Limerick WWTP Upgrade and Regional Bioresource Centre

Appendix 12.1 - Estuarine Ecology Report

June 2026

Formerly JB Barry Partners who became part of Egis in 2023

Estuarine Ecological Survey, Limerick WWTP, Shannon Estuary, Co. Limerick

Produced by

AQUAFACT International Services Ltd. (APEM Group)

On behalf of

JB Barry & Partners

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1. Introduction

AQUAFACT International Services Ltd. (part of the APEM Group) was commissioned by JB Barry and Partners (on behalf of Uisce Éireann) to undertake a biological assessment of the estuarine environment in the vicinity of Limerick Waste Water Treatment Plant (WWTP) prior to an upgrade of the facility.

Figure 1.1 below indicates the location of the Limerick WWTP and outfall within the Shannon Estuary area. The outfall is located in the Lower Shannon Estuary SAC and River Shannon and River Fergus Estuaries SPA, specifically in a freshwater tidal area called Limerick Docks. The Limerick Docks water body comprises the main channel of the River Shannon as it passes through Limerick City as well as the Ballincurra Creek on the south side of the city. The water body stretches from the bridge east of Corbally on Limerick's north side, down as far as Coonagh Point, just below the Limerick Tunnel on the N18. This water body covers a total area of 2.49km^2. Most of this water body is bordered by urban development (Kelly *et al.,* 2015).

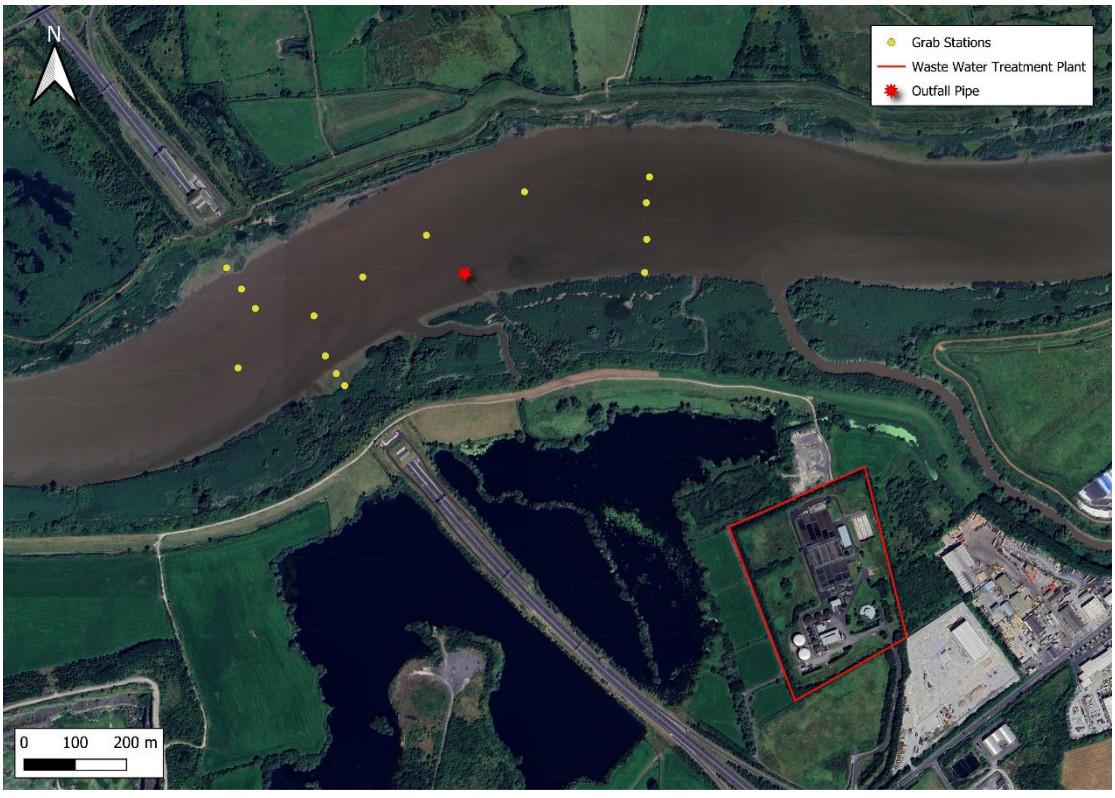

Figure 1.1: Location of the Limerick WWTP and discharge points within the study area of Shannon Estuary, Co. Limerick.

2. Ecological Surveys

2.1. Materials and Methods

2.1.1. Subtidal Survey Procedure

To carry out the subtidal benthic assessment of the area in question, AQUAFACT sampled a total of 6 stations. Sampling took place on the 18th January 2024 from AQUAFACT's 6.8m Lencraft RIB. The weather on both days was clear and frosty. There was a force 1 north-westerly wind blowing. High water was at 12pm (5.32m) at Limerick Docks. The locations of all subtidal stations sampled can be seen in Figure 2.1. Table 2.1 shows the station coordinates.

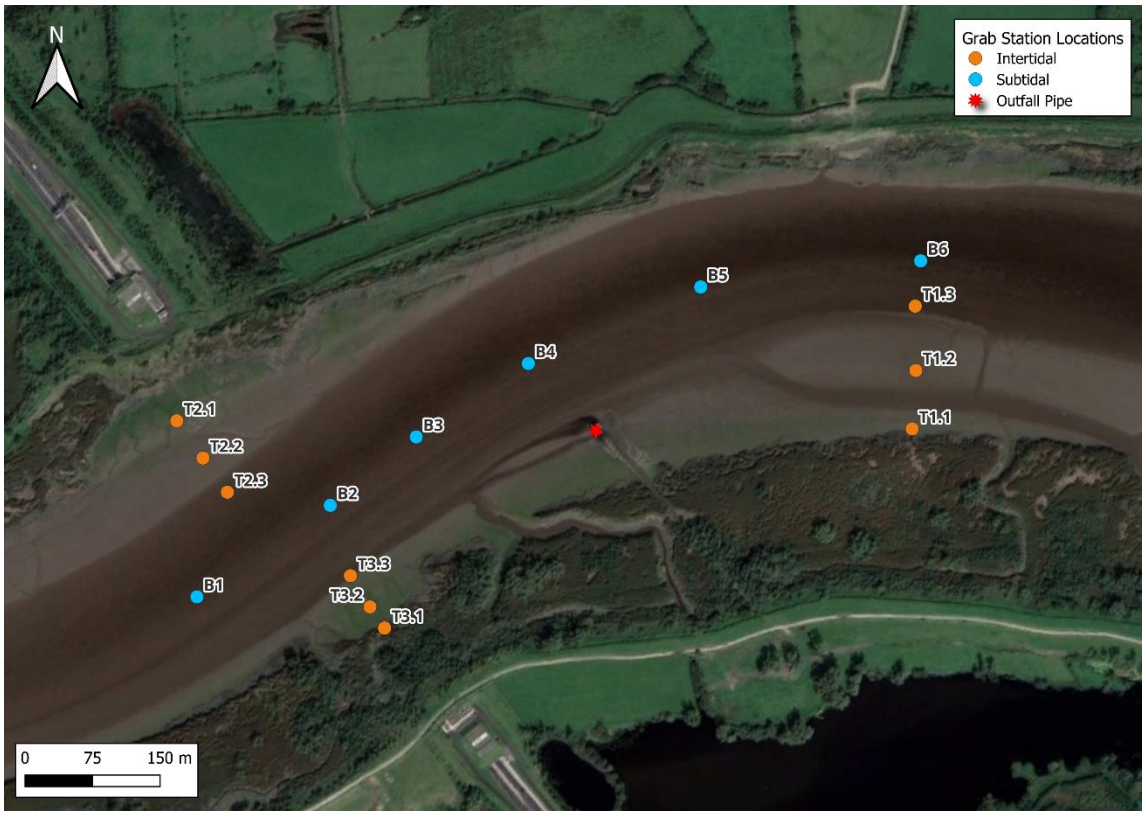

Figure 2.1: Location of grab stations sampled on the 18th January 2024.

Table 2.1: Station coordinates of all 6 subtidal stations sampled on the 18th January 2024.

Station	Latitude	Longitude
B1	52.65426	-8.68785
B2	52.65517	-8.68566
B3	52.65585	-8.68425
B4	52.65658	-8.68241
B5	52.65734	-8.67958
B6	52.65760	-8.67597

AQUAFACT has in-house standard operational procedures for benthic sampling and these were followed for this project. Additionally, the NMBAQC report "Guidelines for processing marine macrobenthic invertebrate samples: a processing requirements protocols" (Worsfold and Hall, 2010) was adhered to.

A 0.025m^2 van Veen grab was used to sample each station and two replicate grab samples were collected at each site. On arrival at each sampling station, the vessel location was recorded using DGPS (Lat/Long & ING). The grab deployment and recovery rates did not exceed 1 metre/sec and were <0.5 m/sec for the last 5 metres for water depths up to 30m and for the last 10m for depths greater than 30m.

A digital image of each sample (including sample label) was taken and its reference number entered in the sample data sheet. These images can be made available on request. The grab sampler was cleaned between stations to prevent cross contamination.

Each grab sample was carefully and gently sieved on a 1mm mesh sieve as a sediment water suspension for the retention of fauna. Great care was taken during the sieving process in order to minimise damage to taxa such as spionids, scale worms, phyllodocids and amphipods. The sample residue was carefully flushed into a pre-labelled (internally and externally) container from below. Each label contained the sample code and date. The samples were stained immediately with Eosin-briebrich scarlet and fixed with 4% w/v buffered formaldehyde solution (10% w/v buffered formaldehyde solution for very organic mud).

An additional grab sample was collected at each station for sediment analysis (organic carbon and granulometry). Each sediment sample was placed in plastic sampling bags and labelled internally and externally. These samples were frozen (<-18ºC) as soon as possible after acquisition.

2.1.2. Sample Processing

All faunal samples were placed in an illuminated shallow white tray and sorted first by eye to remove large specimens and then sorted under a stereo microscope (x 10 magnification). Following the removal of larger specimens, the samples were placed into Petri dishes, approximately one-half teaspoon at a time and sorted using a binocular microscope at x25 magnification.

The faunal samples were sorted into four main groups: Polychaeta, Mollusca, Crustacea, and others. The 'others' group consisted of echinoderms, nematodes, nemerteans, cnidarians, and other lesser phyla. The fauna were maintained in stabilised 70% industrial methylated spirit (IMS) following retrieval and identified to species level where practical using a binocular microscope, a compound microscope and all relevant taxonomic keys. After identification and enumeration, specimens were separated and stored at/to species level.

The sediment granulometric analysis was carried out by AQUAFACT using the traditional granulometric approach. The traditional analysis involved the dry sieving of approximately 100g of sediment using a series of Wentworth graded sieves. The process involved the separation of the sediment fractions by passing them through a series of sieves. Each sieve retained a fraction of the sediment, which were later weighed, and a percentage of the total was calculated. Table 3.2 shows the classification of sediment particle size ranges into size classes. Sieves, which corresponded to the range of particle sizes were used in the analysis. Refer to Appendix 2 for a detailed methodology of this procedure.

Table 2.2: The classification of sediment particle size ranges into size classes (adapted from Buchanan, 1984)

Range of Particle Size	Classification	Phi Unit
<63µm	Silt/Clay	>4 Ø
63-125 µm	Very Fine Sand	4 Ø, 3.5 Ø
125-250 µm	Fine Sand	3 Ø, 2.5 Ø
250-500 µm	Medium Sand	2 Ø, 1.5 Ø
500-1000 µm	Coarse Sand	1 Ø, 1.5 Ø
1000-2000 µm (1 – 2mm)	Very Coarse Sand	0 Ø, -0.5 Ø
>2000 µm (> 2mm)	Gravel	< -1 Ø

Organic carbon analysis was carried out by the by INAB certified ALS Labs in Loughrea using the Loss on Ignition technique.

2.1.3. Intertidal Survey Procedure

AQUAFACT carried out 3 intertidal transects (T1 to T3) on the 18th January 2024. Table 2.3 lists the transect station locations. Transect 1 was upstream of the WWTP outfall and Transects 2 and 3 were downstream of the outfall on opposites banks of the river. Figure 2.1 illustrates their locations. The weather on both days was clear and frosty. There was a force 1 north-westerly wind blowing. High water was at 12pm (5.32m) at Limerick Docks.

A grab survey was necessary to carry out the intertidal survey as the substrate was fine soft mud and it was not possible or safe to stand on the substrate at low water. Instead, the same procedure used for the subtidal benthic survey was employed. A 0.025m^2 Van Veen grab was deployed from AQUAFACT's RIB at high water at each of the of the transects' locations (Upper Shore, Mid Shore and Lower Shore). Replicate grabs were collected at each station as well as an additional grab for sediment analysis. The grabs were preserved and returned to the lab for identification and analysis. The full methodology for the collection and analysis of grab samples is outlined in Sections 2.1.1 and 2.1.2 above.

Table 2.3: Station coordinates of all intertidal transect stations sampled on the 18th January 2024.

Station	Latitude	Longitude
T1.1	52.65593	-8.67611
T1.2	52.65651	-8.67605
T1.3	52.65715	-8.67606
T2.1	52.65601	-8.68818
T2.2	52.65564	-8.68775
T2.3	52.6553	-8.68735
T3.1	52.65395	-8.68477
T3.2	52.65416	-8.68501
T3.3	52.65447	-8.68533

2.2. Data analysis

2.3. Sediment Data

Organic content of sediment samples was determined for each sample by expressing it as a percentage the sediment weight loss following combustion over the initial weight of the sediment. In general, Loss Of carbon Ignition (LOI) correlates with sediment particle size with fine-grained sediments typically containing higher levels of organic matter than coarse sediments.

For the granulometric analysis of sediment samples, the <63 µm (Silt-Clay) fraction was determined by weight loss following wet sieving. Coarser fractions comprising the sediment samples were determined by mechanical dry sieving through a series of Wentworth sieves; >4mm (Fine Gravel), 2-4mm (Very Fine Gravel), 1-2mm (Very Coarse Sand), 0.5-1mm (Coarse Sand), 0.25-0.5mm (Medium Sand), 125-250µm (Fine Sand), 62.5-125µm (Very Fine Sand). For each station, the weight of each fraction of the sediment retained on the sieve was expressed as a percentage of the total sample. The relative proportion of sediments in each fraction was used to classify sediments at the station *sensu* Folk (1954).

2.4. Faunal Data

Uni- and multi-variate statistical analysis of the faunal data was undertaken using PRIMER v.6 (Plymouth Routines in Ecological Research).

2.4.1. Univariate Indices

Using PRIMER the faunal data was used to produce a range of univariate indices. Univariate indices are designed to condense species data in a sample into a single coefficient that provides quantitative estimates of biological variability (Heip *et al*., 1998; Clarke and Warwick, 2001). Univariate indices can be categorised as primary or derived indices.

Primary biological indices used in the current study include:

number of taxa (S) in the samples and

number of individuals (N) in the samples.

Derived biological indices, which are calculated based on the relative abundance of species in samples, used in the study include:

Margalef's species richness index (D) (Margalef, 1958),

$$D = \frac{S-1}{\log_2 N}$$

where: N is the number of individuals and S is the number of species

Margalef's species richness (D) is a measure of the total number of species present for a given number of individuals.

Pielou's Evenness index (J) (Pielou, 1977)

$$J = \frac{H'(observed)}{H'_{max}}$$

where: H'_{max} is the maximum possible diversity, which could be achieved if all species were equally abundant (= $\log_2 S$)

Pielou's evenness is a measure of how evenly the individuals are distributed among different species.

Shannon-Wiener diversity index (H') (Pielou, 1977)

$$H' = -\sum_{i=1}^{S} p_i (\log_2 p_i)$$

where: p_l is the proportion of the total count accounted for by the i^{th} taxa

Shannon-Wiener diversity index takes both species abundance and species richness into account quantify diversity (Shannon & Wiener, 1949).

The Shannon-Wiener based Effective Number of Species (ENS) (Hill, 1973; Jost, 2006)

$$H = \exp(H')$$

where H' is the Shannon-Wiener diversity index.

The Shannon-Wiener index diversity index is converted to ENS to reflect 'true diversities' (Hill, 1973, Jost, 2006) that can then be compared across communities (MacArthur, 1965; Jost, 2006). The ENS is equivalent to the number of equally abundant species that would be needed in each sample to give the same value of a diversity index, *i.e.,* Shannon-Wiener Diversity index. The ENS behaves as one might? intuitively expect when diversity is doubled or halved, while other standard indices of diversity do not (Jost, 2006). If the ENS of one community is twice that of another, then it can be said that the community is twice as diverse as the other.

2.4.2. Multivariate Analysis

The PRIMER programme (Clarke & Warwick, 2001) was used to carry out multivariate analyses on the station-by-station faunal data. All species abundance data from the grab surveys was square root transformed and used to prepare a Bray-Curtis similarity matrix in PRIMER. The square root transformation allows some of the less abundant species to play a part in the similarity calculation. Various ordination and clustering techniques can then be applied to the similarity matrix to determine the relationship between the samples.

Multidimensional scaling (MDS) is a technique that ordinates samples as points in 2D or 3D space based on similarity in species distribution data. MDS performed on the Bray-Curtis similarity matrix produce ordination maps whereby the placement of samples reflects the similarity of their biological communities, rather than their simple geographical location (Clarke & Warwick, 2001). An indication of how well the similarity matrix is represented by the ordination is given by stress values calculated by comparing the interpoint distances in the similarity matrix with the corresponding interpoint distances on the ordinations. Perfect or near perfect matches are rare in field data, especially in the absence of a single overriding forcing factor such as an organic enrichment gradient. Stress values increase, not only with the reducing dimensionality (lack of clear forcing structure), but also with increasing quantity of data (it is a sum of the squares type regression coefficient). Clarke & Warwick (2001) have provided a classification of the reliability of MDS plots based on stress values, having compiled simulation studies of stress value behaviour

and archived empirical data. This classification generally holds well for ordinations of the type used in this study. Their classification is given below:

- Stress value < 0.05: Excellent representation of the data with no prospect of misinterpretation.
- Stress value < 0.10: Good representation, no real prospect of misinterpretation overall structure, but very fine detail may be misleading in compact subgroups.
- Stress value < 0.20: This provides a useful picture, but detail may be misinterpreted, particularly nearing 0.20.
- Stress value 0.20 to 0.30: This should be viewed with scepticism, particularly in the upper part of the range, and discarded for a small to moderate number of points such as < 50.
- Stress values > 0.30: The data points are close to being randomly distributed in the ordination and not representative of the underlying similarity matrix.

Each stress value must be interpreted both in terms of its absolute value and the number of data points. In the case of this study, the moderate number of data points indicates that the stress value can be interpreted more or less directly. While the above classification is arbitrary, it does provide a framework that has proved effective in this type of analysis.

Hierarchical Agglomerative Clustering (HAC) is used to cluster samples based on between-sample similarities into groups in dendrograms. Similarity Profiling (SIMPROF) is used to test if differences between HAC derived similarity-based clusters are significant. Similarity Percentages (SIMPER) analysis can be used to determine the characterising species of each cluster of stations identified either arbitrarily (by eye) from HAC dendrograms or statistically using SIMPROF testing (Clarke and Warwick, 2001; Clarke and Gorley, 2006; Anderson *et al*., 2008).

The species, which are responsible for the grouping of samples in CLUSTER analyses, were identified using the PRIMER programme SIMPER (Clarke & Warwick, 1994). This programme determined the percentage contribution of each species to the dissimilarity/similarity within and between each sample group.

3. Results

3.1. Faunal Results

The taxonomic identification of the benthic infauna across all 6 subtidal benthic stations and 9 intertidal transect stations sampled in the Shannon Estuary yielded a total count of 23 taxa ascribed to 4 phyla. Of the 23 taxa identified, 6 were identified to species level. The remaining 17 could not be identified to species level because they were juveniles, damaged or indeterminate. The full faunal abundance species list can be seen in Appendix 1.

Of the 23 taxa recorded, 7 were annelids (segmented worms), 11 were arthropods (crabs, shrimps, insects *etc.*), 4 were molluscs (mussels, cockles, snails etc.) and 1 was a nematode (round worm).

3.1.1.1. Univariate Analysis

In order to carry out the univariate analyses all replicate data were combined to give a total for each station prior to statistical analysis. Univariate statistical analyses were carried out on the station-by-station faunal data. As the same survey method and replication were used in both the intertidal and subtidal survey, all data was analysed together. The following parameters were calculated and can be seen in Table 3.4; species numbers, number of individuals, richness, evenness, Shannon-Wiener diversity, and Effective Species Number (ENS). Species numbers ranged from 3 (B6) to 14 (T1.1). Number of individuals ranged from 3 (B2 and B6) to 1,385 (T2.1). Richness ranged from 0.54 (T2.1) to 2.06 (T1.1). Evenness ranged from 0.27 (T3.1) to 1.0(B2 and B6). Shannon-Wiener diversity ranged from 0.59 (T3.1) to 1.63 (T1.1). Effective number of species ranged from 1.8 (T3.1) to 5.1 (T1.1), indicating that station T1.1 is over 2.8 times more diverse than T3.1. ENS also indicates that the diversity is broadly similar throughout the stations surveyed. Figure 3.1 shows these community indices in graphical form.

Table 3.1: Univariate measures of community structure.

Station	No. Taxa	No. Individuals	Richness	Evenness	Shannon-Wiener Diversity	Effective Number of Species
	S	N	d	J'	H'(loge)	EXP(H')
B1	7	49	1.54	0.65	1.26	3.51
B2	3	3	1.82	1.00	1.10	3.00
B3	5	36	1.12	0.66	1.06	2.88
B4	5	24	1.26	0.78	1.25	3.49
B5	4	16	1.08	0.87	1.21	3.36
B6	3	3	1.82	1.00	1.10	3.00
T1.1	14	549	2.06	0.62	1.63	5.10
T1.2	5	577	0.63	0.41	0.66	1.93
T1.3	7	566	0.95	0.43	0.84	2.32
T2.1	5	1385	0.55	0.47	0.76	2.15
T2.2	4	269	0.54	0.43	0.59	1.81
T2.3	5	286	0.71	0.57	0.92	2.51
T3.1	9	1094	1.14	0.27	0.59	1.80
T3.2	7	404	1.00	0.38	0.75	2.11
T3.3	5	481	0.65	0.62	1.00	2.71

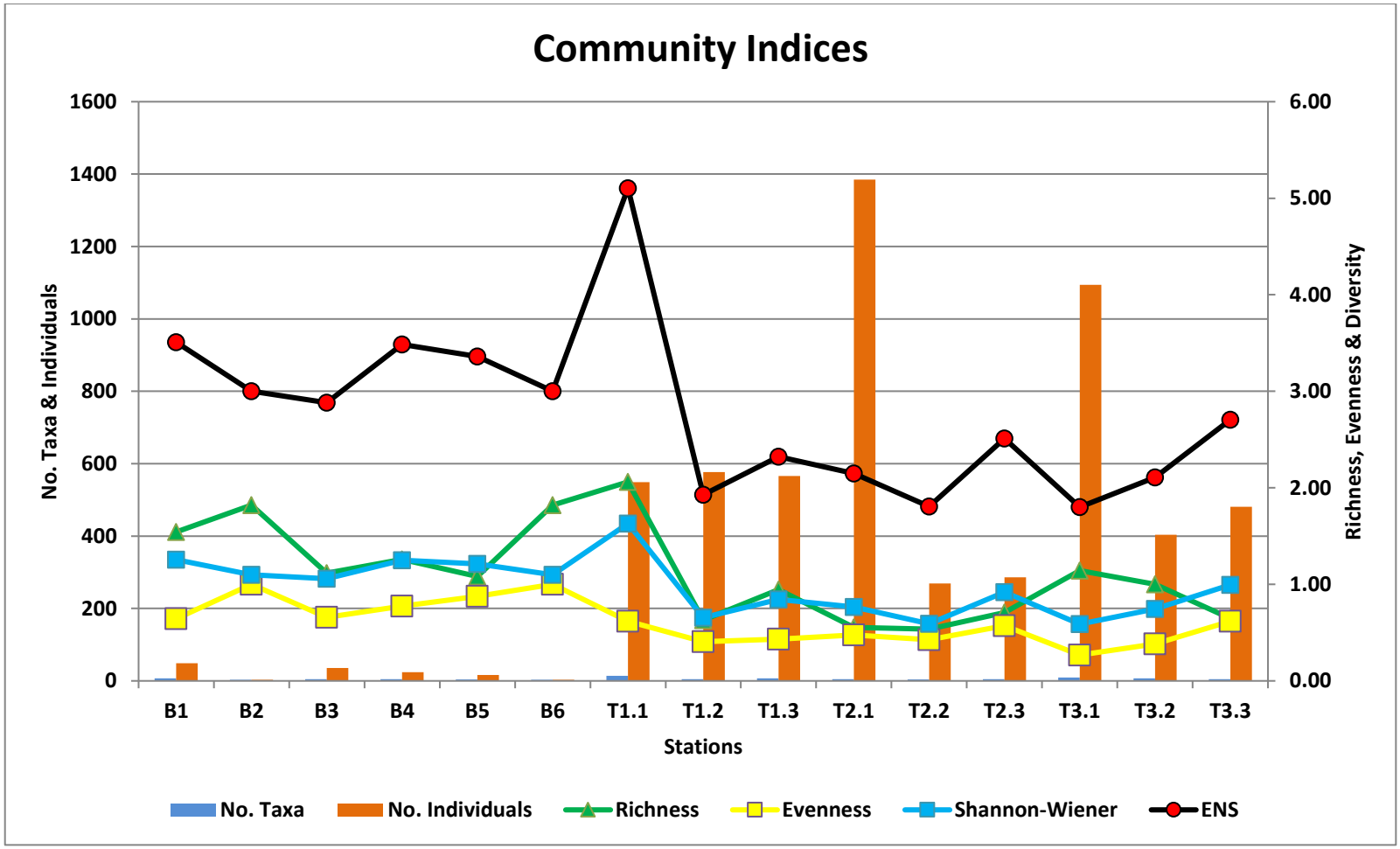

Figure 3.1: Subtidal community diversity indices. Diversity is expressed in Effective Number of Species (ENS) and Shannon-Wiener Diversity index.

3.1.1.2. Multivariate Analysis

The same data set used above for the univariate analyses was also used for the multivariate analyses. The dendrogram and the MDS plot can be seen in Figures 3.2 and 3.3, respectively. SIMPROF analysis revealed 3 statistically significant groupings between the 15 stations (the samples connected by red lines cannot be significantly differentiated). The stress level on the MDS plot indicates an excellent representation of the data with no prospect of misinterpretation of the overall structure.

A clear divide (24.64% similarity) can be seen between the Intertidal Groups (**Groups a** and **b**) and the subtidal **Group c**. A similarly clear divide (43.59% similarity) can be seen between **Group a** and **Group b**.

Group a consisted of station T1.1 (Upper Shore). This group separated from the other intertidal group (**Group b)** at a 56.41% dissimilarity level. T1.1 contained 14 taxa comprising 549 individuals. Of the 14 taxa, five of the taxa were present twice or less. SIMPER analysis could not be undertaken on this group as it only contained one station. Four taxa accounted for over 93% of the faunal abundance: the oligochaetes *Limnodrilus* spp. (183 individuals, 33.33% abundance) and Enchytraeidae (97 individuals, 17.67% abundance), the gastropod *Potamopyrgus antipodarum* (123 individuals, 22.4% abundance) and the amphipod *Corophium multisetosum* (109 individuals, 19.85% abundance). SIMPER analysis could not be carried out on this group as it only contained one station. *Potamopyrgus antipodarum* is indifferent to enrichment and is typically present in low densities with non-significant variations over time. *Corophium multisetosum* are tolerant of disturbance, occurring under normal conditions, but their populations are stimulated by organic enrichment. *Limnodrilus* spp. and Enchytraeidae are first order opportunistic species which proliferate in reduced sediments with high organic content. Species richness and diversity were highest at this station.

Group b consisted of intertidal stations T1.2, T1.3, T2.1, T2.2, T2.3, T3.1, T3.2, and T3.3. This group separated from Group a at 56.41% dissimilarity level and had a within group similarity level of 72.38%. Group b contained 14 taxa comprising 5,062 individuals. Of the 14 taxa, 5 were present twice or less. Three taxa accounted for over 98% of the faunal abundance: *Limnodrilus* spp. (3,083 individuals, 60.9% abundance) and *Tubifex tubifex* (1,815 individuals, 35.86% abundance), and the gastropod *Potamopyrgus antipodarum* (74 individuals, 1.6% abundance). SIMPER analysis

revealed the same three species as the characterising species within this group. *Potamopyrgus antipodarum* is indifferent to enrichment and is typically present in low densities with non-significant variations over time. *Limnodrilus* spp. and *Tubifex tubifex* are first order opportunistic species which proliferate in reduced sediments with high organic content.

Group c contained all 6 subtidal stations (B1 – B6). This group separated from the intertidal groups (**Groups a & b)** at a 75.36% dissimilarity level and had a within group similarity of 46.66%. This group contained 11 taxa comprising 131 individuals. Of the 11 taxa, 5 were present twice or less. Five species accounted for almost 94% of the faunal abundance: the oligochaetes *Tubifex tubifex* (56 individuals, 42.75% abundance) and *Limnodrilus* spp. (47 individuals, 35.88% abundance), the amphipods *Corophium multisetosum* (8 individuals, 6.11% abundance) and *Gammarus zaddachi* (5 individuals, 3.82% abundance), and the gastropod *Potamopyrgus antipodarum* (7 individuals, 5.34% abundance). SIMPER analysis revealed *Tubifex tubifex, Limnodrilus* spp., and *Corophium multisetosum* as the characterising taxa. *Potamopyrgus antipodarum* is indifferent to enrichment and is typically present in low densities with non-significant variations over time. *Corophium multisetosum* and *Gammarus zaddachi* are tolerant of disturbance, occurring under normal conditions, but their populations are stimulated by organic enrichment. *Limnodrilus* spp. and *Tubifex tubifex* are first order opportunistic species which proliferate in reduced sediments with high organic content.

Analysis of the fauna from the subtidal grab stations indicates that these stations can be classified as belonging to one of the seven common benthic community habitat types occurring within the Estuaries Qualifying Interest [1130] within the Lower Shannon Estuary SAC (NPWS, 2012) namely the habitat 'Estuarine subtidal muddy sand to mixed sediment with gammarids community complex'. This community complex occurs at the eastern extreme of the SAC site from Cock Rock to Scarlet Reach in water depths between 2.5 and 7m. The sediment of this community complex is that of muddy sand (silt-clay ranging from 50.7% to 34.4% and very fine sand from 29.3% to 11.2%) with patches of gravel (ranging from 24.1% to 0.5%). This fauna community complex consists of Gammaridae amphipods with *Bathyporeia* sp. *Bathyporeia pelagica*, *Leptocheirus* sp., *Gammarus* sp. and *Gammarus zaddachi* all recorded in low abundances here (NPWS, 2012).

Analysis of the fauna from the intertidal grab stations indicates that these stations cannot be assigned to one of the seven common benthic community habitat types occurring within the

Estuaries Qualifying Interest [1130] within the Lower Shannon Estuary SAC (NPWS, 2012). All intertidal stations (**Group a** and **Group b**), both upstream and downstream of the outfall can be classified as belonging to the JNCC biotope 'SS.SMu.SMuVS.LhofTtub – *Limnodrilus hoffmeisteri, Tubifex tubifex* and *Gammarus* spp. in low salinity infralittoral muddy sediment' (EUNIS code A5.327). This community is found in upper estuary muddy sediments with very low fluctuating salinity, characterized by the oligochaetes *Limnodrilus hoffmeisteri* and *Tubifex tubifex.* Other taxa may include *Marenzellaria* spp. *Gammarus zaddachi, Paranais litoralis* and *Baltidrilus costatus.* The biotope contains elements of both freshwater and brackish communities. It is found in the transitional zone between the freshwater and brackish environments where the tidal currents are sufficiently reduced to allow the deposition of fine silt and the establishment of an infaunal community (Tillin *et al.,* 2023).

Interstitial salinity is an important factor determining the occurrence of the IMU.LimTtub community. Although tidal, the uppermost part of an estuary may predominantly experience freshwater conditions (salinity in Limerick Docks area has been measured between 0.833 psu to 3.2 psu (Kelly *et al.,* 2015)). The infauna consists almost exclusively of the freshwater oligochaetes, *Limnodrilus hoffmeisteri* and *Tubifex tubifex*. Stczynska-Jurewicz (1972) reported that the maximum salinity at which *Tubifex tubifex* could survive was 9 psu and the maximum at which natural egg laying and development occurred was 4 psu. Kennedy (1965) stated that salinity controlled the distribution of *Limnodrilus hoffmeisteri but* gave no precise limits. McLusky *et al.* (1980) found *Tubifex tubifex* in localities with a maximum salinity of 4.1 psu, and *Limnodrilus hoffmeisteri* occurred at salinities of up to 7.7 psu. (Tillin *et al.,* 2023).

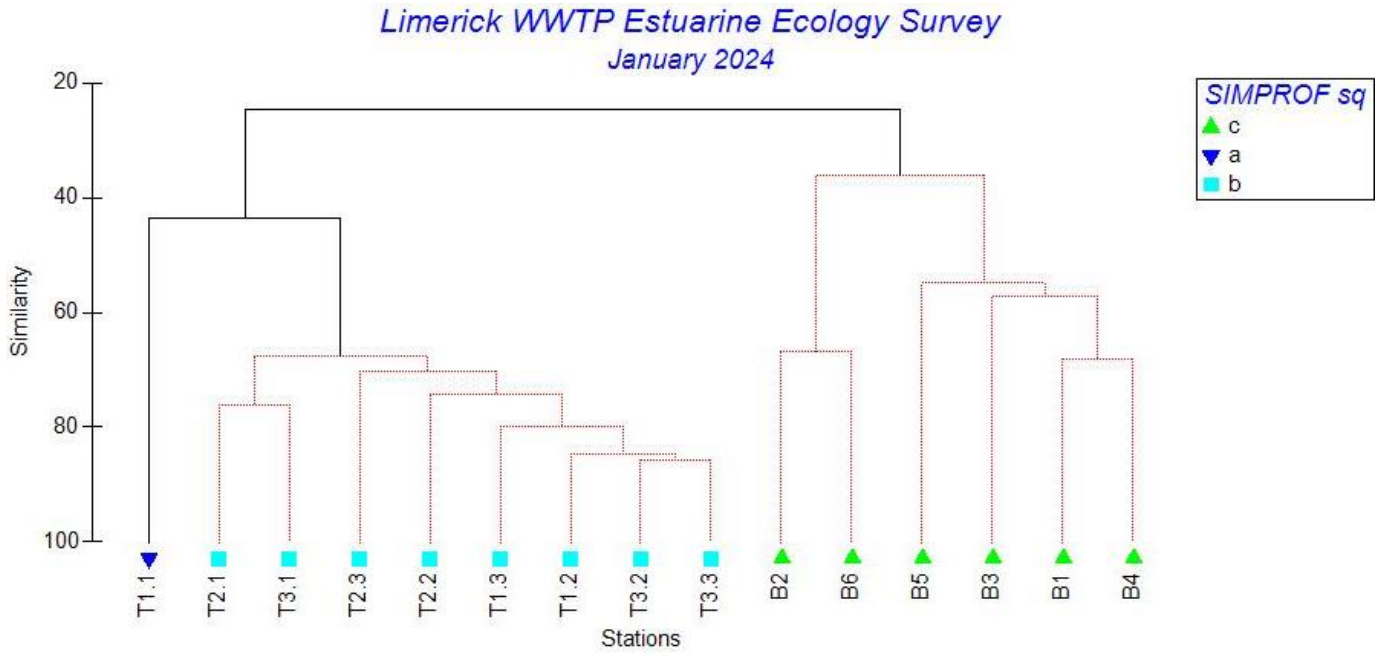

Figure 3.2: Dendrogram produced from Cluster analysis.

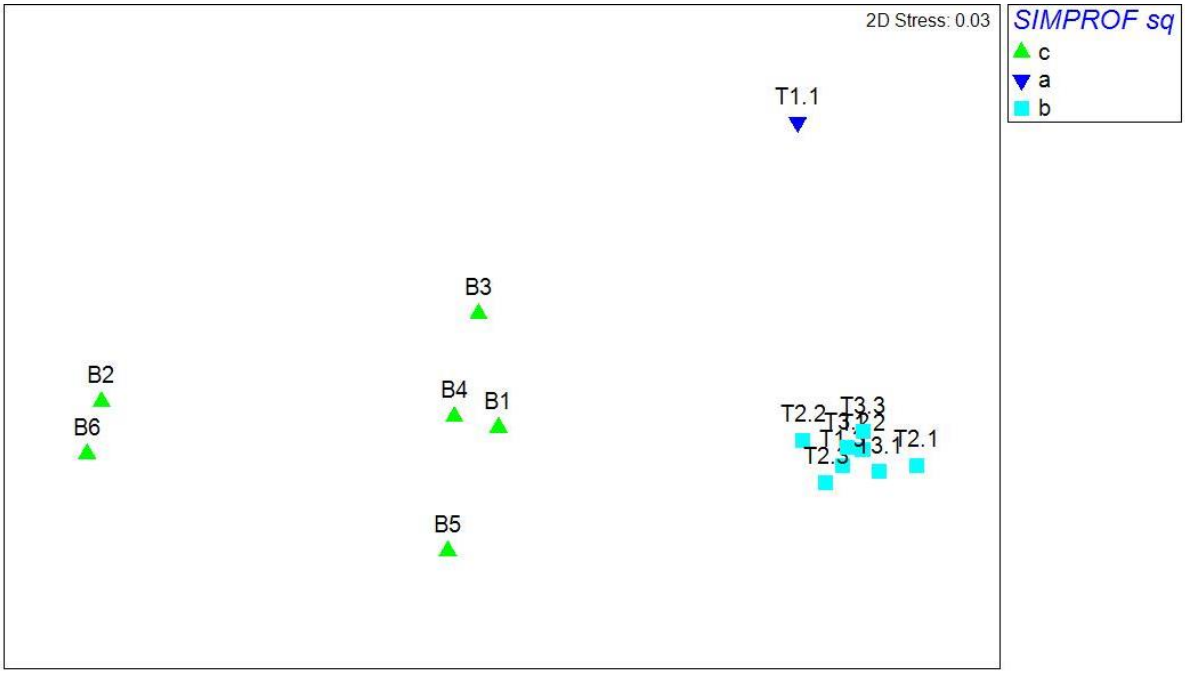

Figure 3.3: MDS plot.

3.2. Sediment Results

Table 3.4 shows the sediment characteristics of the subtidal and intertidal stations surveyed including the granulometry and the percentage organic carbon.

The sediment sampled within the study area was classified as muddy sand, sandy mud, slightly gravelly muddy sand, and slightly gravelly sandy mud, according to Folk (1954). No medium gravel-boulders were recorded. Highest levels of fine gravel were observed at T3.1 and T3.2 (0.4%). Highest levels of very fine gravel and very coarse sand were observed at T3.2 (1% and 20.2 % respectively). Highest levels of coarse sand and medium sand were found at T3.1 (18.1% and 13% respectively). Highest levels of fine sand were found at B6 (10.8%). Highest levels of very fine sand were found at T2.2 (18.9%) and highest levels of silt-clay were found at T2.3 (56.5%). Figure 3.4 shows the breakdown of sediment composition at each station and. Figure 3.5 illustrates the sediment type according to Folk (1954).

Organic matter values ranged from 3.66% (B3) to 6.78% (B1) in the subtidal stations and from 2.59% (T2.3) to 5.31% (T3.1) in the intertidal.

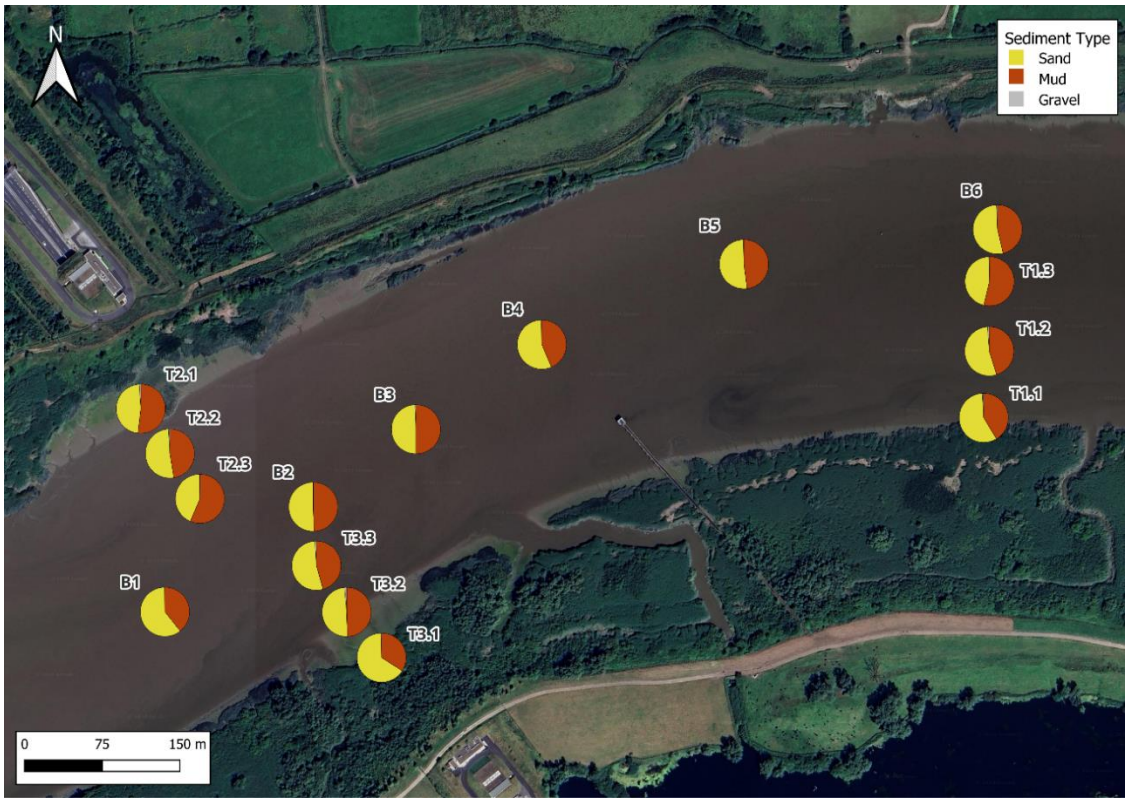

Figure 3.4: A breakdown of sediment type at each station.

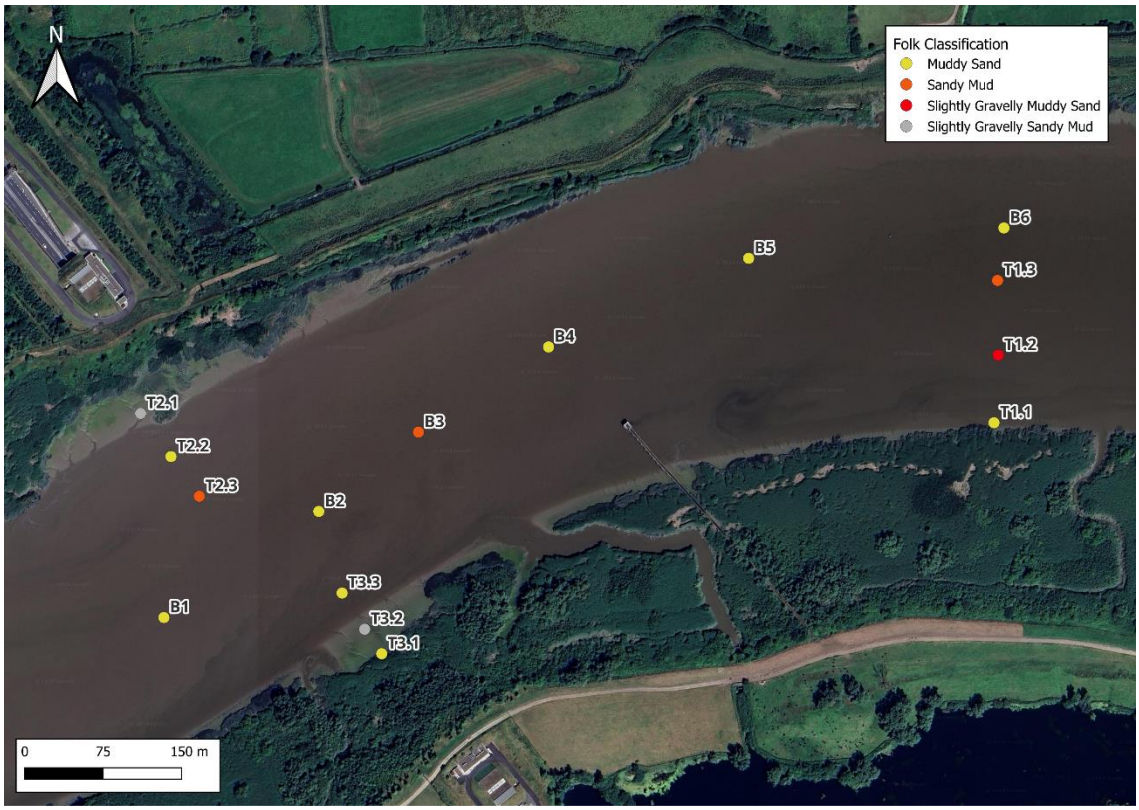

Figure 3.5: Sediment type at each of the subtidal and intertidal stations according to Folk (1954).

Table 3.2: Sediment characteristics of the benthic faunal stations sampled. LOI refers to the % organic carbon loss on ignition.

Station	>8mm	Fine Gravel (4-8mm)	Very Fine Gravel (2-4mm)	Very Coarse Sand (1-2mm)	Coarse Sand (0.5-1mm)	Medium Sand (0.25-0.5mm)	Fine Sand (125-250mm)	Very Fine Sand (62.5-125mm)	Silt-Clay (<63mm)	Folk (1954)	LOI
B1	0	0	0.3	18.7	14.6	8.5	3.7	14.9	39.3	Muddy Sand	6.78
B2	0	0	0.2	19.3	11.3	7.7	5.8	6.2	49.6	Muddy Sand	4.92
B3	0	0.1	0.5	15	10.1	8.2	3	13.1	50	Sandy Mud	3.66
B4	0	0	0.5	17.1	13.4	9.5	6	9.9	43.6	Muddy Sand	4.48
B5	0	0.1	0.3	10.2	9.5	9.3	8.2	14.5	48	Muddy Sand	3.89
B6	0	0.2	0.5	10.4	10.7	9.4	10.8	11.7	46.4	Muddy Sand	4.78
T1.1	0	0	0.9	17.1	15.3	11.2	2.4	11.8	41.3	Muddy Sand	3.92
T1.2	0	0.2	0.9	9.9	14	10.4	3.2	16.2	45.2	Slightly Gravelly Muddy Sand	4.08
T1.3	0	0	0.4	11.6	10.1	6.7	5.2	12.2	53.7	Sandy Mud	2.93
T2.1	0	0.3	0.8	12.8	12.5	8.7	1.7	11.3	51.9	Slightly Gravelly Sandy Mud	4.15
T2.2	0	0.1	0.8	10.7	10	7.4	4.7	18.9	47.4	Muddy Sand	3.79
T2.3	0	0	0.2	7.2	5.3	5.1	5.8	20	56.5	Sandy Mud	2.59
T3.1	0	0.4	0	20.2	18.1	13	6.4	7.8	34.1	Muddy Sand	5.31
T3.2	0	0.4	1	8.3	11.8	11.7	4.9	12.5	49.5	Slightly Gravelly Sandy Mud	4.48

4. Discussion

In order to assess the estuarine biological environment in the vicinity of Limerick Waste Water Treatment Plant (WWTP), prior to an upgrade of the facility, AQUAFACT carried out a series of biological surveys of the intertidal and subtidal estuarine communities upstream and downstream of the Limerick WWTP outfall. This area of the Lower Shannon SAC is known as Limerick Docks and is a freshwater tidal environment with salinity ranging from 0.833 psu to 3.2 psu. The fauna of such areas in upper estuaries is that of a tidal freshwater zone, which leads to a stressed freshwater-seawater interface. Such areas have a reduced faunal composition compared to rivers or the higher salinity upper reaches of an estuary (McLusky *et al.,* 1993).

Multivariate analysis of the fauna identified two distinct biological communities. The subtidal stations are classified as belonging to one of the seven common benthic community habitat types occurring within the Estuaries Qualifying Interest [1130] within the Lower Shannon Estuary SAC namely the habitat 'Estuarine subtidal muddy sand to mixed sediment with gammarids community complex'. This community complex occurs at the eastern extreme of the SAC site from Cock Rock to Scarlet Reach in water depths between 2.5 and 7m (NPWS, 2012). Within this community, the number of species and their abundance was low which is typical of this estuarine community (AQUAFACT, 2011a). Five species accounted for almost 94% of the faunal abundance: the oligochaetes *Tubifex tubifex* and *Limnodrilus* spp., the amphipods *Corophium multisetosum* and *Gammarus zaddachi,* and the gastropod *Potamopyrgus antipodarum.* The oligochaetes and gastropods at these stations are more a reflection of the freshwater influence here than a characteristic of this fauna poor benthic community. The subtidal sediment type was muddy sand and sandy mud according to Folk (1954) classification. Organic carbon values ranged from 3.66% (B3) to 6.78% (B1). The characterising surveys carried out by AQUAFACT (2011a) for the Lower Shannon SAC recorded an organic carbon content range from 17.26% to 18.65% for stations in the same biological community and close vicinity.

The second distinct biological community was recorded at all the intertidal stations, both upstream and downstream of the outfall. Analysis of the fauna from the intertidal grab stations indicates that these stations cannot be assigned to one of the seven common benthic community

habitat types occurring within the Estuaries Qualifying Interest [1130] within the Lower Shannon Estuary SAC (NPWS, 2012). They can be classified as belonging to the JNCC biotope 'SS.SMu.SMuVS.LhofTtub – *Limnodrilus hoffmeisteri, Tubifex tubifex* and *Gammarus* spp. in low salinity infralittoral muddy sediment' (EUNIS code A5.327). This community is found in upper estuary muddy sediments with very low fluctuating salinity, characterized by the oligochaetes *Limnodrilus hoffmeisteri* and *Tubifex tubifex.* Two statistically significant groups can be classified as belonging to this biotope (**Group a** (T1.1) and **Group b** (all other intertidal stations)), with the differences being because of slight species composition variance. However, all intertidal stations are dominated (>90% abundance) by the oligochaetes *Limnodrilus* spp. and *Tubifex tubifex.* Additional species important to note are the gastropod *Potamopyrgus antipodarum* and the amphipod *Corophium multisetosum.* Organic carbon ranged from 2.59% (T2.3) to 5.31% (T3.1).

Limnodrilus spp. and *Tubifex tubifex* were the two most dominant taxa in both the subtidal and intertidal stations, accounting for over 90% of the faunal abundance. *Limnodrilus* spp. has a wide ecological range and inhabits cohesive muds in all types of waters including polluted, but it cannot tolerate oxygen deficiency. Although it is a freshwater species it is found further seaward than any other freshwater aquatic oligochaete species and is found in habitats likely to be exposed to very low salinity, *e.g.,* upper estuaries where interstitial salinity is less than 5 psu (Budd, 2003). *Tubifex tubifex* inhabits cohesive muds in a variety of habitats and is tolerant of oxygen deficiency. It is especially abundant in polluted sediments and marginal habitats not occupied by many other species, *e.g.,* upper estuaries where interstitial salinity is less than 5 psu (Budd, 2005).

In a study on the intertidal and subtidal soft sediment macrofauna of the upper Forth estuary, Scotland, McLusky *et al.* (1993) recorded very high abundances of these taxa in an area which historically received large quantities of organic waste from both sewage and industrial discharge. In 1977, areas in the upper Forth Estuary with comparable salinity and grain size as the intertidal stations in the Limerick Dock area had *Limnodrilus* spp. recorded at 217,600 per m^2, and *Tubifex tubifex* recorded at 127,400 per m^2 with organic carbon levels from 8% to 19.8%. In 1988/89 surveys in the same locations, following substantial sewage treatment works upstream at Stirling and Alloa, the organic carbon levels had reduced to between 3.6% and 8.6% and a corresponding near 10-fold reduction in the density of oligochaetes.

In a similar study of the spatial distribution of oligochaetes in the tidal freshwater and brackish parts of the Schelde Estuary, Belgium, Seys *et al.* (1999) record densities of oligochaetes in similar oligohaline to freshwater zones at between 3,750 per m^2 to 216,032 per m^2 respectively. They report that in addition to organic content the total density and total biomass of these oligochaetes are significantly correlated with sediment and preferring fine silt sediments.

Within the present study, highest densities of oligochaetes were *Limnodrilus* spp. recorded at 11,610 per m^2 (T3.1) and *Tubifex tubifex* recorded at 10,397 per m^2 (T2.1), with organic carbon levels ranging from 2.59% (T2.3) to 5.31% (T3.1). This biotope is common in the upper tidal reaches of estuaries. The nutrient and organic enrichment from agriculture and siltation from the Shannon catchment have a strong influence on the benthic communities in the Shannon Estuary. It is probable that this influence across the area surveyed is more important than that of the treated waters released from the outfall.

5. Conclusion

The Estuarine subtidal muddy sand to mixed sediment with gammarids community complex is a species poor biotope reflective of its location in the upper estuary with a freshwater tidal influence. In the Limerick Docks area in the vicinity of the Limerick WWTP outfall there is a slightly higher diversity than recorded for this biotope for the Lower Shannon SAC (AQUAFACT, 2011a) and this is due to the presence of freshwater species of oligochaetes that may not be present in this community further downstream. The taxa characterizing this community are amphipods indifferent to organic enrichment (*i.e.,* gammarids, *Leptocheirus* sp.) and those sensitive to organic enrichment (*Bathyporiea* spp). Changes in water quality (*i.e.,* decrease in organic content in outfall discharge) by the Limerick WWTP upgrade are unlikely to have a negative effect on the biotope at this location.

The intertidal biotope SS.SMu.SMuVS.LhofTtub – *Limnodrilus hoffmeisteri, Tubifex tubifex* and *Gammarus* spp. in low salinity infralittoral muddy sediment is present in the survey area both upstream and downstream of the outfall. It is dominated by the oligochaetes *Limnodrilus* spp. and *Tubifex tubifex.* This biotope is not considered a QI for the SAC. It is not negatively affected by

changes in nutrient or organic enrichment however the population density may decrease with reduction in organic enrichment. However, considering the strong influence of the nutrient and organic enrichment from the Shannon catchment, reductions in the organic content of outfall discharges should have little impact when considering the wider influence of upstream loading.

6. References

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Appendix I

Species Lists

P12420 Limerick WWTP- Subtidal																	
Phylum	**Class**	**Order**	**Family**		**AphiaID**	**B1a**	**B1b**	**B2a**	**B2b**	**B3a**	**B3b**	**B4a**	**B4b**	**B5a**	**B5b**	**B6a**	**B6b**
Nematoda				Nematoda	799							1					
Annelida	Clitellata			Oligochaeta	2036							pres					
Annelida	Clitellata	Haplotaxida	Naididae	Naididae	2039					3							
Annelida	Clitellata	Haplotaxida	Naididae	*Limnodrilus*	137388	17	11	1			5	8			5		
Annelida	Clitellata	Haplotaxida	Naididae	*Tubifex tubifex*	137569	5	7		1	10	14	11		3	4		1
Annelida	Clitellata	Haplotaxida	Naididae	*Tubificoides pseudogaster*	137582					1							
Annelida	Clitellata	Enchytraeida	Enchytraeidae	Enchytraeidae	2038	1											
Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammaridae juvenile	101383									1			
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus zaddachi*	102299	2							3				
Arthropoda	Malacostraca	Amphipoda	Corophiidae	Corophiidae	101376				pres								
Arthropoda	Malacostraca	Amphipoda	Corophiidae	*Corophium multisetosum*	102096		2	1			3		1			1	
Mollusca	Gastropoda	Cycloneritida	Neritidae	*Theodoxus fluviatilis*	153558		1										
Mollusca	Gastropoda	Littorinimorpha	Tateidae	*Potamopyrgus antipodarum*	147123	2	1								3	1	

P12420 Limerick WWTP – Intertidal Transect 1											
Phylum	**Class**	**Order**	**Family**		**AphiaID**	**T1.1a**	**T1.1b**	**T1.2a**	**T1.2b**	**T1.3a**	**T1.3b**
Nematoda				Nematoda	799						
Annelida	Polychaeta	Terebellida	Ampharetidae	Ampharetidae	981		3				
Annelida	Clitellata			Oligochaeta	2036		3				
Annelida	Clitellata	Haplotaxida	Naididae	Naididae	2039						
Annelida	Clitellata	Haplotaxida	Naididae	*Limnodrilus*	137388	77	106	273	166	165	128
Annelida	Clitellata	Haplotaxida	Naididae	*Tubifex tubifex*	137569	5	11	62	58	146	111
Annelida	Clitellata	Enchytraeida	Enchytraeidae	Enchytraeidae	2038	95	2				
Arthropoda	Arachnida			Acari	292684	1					
Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammaridae	101383						1
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus zaddachi*	102299					4	
Arthropoda	Malacostraca	Amphipoda	Corophiidae	*Corophium*	101489			1			
Arthropoda	Malacostraca	Amphipoda	Corophiidae	*Corophium multisetosum*	102096	20	89			3	
Arthropoda	Insecta	Diptera		Diptera	118088						
Arthropoda	Insecta	Diptera		Diptera larva	118088	1	2				
Arthropoda	Insecta	Diptera	Ceratopogonidae	Ceratopogonidae	150940		1				
Arthropoda	Insecta	Diptera	Chironomidae	Chironomidae	118100			1			
Arthropoda	Insecta	Diptera	Chironomidae	Chironomidae larva	118100	3	4			3	
Arthropoda	Insecta	Diptera	Psychodidae	Psychodidae	391173						
Arthropoda	Insecta	Ephemeroptera		Ephemeroptera	235812	1					
Arthropoda	Collembola			Collembola	118086	1					
Mollusca	Gastropoda			Gastropoda	101		1				
Mollusca	Gastropoda	Littorinimorpha	Hydrobiidae	Hydrobiidae	120						
Mollusca	Gastropoda	Littorinimorpha	Hydrobiidae	Hydrobiidae juvenile	120						
Mollusca	Gastropoda	Littorinimorpha	Tateidae	*Potamopyrgus antipodarum*	147123	6	117	9	7	5	

P12420 Limerick WWTP – Intertidal Transect 2											
Phylum	**Class**	**Order**	**Family**		**AphiaID**	**T2.1a**	**T2.1b**	**T2.2a**	**T2.2b**	**T2.3a**	**T2.3b**
Nematoda				Nematoda	799	6	11			2	
Annelida	Polychaeta	Terebellida	Ampharetidae	Ampharetidae	981						
Annelida	Clitellata			Oligochaeta	2036						
Annelida	Clitellata	Haplotaxida	Naididae	Naididae	2039			4			
Annelida	Clitellata	Haplotaxida	Naididae	Limnodrilus	137388	256	327	117	99	9	103
Annelida	Clitellata	Haplotaxida	Naididae	Tubifex tubifex	137569	339	441	23	23	9	147
Annelida	Clitellata	Enchytraeida	Enchytraeidae	Enchytraeidae	2038						
Arthropoda	Arachnida			Acari	292684						
Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammaridae	101383						
Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammarus zaddachi	102299						
Arthropoda	Malacostraca	Amphipoda	Corophiidae	Corophium	101489						
Arthropoda	Malacostraca	Amphipoda	Corophiidae	Corophium multisetosum	102096					11	2
Arthropoda	Insecta	Diptera		Diptera	118088						
Arthropoda	Insecta	Diptera		Diptera larva	118088						
Arthropoda	Insecta	Diptera	Ceratopogonidae	Ceratopogonidae	150940						
Arthropoda	Insecta	Diptera	Chironomidae	Chironomidae	118100	1					2
Arthropoda	Insecta	Diptera	Chironomidae	Chironomidae larva	118100		2			1	
Arthropoda	Insecta	Diptera	Psychodidae	Psychodidae	391173						
Arthropoda	Insecta	Ephemeroptera		Ephemeroptera	235812						
Arthropoda	Collembola			Collembola	118086						
Mollusca	Gastropoda			Gastropoda	101						
Mollusca	Gastropoda	Littorinimorpha	Hydrobiidae	Hydrobiidae	120						
Mollusca	Gastropoda	Littorinimorpha	Hydrobiidae	Hydrobiidae juvenile	120						
Mollusca	Gastropoda	Littorinimorpha	Tateidae	Potamopyrgus antipodarum	147123		2	1	2		

P12420 Limerick WWTP – Intertidal Transect 3											
Phylum	**Class**	**Order**	**Family**		**AphiaID**	**T3.1a**	**T3.1b**	**T3.2a**	**T3.2b**	**T3.3a**	**T3.3b**
Nematoda				Nematoda	799		2		1	2	
Annelida	Polychaeta	Terebellida	Ampharetidae	Ampharetidae	981						
Annelida	Clitellata			Oligochaeta	2036	1					
Annelida	Clitellata	Haplotaxida	Naididae	Naididae	2039			2			
Annelida	Clitellata	Haplotaxida	Naididae	*Limnodrilus*	137388	607	264	199	78	162	130
Annelida	Clitellata	Haplotaxida	Naididae	*Tubifex tubifex*	137569	160	45	60	57	38	96
Annelida	Clitellata	Enchytraeida	Enchytraeidae	Enchytraeidae	2038						
Arthropoda	Arachnida			Acari	292684						
Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammaridae	101383						
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus zaddachi*	102299						
Arthropoda	Malacostraca	Amphipoda	Corophiidae	*Corophium*	101489						
Arthropoda	Malacostraca	Amphipoda	Corophiidae	*Corophium multisetosum*	102096						pres
Arthropoda	Insecta	Diptera		Diptera	118088		1				
Arthropoda	Insecta	Diptera		Diptera larva	118088						
Arthropoda	Insecta	Diptera	Ceratopogonidae	Ceratopogonidae	150940						
Arthropoda	Insecta	Diptera	Chironomidae	Chironomidae	118100	1	2				1
Arthropoda	Insecta	Diptera	Chironomidae	Chironomidae larva	118100			2		17	
Arthropoda	Insecta	Diptera	Psychodidae	Psychodidae	391173		1				
Arthropoda	Insecta	Ephemeroptera		Ephemeroptera	235812						
Arthropoda	Collembola			Collembola	118086						
Mollusca	Gastropoda			Gastropoda	101						
Mollusca	Gastropoda	Littorinimorpha	Hydrobiidae	Hydrobiidae	120	1					
Mollusca	Gastropoda	Littorinimorpha	Hydrobiidae	Hydrobiidae juvenile	120				1		
Mollusca	Gastropoda	Littorinimorpha	Tateidae	*Potamopyrgus antipodarum*	147123	4	5	2	2	35	

Appendix 2

Sediment Analysis Methodology

Particle size analysis (PSA)

AQUAFACT will carry out the Particle Size Analysis using the traditional granulometric technique. We have all of the necessary equipment required *e.g.,* Wentworth graded sieves, Easysize and GRADISTAT computer software, hydrogen peroxide, sodium hexametaphosphate, drying oven, beakers, mixers, electronic scales. We have carried out sediment analysis for all subtidal sampling programmes that we have been involved in.
AQUAFACT will employ the following methodology for the granulometric analysis:

1. Approximately 100g of dried sediment (previously washed in distilled water and dried) is weighed out and placed in a labelled 1L glass beaker to which 100ml of a 6 percent hydrogen peroxide solution is then added. This is allowed to stand overnight in a fume hood.
2. The beaker is placed on a hot plate and heated gently. Small quantities of hydrogen peroxide are added to the beaker until there is no further reaction. This peroxide treatment removes any organic material from the sediment which can interfere with grain size determination.
3. The beaker is then emptied of sediment and rinsed into a 63µm sieve. This is then washed with distilled water to remove any residual hydrogen peroxide. The sample retained on the sieve is then carefully washed back into the glass beaker up to a volume of approximately 250ml of distilled water.
4. 10ml of sodium hexametaphosphate solution is added to the beaker and this solution is stirred for ten minutes and then allowed to stand overnight. This treatment helps to dissociate the clay particles from one another.
5. The beaker with the sediment and sodium hexametaphosphate solution is washed and rinsed into a 63µm sieve. The retained sampled is carefully washed from the sieve into a labelled aluminium tray and placed in an oven for drying at 100ºC for 24 hours.
6. The dried sediment should then be passed through a Wentworth series of analytical sieves (>8,000 to 63µm; single phi units). The weight of material retained in each sieve is weighed and recorded. The material passing through the 63µm sieve is also weighed and the value added to the value measured in Point 5 above.
7. The total silt/clay fraction is determined by subtracting all weighed fractions from the initial starting weight of sediment as the less than 63µm fraction was lost during the various washing stages.
8. The reporting of sediment samples will be as percentages within the following range of particle sizes:
    - PSA % <63
    - PSA % 63<125
    - PSA % 125<250
    - PSA % 250<500
    - PSA % 500<1000
    - PSA % 1000<2000
    - PSA % 2000<4000
    - PSA % 4000<8000
    - PSA % ≥8000

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