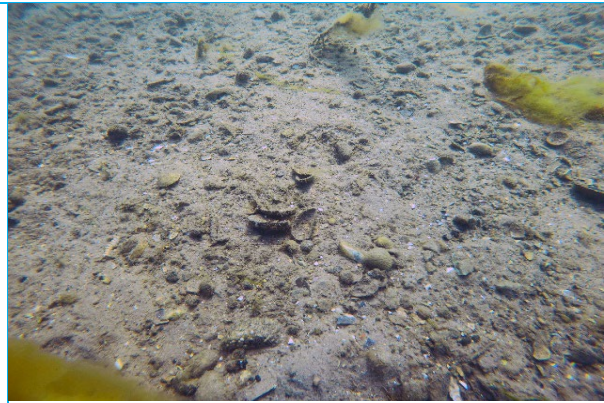
LINDISFARNE DAY 1 TEST LOWER



LINDISFARNE DAY 1 TEST MIDDLE



LINDISFARNE DAY 1 TEST UPPER



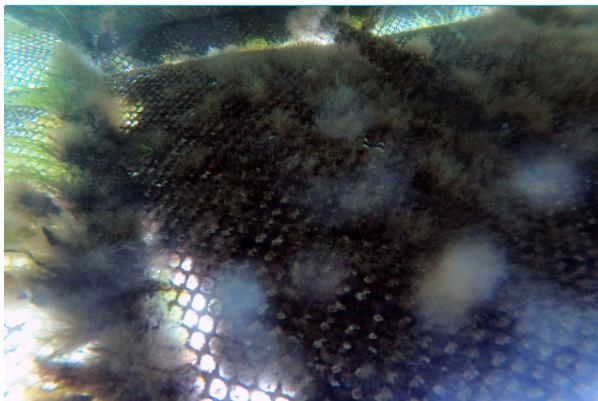
LINDISFARNE DAY 2 CONTROL LOWER



LINDISFARNE DAY 2 CONTROL UPPER



LINDISFARNE DAY 2 TEST LOWER



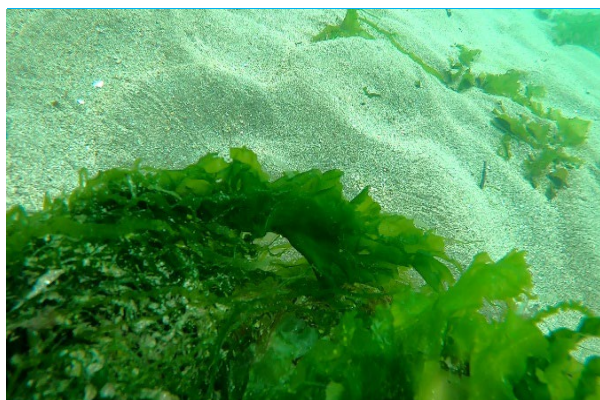
LINDISFARNE DAY 2 TEST MIDDLE



LINDISFARNE DAY 2 TEST UPPER

Table 20.

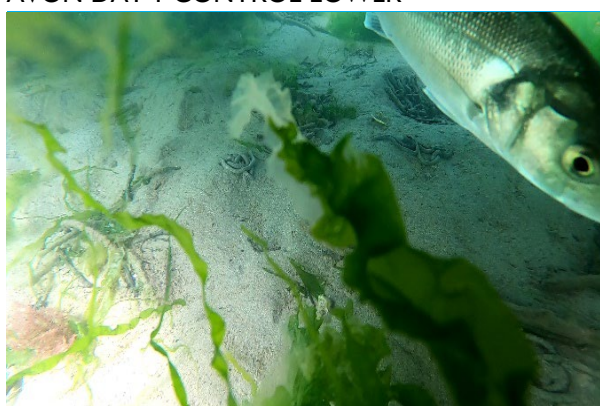
Sample imagery from each camera set up during River Avon oyster farm survey, July 2023.



AVON DAY 1 CONTROL LOWER



AVON DAY 1 CONTROL UPPER



AVON DAY 1 TEST LOWER



AVON DAY 1 TEST MIDDLE



AVON DAY 1 TEST UPPER



AVON DAY 2 CONTROL LOWER



AVON DAY 2 CONTROL UPPER



AVON DAY 2 TEST LOWER



AVON DAY 2 TEST MIDDLE



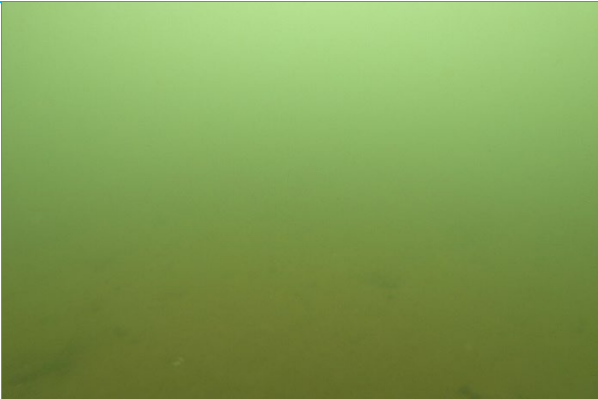
AVON DAY 2 TEST UPPER

Table 21.

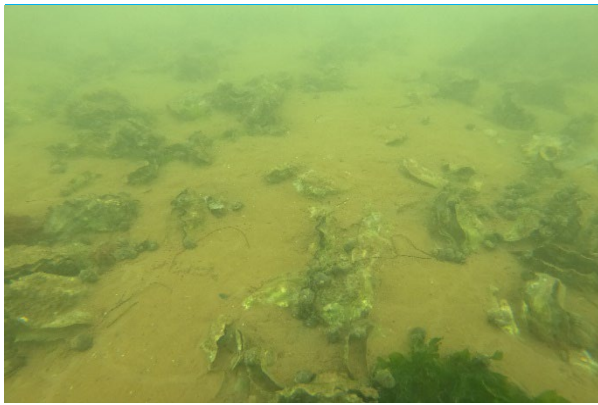
Sample imagery from each camera set up during River Exe naturalised oyster site survey, July 2023.



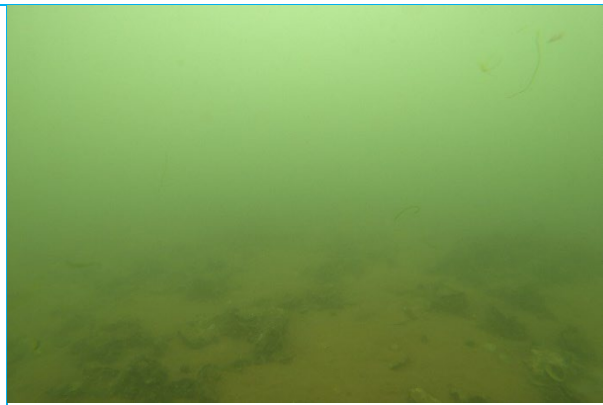
EXE DAY 1 CONTROL LOWER



EXE DAY 1 CONTROL UPPER



EXE DAY 1 TEST LOWER



EXE DAY 1 TEST UPPER



EXE DAY 2 CONTROL LOWER



EXE DAY 2 CONTROL UPPER



EXE DAY 2 TEST LOWER



EXE DAY 2 TEST UPPER

8. References

- ⁱ Herbert R.J.H., Roberts C., Humphreys J., Fletcher S. (2012). The Pacific oyster (*Crassostrea gigas*) in the UK: economic, legal and environmental issues associated with its cultivation, wild establishment and exploitation. Report for the Shellfish Association of Great Britain
- ⁱⁱ Syvret, M. & Woolmer, A., (2015). Native oyster, *Ostrea edulis* - Subtidal Cultivation Handbook. Report for the Mumbles Oyster Company Ltd., 49p
- ⁱⁱⁱ FISP ECM_65323 Mumbles Oyster Site 2022 Survey Report (2023). Prepared by Tethys Oysters / Envision Marine Ltd. / University of Essex for DEFRA FISP & Fishmongers' Company.
- ^{iv} FISP ECM_65323 Mumbles Oyster Site 2023 Survey Report (2024). Prepared by Tethys Oysters / Envision Marine Ltd. / University of Essex for DEFRA FISP & Fishmongers' Company.
- ^v FISP ECM_65323 Pembrokeshire Oyster Farm Survey Report (2024). Prepared by Tethys Oysters / Envision Marine Ltd. / University of Essex for DEFRA FISP & Fishmongers' Company.
- ^{vi} FISP ECM_65323 Lindisfarne Oyster Farm Survey Report (2024). Prepared by Tethys Oysters / Envision Marine Ltd. / University of Essex for DEFRA FISP & Fishmongers' Company.
- ^{vii} FISP ECM_65323 River Avon Oyster Farm Survey Report (2024). Prepared by Tethys Oysters / Envision Marine Ltd. / University of Essex for DEFRA FISP & Fishmongers' Company.
- ^{viii} FISP ECM_65323 River Exe Naturalised Oyster Site Survey Report (2024). Prepared by Tethys Oysters / Envision Marine Ltd. / University of Essex for DEFRA FISP & Fishmongers' Company.
- ^{ix} Ma, D., Whitby, C. and Cameron, T. (Manuscript in preparation). Nutrient and functional nitrogen cycle microbial analysis of oyster aquaculture sites across the United Kingdom,
- ^x Worsfold, T.M., Hall, D.J. & O'Reilly, M. (Ed.). 2010. Guidelines for processing marine macrobenthic invertebrate samples: a Processing Requirements Protocol: Version 1.0, June 2010. Unicomarine Report NMBAQCMbPRP to the NMBAQC Committee. 33pp
- ^{xi} Davies, J., Baxter, J., Bradley, M., Connor, D., Khan, J., Murray, E., Sanderson, W., Turnbull, C. & Vincent, M. (eds). (2001). Marine Monitoring Handbook, JNCC, Peterborough, ISBN 1 86107 5243
- ^{xii} Cefas (2002). Aquatic Environment Protection Analytical Methods Number 13. Review of Standard Operating Procedures (SOPs). Sci. Ser., Aquat. Environ. Prot.: Analyt. Meth., CEFAS Lowestoft, (13): 57pp
- ^{xiii} Zhang, X., Yang, C., Zhang, B., Tan, W., Gong, J., Wang, G., Zhao, J. and Lin, X. (2023). Dynamics of Benthic Nitrate Reduction Pathways and Associated Microbial Communities Responding to the Development of Seasonal Deoxygenation in a Coastal Mariculture Zone. Environ Sci & Technol, 57(40), 15014–15025

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- ^{xiv} Rose, J.M., Bricker, S.B., Tedesco, M.A. and Wikfors, G.H. (2014). A Role for Shellfish Aquaculture in Coastal Nitrogen Management. *Environ Sci & Technol*, 48(5), 2519-2525.
- ^{xv} Long, D. (2006). BGS detailed explanation of seabed sediment modified folk classification https://www.researchgate.net/publication/284511408_BGS_detailed_explanation_of_seabed_sediment_modified_folk_classification
- ^{xvi} Clarke, K.R. & Gorley, R.N. (2015). PRIMER v7: User manual/tutorial. PRIMER-E Ltd.
- ^{xvii} R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (R version 4.3.3) URL <https://www.R-project.org/>.
- ^{xviii} BS EN 16260:2012, Water quality – Visual seabed surveys using remotely operated and/or towed observation gear for collection of environmental data.
- ^{xix} Turner, J.A., Hitchin, R., Verling, E., van Rein, H. 2016. Epibiota remote monitoring from digital imagery: Interpretation guidelines.
- ^{xx} The Conservation of Habitats and Species Regulations 2017 & European Union Council Directive 92/43/EEC
- ^{xxi} Marine Environmental Data and Information Network. <https://medin.org.uk/>
- ^{xxii} Scarlett K, Denman S, Clark DR, Forster J, Vangelova E, Brown N, Whitby C (2021). Relationships between nitrogen cycling microbial community abundance and composition reveal the indirect effect of soil pH on oak decline. *Isme J* 15:623-635.
- ^{xxiii} Beddow J, Stolpe B, Cole PA, Lead JR, Sapp M, Lyons BP, Colbeck I, Whitby C (2017). Nanosilver inhibits nitrification and reduces ammonia-oxidizing bacterial but not archaeal amoA gene abundance in estuarine sediments. *Environ Microbiol* 19: 500-510.
- ^{xxiv} Hitchin, R., Turner, J.A., Verling, E. 2015. Epibiota remote monitoring from digital imagery: Operational guidelines
- ^{xxv} JNCC, 2018. Remotely Operated Vehicles for use in marine benthic monitoring. Marine Monitoring Platform Guidelines No. 1. JNCC, Peterborough. ISSN 2517-7605
- ^{xxvi} Davies, J., Baxter, J., Bradley, M., Connor, D., Khan, J., Murray, E., Sanderson, W., Turnbull, C., & Vincent, M. (Eds.) (2001). *Marine Monitoring Handbook*. Joint Nature Conservation Committee
- ^{xxvii} Hyland, J., L. Balthis, I. Karakassis, P. Magni, A. Petrov, J. Shine, O. Vestergaard, and R. Warwick. "Organic Carbon Content of Sediments as an Indicator of Stress in the Marine Benthos." *Marine Ecology Progress Series* 295 (2005): 91–103. <http://www.jstor.org/stable/24868595>.