Feasibility Study: Assessing Water Quality to Determine the Habitat Suitability for Seagrass Restoration near Roosecote Sands

Alice Parker, North West Marine Futures Intern



North West Marine Futures Internship 2024

Contents

Glossary of Abbreviations	2
1. Introduction	3
1.1 Project Rationale	3
1.2 Background	3
1.2.1 Seagrass Restoration	3
1.2.2 Seagrass Ecology	4
1.2.3 Effect of Water Quality on Seagrass	4
1.2.4 Local Water Quality Concerns	5
2. Aims and Objectives	7
2.1 Aims	7
2.2 Objectives	7
3. Methods	8
3.1 Pre-Sampling Test	8
3.1.1 Pre-Sampling Test: Method	8
3.1.2 Pre-Sampling Test: Results	8
3.2 Pilot Water Quality Assessments	8
3.2.1 Sample Strategy	8
3.2.2 Sampling Procedure	11
3.2.3 Testing Water Quality Parameters	12
4. Results	13
4.1 Nutrient Status	13
4.1.1 Phosphate	13
4.1.2 Ammonia	16
4.1.3 Nitrate	18
4.2 Physio-Chemical Status	20
4.2.1 pH	20
4.2.2 Salinity	21
4.2.3 Temperature	22
5. Discussion	23
5.1 Study Limitations	23
5.2 Justification for Further Water Quality Assessment	24
5.3 Evaluation of Methods	26
6. Summary of Recommendations for Future Water Quality Assessment	26
7. Conclusion	28
Acknowledgements	28
References	29
Appendices	35

Disclaimer

The author of this report confirms that the views expressed within are solely their own and are not necessarily representative of the North West Wildlife Trusts or other organisations involved in the project.

BWWTW	Barrow Wastewater Treatment Works
СВ	Concle Bank seagrass bed
DEM	Digital elevation model
EA	Environment Agency
LOD	Limit of Detection (i.e., the lower limit of detection)
NWWT	North West Wildlife Trusts
PRA	Potential restoration area
RS	Roosecote Sands seagrass bed
WFD (2000)	The Water Framework Directive, i.e., EU 2000 Directive 2000/60/EC of the
	European Parliament and of the Council of 23 October 2000 establishing a
	framework for community action in the field of water policy, Official Journal of
	European Communities L 327 1 72.

Glossary of Abbreviations

1. Introduction

1.1 Project Rationale

The North West Wildlife Trusts (NWWT) requested this study to investigate the feasibility of carrying out a water quality assessment to inform their approach to seagrass restoration.

The NWWT has been investigating the potential for seagrass restoration near Roosecote Sands, Barrow, Cumbria, as part of the Morecambe Seascape Restoration Project. Both seagrass species native to the UK are found in this area (Peyton-Jones and Rounce, 2023). Based on historical extent data, the NWWT has identified a potential restoration area (PRA) adjacent to the existing seagrass beds.

Previous reports from NWWT outline the historical and recent extent of seagrass along the Cumbria coast and detail the selection of the PRA (Cale and Churn, 2021; Clifford, 2021; Gould, 2022a; 2022b; Peyton-Jones and Rounce, 2023). The NWWT is collecting *Zostera noltii* seeds this year, with the aim to begin its first trials of seagrass restoration in 2024-2025. The NWWT recognises that water quality is a significant variable to be considered prior to restoration. However, given resource and time constraints it is important to determine if assessing water quality would be necessary, cost-effective and an appropriate use of resources.

1.2 Background

1.2.1 Seagrass Restoration

Globally, human activities continue to degrade ecosystems that sustain life on Earth (IPBES, 2018; Díaz et al., 2019; Dhiman, 2022; UN, 2023). Seagrass meadows can provide numerous vital ecosystem services, including carbon sequestration (McLeod et al., 2011; Fourqurean et al., 2012; Potouroglou et al. 2021; Serrano et al., 2021; WWF, RSPB and The Wildlife Trusts, 2024), habitat provisioning for economically significant fish species (Nordlund et al., 2017; Erzini et al., 2022) and natural protection from coastal erosion and inundation (Christianen et al., 2013; Ondiviela et al., 2014; Jacob et al., 2023). Meta-analyses report extensive seagrass loss internationally during the last century (Waycott et al., 2009; Dunic et al., 2021). Although the lack of historical data impedes accurate evaluations, seagrass decline in the UK is estimated at between 44% (high certainty) and 94% (low certainty) (Green et al., 2021). Suggested drivers of this decline include the outbreak of wasting disease (*Labyrinthula zosterae*) in the 1930s and anthropogenic pressures such as physical disturbances (e.g., bottom trawling), climate change impacts (e.g., increased storminess and rainfall elevating turbidity in the water column) and water pollution (Jones and Unsworth, 2016; Potouroglou et al., 2021).

As a result, seagrass restoration is increasingly prominent; it is promoted as a mitigation approach to tackling the entwined climate and biodiversity crises (Gamble et al., 2021; Unsworth et al., 2022). In recent years, various seagrass restoration projects have emerged within the UK (Ward et al., 2023). However, there is a widespread consensus amongst academics, policymakers and practitioners that further implementation and research into marine restoration is necessary if its potential to facilitate socio-environmental sustainability is to be achieved (Garbutt et al., 2024).

Assessing habitat suitability before implementation is widely recommended for effective seagrass restoration (Kent et al., 2021; Preston et al., 2021). Although habitat suitability assessments or models cannot guarantee restoration success, they provide crucial insights that can increase the likelihood of ecosystem recovery and can inform wider management approaches (Preston et al., 2021). Assessing the water quality of restoration sites pre-planting is a vital part of determining habitat suitability, since poor water quality can inhibit seagrass growth (Unsworth et al., 2018; Preston et al., 2021).

1.2.2 Seagrass Ecology

Seagrasses are a group of marine angiosperms (Nordlund et al., 2017), with two species found in the UK (Table 1).

Table 1. Summary of the two species of seagrass found in the UK, information based on Potouroglou and Unsworth (2021) and Gamble et al. (2021).

Common name	Common eelgrass	Dwarf eelgrass	
Taxonomic name	Zostera marina	Zostera noltei or noltii	
Predominant habitat niche	Sublittoral	Intertidal	
Typical position on shoreline	Lower shore	Higher shore	
Relative light toleranceLower light tolerance		Higher light tolerance	
Height	>1.5 mm <1.5 mm		
Reproductive strategies	Pollination of flowers (sexual) and rhizomes (asexual)		

1.2.3 Effect of Water Quality on Seagrass

Poor water quality is widely accepted as a major cause of seagrass decline around the UK and internationally (Griffiths et al., 2020; Zhang et al., 2023; Unsworth et al., 2024). More specifically, increased nutrient inputs in coastal systems presents one of the most significant threats to seagrass meadows in Britain (Jackson et al., 2013; Jones and Unsworth, 2016). A recent study from the Blue Marine Foundation, Project Seagrass and Surfers Against Sewage assessed the elemental tissue of *Z. marina* in 46 sites and *Z. noltii* in 16 sites around the UK coastline. Average elemental nitrogen and phosphate in the seagrass was found to above the global average in 30 sites and 16 sites across the UK, respectively (Unsworth et al., 2024). This followed Natural England's report which found extreme nutrient enrichment of *Z. noltii* on the East Coast of England compared to global averages (Fox et al., 2024). However, further research into the direct impacts of water pollutant on seagrass is needed (Unsworth et al., 2024).

Nutrient enrichment typically arises from agricultural run-off and wastewater treatment discharges (Nie et al., 2018; Aniebone et al., 2024). Nutrients are essential elements for seagrass and are typically limiting factors on productivity in coastal ecosystems. As such, moderate increases are associated with increased seagrass growth (Jiménez-Ramos et al., 2022). However, elevated levels can limit seagrass primary productivity via two means. Firstly, by stimulating phytoplankton and ephemeral algal growth and increasing water turbidity, which singly or combined can result in reduced underwater irradiance that supresses the photosynthetic rate of seagrass. Secondly, high nutrient levels can result in direct toxicity (Brun et al., 2002; 2008; Moreno-Marín et al., 2016). Laboratory studies have observed declines in Zostera spp. due to the effect of the nitrate (Burkholder et al. 1992; 1994) and ammonium (van Katwijk et al., 1997). The toxicity mechanisms are not fully understood (Brun et al., 2008). Yet, previous research on toxicity mechanisms in similar plants identify a combination of physiological processes (Brun et al., 2008), including ammonia molecules causing the uncoupling of the ATP production during photosynthetic electron transport (Goyal et al. 1982; Marschner, 1995), the increase of intracellular inorganic anions (e.g., phosphate) resulting in the intracellular depletion of essential cations (e.g., potassium) (Kirkby, 1968; van Katwijk et al. 1997) and higher energetic expenditure associated with pumping out intracellular ammonia (Britto et al., 2001).

To assess the habitat suitability of water quality for seagrass, it is crucial to investigate different nutrients and wider environmental conditions in combination (Preston et al., 2021). Nutrients interact in the water column and seagrass nutrient levels depend on the availability and interactions between different nutrients (Touchette and Burkholder, 2000). For instance, *Zostera spp*. are able to utilise

nitrogen as nitrate and ammonium, in contrast to some terrestrial plants (Tennant, 2006). Seagrasses normally exhibit greater uptake of ammonium, since nitrate uptake requires more energy expenditure (Jiménez -Ramos et al., 2022). When ammonia (NH₃) dissolves in water, some converts to ammonium ions (NH₄). The proportion of ammonia converted to ammonium is dependent on environmental conditions, e.g., temperature and pH (Bower and Bidwell, 1978). In seawater (with a typical pH of 8.1), the majority of total ammonia will be in the form of ammonium (Bell et al., 2007). Moreover, photosynthetic organisms need both nitrogen and phosphate, though uptake of nitrogen is affected by the availability of phosphorous (Tennant, 2006). Short (1983) reported Z. marina tissues contain N/P ratio of 23:1, with ambient environment N/P ratios ideally reflecting the Redfield ratio (16:1). N/P ratios below 5:1 are shown to limit Z. marina (Thom and Albright, 1990; Murray et al., 1992). Consequently, phosphate deficiency has been found to increase the vulnerability of Z. noltii to elevated levels of ammonium (Brun et al., 2008). Likewise, environmental conditions can exacerbate or mediate the effects of nutrient levels (Alexandre et al., 2020; Jiang et al., 2024). For instance, van Katwijk et al. (1997) found ammonium toxicity in Z. marina was greatest at higher temperatures, attributed to increased metabolic activity in warmer conditions. van Katwijk et al. (1999) observed that the effects of nitrate, ammonium and phosphate enrichment on Z. marina varied with the salinity of ambient water. As with nutrient concentrations, seagrass species will have tolerance thresholds for pH, salinity and temperature and ranges within which their growth is optimised. However, this is dependent on species acclimatisation to localised conditions and site-specific environmental factors, which necessitates site-by-site investigations to determine the habitat suitability for seagrass restoration (Lee et al., 2007; Nejrup and Pedersen, 2008; Gamble et al., 2021).

1.2.4 Local Water Quality Concerns

There is little evidence of previous water quality testing within the area of the NWWT's PRA and surrounding seagrass beds. Figure 1 shows the nearest locations sampled regularly by the Environment Agency (EA). The sampling point Poaka (Mill) Beck into Cavendish Dock (NW-88004904) presents the nearest EA sampling location, with annual readings for various nutrient and physiochemical variables (EA, 2024). Sampling closer to the PRA and existing seagrass meadows is necessary for more detailed analysis of water quality.

There are several potential pollution sources feeding into the site that may be of concern (Figure 1). A particular concern is the storm outflow from Barrow-in-Furness Wastewater Treatment Works (BWWTW): regular reviews of the United Utilities' (2024) online *Storm Overlap Map* suggests this storm outflow is occasionally discharging directly into the PRA.



Figure 1. Map showing the study area, including the potential restoration area (PRA) and seagrass extent (based on the NWWT's 2023 extent surveys), with potential pollution sources and EA water quality sampling points highlighted; the EA sampling point labelled 1 is known as Poaka Beck (Mill) into Cavendish Dock.

2. Aims and Objectives

2.1 Aims

This project aims to:

- Investigate the feasibility of carrying out a water quality assessment to inform the NWWT's approach to seagrass restoration.
- Conduct pilot assessments to evaluate different methods for conducting a water quality assessment of the PRA and existing seagrass beds.
- Analyse results from these pilot assessments to evaluate if further water quality assessments would be worthwhile.
- Provide recommendations for future water quality assessments in relation to the NWWT's seagrass restoration project.

2.2 Objectives

To achieve these aims, the following objectives were set:

- (1) Conduct pilot assessments to develop a method for assessing water quality within the existing seagrass beds, PRA and relevant freshwater inflows feeding into these sites.
- (2) Compare the results for between the existing seagrass beds and PRA from the pilot assessments to identify any indications of differences between the two sites.
- (3) Analyse the results from the pilot assessments to determine if there are any indications that further water quality assessments would be appropriate to inform restoration efforts.
- (4) Utilise these findings to provide recommendations for future monitoring of water quality.

3. Methods

3.1 Pre-Sampling Test

The proposed method was to collect water samples, then conduct the tests to quantify the water quality parameters the following day. This was intended to optimise the sampling effort, thereby allowing for a larger sample size and more robust results. However, a literature review found that the accuracy of water quality readings may be affected by storing samples overnight (Gardolinski et al., 2001; Reed et al., 2023). Thus, a pre-sampling test was conducted to determine the effect of storing the samples.

3.1.1 Pre-Sampling Test: Method

Samples were collected from the freshwater inflows and seawater pools within the vicinity of the existing seagrass beds at low tide. The samples were filtered immediately after collection and ammonia (NH₃/NH₄⁺), nitrate (NO₃⁻) and phosphate (PO₄³⁻) concentrations were tested immediately at the shore with the HI-784 Marine Ammonia Checker, HI-782 Marine Nitrate Checker for Marine and the HI-713 Phosphate Checker, respectively. The samples were transported to the storage facility (within approximately 1-2 hours) and stored in a refrigerated for approximately 12 hours. Thereafter, ammonia, nitrate and phosphate concentrations were retested using the same process. Refrigeration is used because cooler temperatures reduce microbial activity, resulting in slower nutrient degradation: this should minimise the change in nutrient concentrations overtime (Kirkwood, 1996; Lloyd et al., 2022).

3.1.2 Pre-Sampling Test: Results

The results indicated the difference between the readings taken pre- and post-storage was within or close to the accuracy range of the given meter used. Hence, it was determined samples could be collected then tested the next day.

3.2 Pilot Water Quality Assessments

3.2.1 Sample Strategy

Two pilot assessments were undertaken and compared to evaluate the most effective method.

The first was a watercraft-based assessment: water samples were collected from kayaks or paddleboards. This allowed samples to be collected directly from over the PRA and existing seagrass beds. A shore-based assessment was also conducted: water samples were collected from the shore. Although samples were not collected directly from within the PRA or over the existing seagrass beds, the shore-based method was proposed as a simpler, faster and safer approach. Table 2 and 3 detail the equipment used.

Table 2. Equipment list of equipment used for both assessments.

Equipment	Reason for use
1 x container (i.e., clean glass jar) for each sample	To collect water samples
1 x Garmin GPS device per team of samplers	To record location of sampling point
HI-784 Marine Ammonia Checker	To test ammonia concentration of samples
HI-782 Nitrate High Range Checker for Marine	To test nitrate concentration of samples
HI-781 Handheld Colorimeter Marine Nitrate LR	To test nitrate concentration of samples
HI-713 Handheld Colorimeter Phosphate Low Range	To test phosphate concentration of samples
HI-98319 Marine Waterproof Salinity Tester	To test salinity concentration of samples and
	temperature at the sampling point
Thermoscientific Waterproof ELITE PCTS	To test pH samples and temperature at the
	sampling point
10 x filter funnels	
1 x filter paper sheet per sample (> 10 μm particle	To filter samples
retention, Stonylab Quantitative Filter Paper	
1 x container to filter into/from for each sample	

Table 3. Additional equipment required for the watercraft-based assessment.

Equipment	Reason for use
1 x kayak/paddle-board per sampler	To access the marine sampling points
PPE for each sampler onboard watercraft (buoyancy	For the health and safety of the samplers
aids, wet suits, etc.)	

In each case, samples were taken to represent water quality conditions within the PRA, the existing seagrass beds and freshwater inflows. Samples were taken from the existing seagrass beds to allow for a comparison with conditions within the PRA, and determine any difference between the two which may indicate why the PRA currently exhibits less extensive seagrass growth and, thus, why PRA may be unsuitable for seagrass restoration. Two existing seagrass beds were selected for sampling. Roosecote Sands seagrass bed (RS) was selected because it is the existing seagrass bed closest to the PRA. Concle Bank seagrass bed (CB) was sampled to act as an additional control: it is southward of the spit and, therefore, may not be so significantly affected by the freshwater inflows identified as potential pollution sources for the PRA (Figure 2).

Four sampling points were selected for each sampling area (i.e., PRA, RS and CS). This was deemed the maximum that could feasibly be sampled and then tested within 24 hours of sampling. The colorimetric tests used to determine the nutrient concentrations rely on time-dependent reactions to quantify the nutrient concentrations. For each sample, the test for ammonia, nitrate and phosphate had a reaction time of 15 minutes, 8 minutes or 7 minutes and 3 minutes, respectively, with additional time required for setting up the tests (see section 3.2.3). A total of 12 tests plus 2 control tests was estimated to take at least 7 hours (one working day).

Figure 2 shows the sample collection points from for each pilot assessment. The freshwater sampling points were the same for both surveys and samples from these sites were collected on foot. In each case, one sample was collected from each sampling point.



Figure 2. Sampling plan with sampling points for the shore-based and watercraft-based assessments.

For the watercraft-based sampling, the marine sampling points were predetermined using QGIS. To minimise the confounding effect of having sampling areas of different sizes, a standardised sampling area of 90,000 m² was drawn within the extent of the PRA, RS and CB. Four randomised sampling points within these sampling areas were generated in QGIS. These points were found on site using the MerginMaps mobile phone app and Garmin GPS devices.

For the shore-based sampling, marine sampling points were taken from the shore adjacent to the sampling areas: PRA, RS and CB. A main goal of this pilot assessment was to determine safe access points to collect marine water samples from the shore. Hence, approximate sampling points were selected prior to surveying; then, the locations of the sampling points were finalised in the field and recorded using the Garmin GPS devices for future reference.

Watercraft-based Assessment

Samples for the watercraft-based assessment were collected on September 29th 2024 (high water at 10:29 am, 7.77 metres). For several days prior to this, United Utilities (2024) was reporting that the storm outflow had not be discharging. For the watercraft-based assessment, three surveyors collected the marine samples (working in two teams), with a land-based observer acting as safety cover. Team 1 collected samples from all the sampling points for the PRA and CB3 and Team 2 collected the sampling points for RS and the remaining CB sampling points (Figure 2). The marine water samples were collected during the two hours around high tide. A date close to the neap tide was selected for the watercraft-based assessment as it was thought this would make for calmer sea conditions. Also, neap tides theoretically present a 'worst case scenario' where there is less tidal influence affecting pollutants flowing into the estuary from freshwater inflows, resulting in a lower dilution factor (Cereja et al., 2022). After the marine samples had been collected, the freshwater samples were collected on foot by approach points from the shore (approximately 1-2 hours after high tide).

Shore-based Assessment

Samples for the shore-based assessment were collected on October 29th 2024 (high water at 09:32 am, 8.12 meters). United Utilities (2024) was reporting that the storm outflow had been discharging for a at least 10 hours prior to this. For the shore-based assessment, four surveyors collected the marine samples (working in two teams). Team 1 collected the samples from the sampling points for PRA and RS in addition to collecting the samples from freshwater sampling points 1,2 and 3 (F1, F2 and F3) (Figure 2). Team 2 collected the samples from the sampling points for CB and freshwater sampling point 4 (F4) (Figure 2). All samples were collected between 09:30 and 11:30 (the two hours after high tide).

3.2.2 Sampling Procedure

For each marine sampling point, the approximate locations of where the sample was collected was recorded using a Garmin GPS device or the MerginMaps App. This may have deferred slightly from the planned sampling points, e.g., due to drift on the watercraft whilst collecting or if points were changed when on site to more accessible locations. For the watercraft-based assessment, the surface water temperature of all sampling points was tested using the HI-98319 Marine Waterproof Salinity Tester or Thermoscientific Waterproof ELITE PCTS. (This equipment was not available to test salinity and temperature for the shore-based assessment).

For all sampling points, the water sample (approximately 300 ml) was collected in a clean glass jar. Marine samples were taken from the greatest depth the surveyor could safely reach, in an attempt to collect water that is most representative of the conditions for seagrass rooted on the seabed. Before collecting the sample, the jar was rinsed several times with the water from the water body which was being sampled. Jars were labelled and wrapped in a protective layer (i.e., a sock) to prevent breakages.

As soon as possible after collection (i.e., after collecting all the samples), the samples were filtered using filter paper (> 10 μ m particle retention, Stonylab Quantitative Filter Paper) and funnels. Filtering was undertaken to minimise the likelihood of silt or other particles affecting the colorimetric tests for determining nutrient concentrations. The samples were transported to the storage facility (within approximately 1-2 hours), refrigerated overnight (for approximately 12 hours) and tested the following day.

3.2.3 Testing Water Quality Parameters

The samples were mixed before testing and the readings were recorded when the given meter had stabilised. At least two blank controls were tested with each set of samples (i.e., the samples from the watercraft-based and shore-based assessment, respectively) as a quality control measure to identify sample contamination or other methodological inaccuracies. Deionised water was used for these analytical blanks. The lower Limit of Detection (LOD) for the nutrient colorimeters was taken as the accuracy range of the given meter based on the manufacturer's handbook (Appendix 1), given no standard solutions were available to verify the nutrient responses at lower concentrations. For example, for the phosphate colorimeters' accuracy range is ± 0.04 ppm, thus the LOD was taken as 0.04 ppm and any readings ≤ 0.04 were recorded as 0 ppm.

The pH of each water sample was tested using a calibrated Thermoscientific Waterproof ELITE PCTS pH/Conductivity/TDS/Salinity/Temp Meter with ATC. The salinity was tested using a calibrated HI-98319 Marine Waterproof Salinity Tester for samples from watercraft-based assessment. (The salinity tester was unavailable for testing samples from the shore-based assessment.)

All marine samples were tested for ammonia, nitrate and phosphate. The ammonia and nitrate checkers were not applicable to freshwater conditions, so the freshwater samples were only tested for phosphate. Nutrient concentrations were determined using Hanna Instruments colorimetric checkers, as per the manufacturer's instructions. Ammonia (including ammonium, NH_3/NH_4^+) concentration was tested using the HI-784 Marine Ammonia Checker. Phosphate (PO_4^{3-}) concentration was tested using the HI-784 Marine Ammonia Checker. Phosphate (PO_4^{3-}) concentration was tested using the HI-784 Marine Ammonia Section Range. In trials, the HI-782 Nitrate High Range Checker for Marine only gave zero readings, within its accuracy range (2.0 ppm). Therefore, the nitrate (NO_3^-) concentrations of the samples from the pilot assessments were tested using the HI-781 Handheld Colorimeter Marine Nitrate LR (with an accuracy range of 0.25 ppm), and only tested using the HI-782 Nitrate High Range Checker if there were not enough reagents for HI-781 Handheld Colorimeter LR or the HI-781 Handheld Colorimeter Marine Nitrate LR gave persistent error readings.

4. Results

See Appendix 2 for the full results.

4.1 Nutrient Status

Nutrient readings less than or equal to the LOD were taken as 0 ppm.

4.1.1 Phosphate

Figure 3 shows the phosphate concentrations recorded for the marine samples.

For the watercraft-based assessment, the phosphate concentrations recorded for PRA appear to be higher than that of CB and RS. This is due to two positive readings for PRA (0.07 ppm PO_4 and 0.05 ppm PO_4) with only zero readings recorded for CB and RS.

Likewise, for the shore-based assessment the phosphate concentrations recorded for PRA appear higher than that of CB and RS. The PRA samples gave three positive readings (0.2 ppm PO₄, 0.13 ppm PO₄ and 0.08 ppm PO₄). RS also gave two positive readings, though within a slightly lower range (0.06 ppm PO₄ and 0.12 ppm PO₄). Only zero readings were recorded for CB.

Comparing the two assessments overall, the average readings for all marine samples is greater for the shore-based assessment (0.05 ppm PO₄) than the watercraft-based assessment (0.00 pm PO₄). Samples from the shore-based assessment gave five positive readings for phosphate, whereas the watercraft-based assessment only have two positive readings. Also, the average readings for the shore-based assessment are higher for PRA (0.1 ppm PO₄) and RS (0.045 ppm PO₄) than for the watercraft-based assessment (0.03 ppm PO₄ and 0 ppm PO₄ for PRA and RS, respectively).



Figure 3. Phosphate concentrations recorded for the marine samples collected during the watercraft-based and shore-based assessment for the potential restoration area (PRA) and existing seagrass beds: Roosecote Sands (RS) and Concle Bank (CB).

Figure 4 shows the phosphate concentrations recorded from the freshwater samples in relation to the Water Framework Directive (WFD) status thresholds for freshwater samples (WFD, 2000). For both assessments the freshwater samples show a similar pattern. The F1 samples from each assessment read as 0 ppm PO₄. The F2 samples are good or moderate based on the WFD status thresholds in both cases (0.09 ppm PO₄ for watercraft-based assessment and 0.13 ppm PO₄ for shore-based assessment). The F3 samples gave the highest readings for the watercraft-based assessment (1.06 ppm PO₄) and shore-based assessment (0.43 ppm PO₄), both being within the range of poor status. The F4 samples

gave lower readings than the F3 samples for both assessments, though both are still in the range of poor status (0.27 ppm PO₄ and 0.33 ppm PO₄ for the watercraft-based and shore-based assessment, respectively). The F3 sample from the watercraft-based assessment gave a markedly higher reading than that of any other sample (1.06 ppm PO₄), almost reaching the range of bad status.



Figure 4. Phosphate concentrations recorded from the freshwater samples collected during the watercraft-based and shorebased assessment, in relation to the Water Framework Directive (WFD) status thresholds for surface freshwater bodies (WFD, 2000).

Figure 5 shows the spatial distribution of the phosphate concentrations recorded for the samples from (a) the watercraft-based and (b) the shore-based assessments. The path of the outflow channel is based on a digital elevation model (DEM) (Ladd, C., personal communication, 2024).

For the watercraft-based assessment, the phosphate concentrations of the marine samples appear relatively consistent across all three sample areas, with only one slightly higher phosphate reading highlighted within PRA (PRA 1 reading 0.07 ppm PO₄). This is distant from the two high phosphate readings from the freshwater outflows, F3 and F4, on the east shore of the sampling areas (F3 reading 1.06 ppm PO₄ and F4 reading 0.27 ppm PO₄). The marine samples closet to the outflow channels of these two freshwater inputs sampled (RS 4 and RS 3) are not higher than more distant marine sample readings.

For the shore-based assessment, the phosphate concentrations of the marine samples appear more variable due to the higher readings around the PRA (SPRA 1, SPRA 2 and SPRA 4) and two higher readings taken for RS (SRS 4 and SRS 1 reading). Again, the freshwater outflows F3 and F4 gave the highest readings (0.43 ppm PO₄ and 0.33 PO₄, respectively). The marine samples closet to these freshwater outflows (SPRA 1 and SRS 4) gave high phosphate readings relative to the rest of the marine samples (0.2 ppm PO₄ and 0.12 ppm PO₄ for SPRA 1 and SRS 4, respectively). The reading for the freshwater inflow feeding from the BWWTW storm discharge (F2) was slightly higher for the shore-based assessment than the watercraft-based assessment (0.13 ppm PO₄ compared to 0.09 ppm PO₄), though this is within the accuracy range of the phosphate meter (0.04 ppm). The one other higher marine sample (SPRA 4 reading 0.13 ppm PO₄) is closest to F2.



Sampling points for each sampling area are labelled:

4 for the potential restoration

RS 1, RS 2, RS 3 and RS 4 for Roosecote Sands seagrass bed

CB 1, CB 2, CB 3 and CB 4 for Concle Bank seagrass bed (CB)

for samples from the shore-based

Path of outflow channel (based on DEM)

sampling area are labelled:

SPRA 1, SPRA 2, SPRA 3 and SPRA 4 for the potential restoration

SRS 1, SRS 2, SRS 3 and SRS 4 for Roosecote Sands seagrass bed

SCB 1, SCB 2, SCB 3 and SCB 4 for Concle Bank seagrass bed (CB)

Figure 5. Spatial distribution of the phosphate concentrations recorded for the samples collected during the (a) watercraftbased assessment and (b) shore-based assessment, with labels naming each of the four sampling points for each sampling area.

4.1.2 Ammonia

Figure 6 shows the ammonia concentrations recorded for the marine samples.

For the watercraft-based assessment, the ammonia concentrations determined for the PRA and CB samples are similar, with almost equal ranges (of 0.09 ppm NH₃/NH₄ and 0.08 ppm NH₃/NH₄, respectively) and averages (means of 0.06 ppm NH₃/NH₄ and 0.04 ppm NH₃/NH₄, respectively). The ammonia concentrations of RS samples appear to be slightly higher, with a greater average (mean of 0.19 ppm NH₃/NH₄⁺) and greater variability (with a range of 0.25 ppm NH₃/NH₄), due to two samples with uniquely high ammonia concentrations (0.32 ppm NH₃/NH₄ and 0.28 ppm NH₃/NH₄).

For the shore-based assessment, the ammonia concentration determined for the PRA samples have a much greater range than the CB and RS samples, due to two high readings (0.37 ppm NH_3/NH_4 and 1.29 ppm NH_3/NH_4); one of which is ten times greater than the highest readings for the CB and RS samples (i.e., 1.29 ppm NH_3/NH_4).

Comparing the two assessments, the results show an inconsistent pattern. For the shore-based assessment, the PRA samples gave much more variable and some markedly higher readings than the lower readings for the PRA samples from the watercraft-based assessment. The RS samples gave the highest readings for the shore-based assessment and lower readings than the PRA samples for the watercraft-based assessment. Yet, the CB samples are similar and consistently low for both assessments.



Figure 6. Ammonia concentrations recorded for the marine samples collected during the watercraft-based and shore-based assessment from the potential restoration area (PRA) and existing seagrass beds: Roosecote Sands (RS) and Concle Bank (CB).

Figure 7 shows the spatial distribution of the ammonia concentrations recorded for the samples from the watercraft-based assessment and the shore-based assessment.

For the watercraft-based assessment, the ammonia concentrations of marine samples within PRA and CB are relatively consistent and low (ranging from 0 ppm to 0.9 ppm NH₃/NH₄). The two higher ammonia readings from within RS (RS 3 reading 0.28 ppm NH₃/NH₄ and RS 4 reading 0.32 ppm NH₃/NH₄) are close to the freshwater outflow channel sampled at F4. However, sampling point RS 4 is close to this outflow channel (being further inshore) and gave a lower ammonia reading (0.07 ppm NH₃/NH₄).

For the shore-based assessment, the ammonia concentrations of the marine samples appear more variable. The highest readings are from the samples clustered together adjacent to the PRA, opposite

the gas terminal (SPRA 2 reading 0.15 ppm NH₃/NH₄, SPRA 3 reading 0.37 ppm NH₃/NH₄ and SPRA 1 reading 1.29 ppm NH₃/NH₄). The two RS samples that gave slightly higher ammonia concentrations (SRS 1 and SRS 4 reading 0.11 ppm NH₃/NH₄, respectively) are not clustered together and the RS sample between these samples (SRS 2) gave a zero reading.



Ammonia concentrations recorded for samples from the watercraft-based assessment (ppm) ▲ 0.05 - 0.1 A 0.25 - 0.3 0.3 - 0.35

Potential restoration area

Seagrass extent 2023

Path of outflow channel

Freshwater sampling points (labelled F1, F2, F3 and F4)

OpenStreetMap

Sampling points for each sampling area are labelled:

PRA 1, PRA 2, PRA 3 and PRA 4 for the potential restoration area

RS 1, RS 2, RS 3 and RS 4 for Roosecote Sands seagrass bed

CB 1, CB 2, CB 3 and CB 4 for Concle Bank seagrass bed (CB)

Ammonia concentration recorded for samples from the shore-based

- 0.1 0.15
- 0.35 0.4

1.25 - 1.30

Potential restoration area

Seagrass extent 2023

- Path of outflow channel (based on DEM)
- Freshwater sampling points (labelled F1, F2, F3 and F4)

Sampling points for each sampling area are labelled:

SPRA 1, SPRA 2, SPRA 3 and SPRA 4 for the potential restoration

SRS 1, SRS 2, SRS 3 and SRS 4 for Roosecote Sands seagrass bed

SCB 1, SCB 2, SCB 3 and SCB 4 for Concle Bank seagrass bed (CB)

Figure 7. Spatial distribution of the ammonia concentrations recorded for the samples collected during the (a) watercraftbased assessment and (b) shore-based assessment, with labels naming each of the sampling points for each sampling area.

4.1.3 Nitrate

For the watercraft-based assessment, all marine samples were tested with the HI-781 Handheld Colorimeter Marine Nitrate LR Checker (accuracy range of 0.25 ppm) and all results were below the LOD (≤ 0.25 ppm).

For the shore-based assessment, all RS and CB samples were tested with the HI-782 Nitrate High Range Checker for Marine (accuracy range of 2.00 pm) and all these readings were below the LOD (\leq 2.00 ppm). For the shore-based assessment, all PRA samples were tested with the HI-781 Handheld Colorimeter Marine Nitrate LR, with positive results (above the LOD). Given different meters were used with different LODs for RS and CB samples from the watercraft-based and shore-based assessments, respectively, only the PRA samples can be used for comparison.

Figure 8 shows the nitrate concentrations recorded for the PRA samples. The results show higher phosphate readings for the shore-based assessment than the watercraft-based assessment: for the latter all PRA samples gave zero readings, whilst samples from shore-based assessment gave three positive results (0.33 ppm NO₃, 0.57 ppm NO₃ and 0.95 ppm NO₃).



Figure 8. Nitrate concentrations recorded for the marine samples collected during the watercraft-based and shore-based assessment from the potential restoration area (PRA).

Figure 9 shows the spatial distribution of the nitrate concentrations recorded for the PRA samples from the watercraft-based assessment and the shore-based assessment. For the watercraft-based assessment, the PRA samples consistently gave zero readings. For the shore-based assessment, the highest nitrate reading (0.95 ppm NO₃) was from SPRA 4, the sampling point closest to F2. The two other positive nitrate readings are on the east shore (SPRA 3 and SPRA 1), closer to the outflow from the gas terminal (F3). However, between the sample point between these two high readings gave a zero reading (SPRA 2).



Figure 9. Spatial distribution of the nitrate concentrations recorded for the samples collected during the (a) watercraft-based assessment and (b) shore-based assessment, with labels naming each of the sampling points for each sampling area.

4.2 Physio-Chemical Status

4.2.1 pH

Figure 10 shows the pH recorded for the marine samples. Comparing the watercraft-based assessment and the shore-based assessment results, the readings appear relatively consistent for each sampling area. In both cases, the pH values recorded for the PRA samples appear to be higher, more variable and exhibit no overlap with the pH values for the CB and RS samples. Overall, the pH values of all the marine samples range from pH 7.9 to 8.59.



Figure 10. pH readings recorded for the marine samples collected during the watercraft-based and shore-based assessment from the potential restoration area (PRA) and existing seagrass beds: Roosecote Sands (RS) and Concle Bank (CB).



Figure 11 shows the pH recorded for all the freshwater samples are similar and within the range of good status based on the WFD (2000) thresholds.

Figure 11. pH readings recorded for the freshwater samples taken during the watercraft-based and shore-based assessment with the green lines showing the Water Framework Directive (WFD) status threshold for high/good status (i.e., pH 6-9).

4.2.2 Salinity

Salinity was only sampled for the watercraft-based assessment (Figure 12). For this assessment, the sodium chloride concentration recorded for the marine samples are similar across all three sites, with near-equal averages (of 35.3 ppt, 35.5 ppt and 35.4 ppt for PRA, CB and RS samples respectively). Overall, the sodium chloride readings range from 34.7 ppt to 36.2 ppt.



Figure 12. Salinity recorded for the marine samples collected during the watercraft-based assessment from the potential restoration area (PRA) and existing seagrass beds: Roosecote Sands (RS) and Concle Bank (CB).

The freshwater samples gave low sodium chloride readings (≤ 2.55 ppt) (Figure 13). These readings are relatively consistent (with a range of 2.15 ppt), though the reading from F4 (0.04 ppt) was noticeably less saline the other sampling points (recorded as 2.55 ppt, 1.77 ppt and 2.56 ppt for F1, F2 and F3, respectively).



Figure 13. Salinity recorded for the freshwater samples collected during the watercraft-based assessment.

4.2.3 Temperature

Temperature was only sampled for the watercraft-based assessment (Figure 14). The surface water temperatures recorded for the PRA samples appear to be slightly lower (mean of 12°C) and more variable (range of 2°C), than that of the CB samples (mean of 12.52°C, range of 0.3°C) and the RS samples (mean of 12.35°C, range of 0.8°C). Overall, the temperatures recorded for all the marine samples are relatively consistent, only ranging by 2°C.



Figure 14. Sea surface temperature recorded for the marine samples collected during the watercraft-based assessment from the potential restoration area (PRA) and existing seagrass beds: Roosecote Sands (RS) and Concle Bank (CB).

The surface water temperatures recorded from the freshwater samples ranged by 1.3°C (Figure 15). The highest temperature was recorded at F4 (13.5°C) and the lowest freshwater temperature was recorded at F1 (12.4°C).



Figure 15. Water surface temperature recorded for the freshwater samples collected during the watercraft-based assessment.

5. Discussion

5.1 Study Limitations

Given the low sample size for the pilot assessments, the results do not provide strong evidence of the extent of nutrient pollution within the PRA or existing seagrass beds. Also, the lack of standard solutions to verify the precision, accuracy and LOD of the colorimeters limits the robustness of the nutrient results. Acknowledging these limitations, the results are analysed to identify indications of pollution that would justify further assessments and to provide recommendations for future assessments.

The filtering process employed could have affected the nutrient test results. Reed et al. (2023) tested ammonium (NH4⁺) and orthophosphate (ortho-P) concentrations of water samples filtered with filter paper of three different sizes: 0.22, 0.45, and 0.70 μ m. Reed et al. (2023) found no predictable direction of change in nutrient concentration between the different pore sizes or overtime. They recommend filtering water samples with 0.22 μ m filter paper immediately upon collection to extract microorganisms from the sample that may uptake nutrients. Samples collected for the pilot assessments were filtered with 10 μ m filter paper, with up to 2 hours between sample collection and filtering. Although immediate filtering of samples was not considered feasible, further investigation into the effect of filter pore size could increase the accuracy of results.

When testing the samples from both assessments, the ammonia checker gave a positive reading above the accuracy range (± 0.05 ppm) for one blank control, i.e., an analytical blank of deionized water read 0.08 ppm NH₃/NH₄ and 0.06 ppm NH₃/NH₄ for the shore-based and watercraft-based assessment, respectively. The ammonia results from the samples were accepted, since these control readings are only slightly above the accuracy range of the meter and the other blank controls gave readings below the accuracy range. However, this could indicate a slight methodological error that may have affected the sample results.

With only marine ammonia and nitrate checkers available, the freshwater samples could not be tested for these nutrients. Hence, the results cannot provide an indication if the freshwater inputs to the site are sources of ammonia and nitrate.

It was challenging to determine specific nutrient thresholds that would cause concern for seagrass restoration, given the limited literature on seagrass nutrient tolerances. Available literature generally relies on laboratory exposure tests to determine thresholds of *Z. noltii* tolerance that are not directly comparable to ex situ conditions (Brun et al., 2002; 2008; Burkholder et al. 1992, 1994; van Katwijk et al., 1997). The EA only gives thresholds for selected nutrients in specific types of water bodies for determining water quality status (Bilous, Environment Agency, personal communication, 2024). These specific thresholds are used for comparison with the results where possible. However, Unsworth et al. (2024) recently found current water quality thresholds employed by statutory regulators insufficiently assessed water quality in terms of habitat suitability for seagrass growth in the UK. Moreover, various institutions present varying recommended nutrient thresholds for indication of water quality status. Ultimately, determining nutrient thresholds to indicate water quality status is complex, given these are dependent on the ambient conditions and limited by the available literature on species-specific responses (Johnson et al., 2007; Batley and Simpson, 2009).

5.2 Justification for Further Assessments

Testing the PRA samples from the watercraft-based assessment and shore-based assessment gave positive readings for phosphate, higher than the averages from RS and CB in both cases. This may indicate elevated phosphate levels in the PRA compared to the neighbouring seagrass beds. The reported thresholds for excess phosphorous vary and are affected by salinity (EPA, 2016). The salinity results for all three marine sampling areas (averages of 35.3 ppt, 35.4 ppt and 35.5 ppt for the PRA, RS and CB, respectively) indicate typical seawater conditions; salinities of UK shelf seas are reported between 34 to 35 ppt (Huthnance, 2010). The EPA (2016) for Ireland suggests a threshold of 0.04 ppm of phosphorous to indicate excess levels for full salinity waters. In both assessments, phosphate results are higher than this threshold: two readings for the PRA from the watercraft-based assessment samples (0.07 ppm PO₄ and 0.05 ppm PO₄) and three readings for the PRA from the shore-based assessment (0.13 ppm, 0.08 ppm PO₄ and 0.2 ppm PO₄). Therefore, these results indicate the PRA and neighbouring bed, RS, could be affected by phosphate pollution.

Higher readings from the PRA than the RS and CB from both assessments suggests the PRA is more affected by excessive phosphate levels than the neighbouring seagrass beds. This is expected to be due to the closer proximity of the PRA to storm discharge outflow from BWWTW (sampled at F2), given sewage effluent is a main source of phosphorous pollution (EA, 2019). As such, the average phosphate readings for PRA and RS were greater for the watercraft-based assessment than the shorebased assessment. The BWWTW storm outflow had been discharging for at least 10 hours previous to the shore-based assessment, though was not discharging for at least a few days prior to the watercraft-based assessment. This may indicate the phosphate pollution is arising from the BWWTW storm outflow. However, the freshwater phosphate readings were within the range of good or moderate status for freshwater inflow feeding from the storm discharge outflow (F2 samples read 0.09 ppm PO₄ and 0.13 ppm PO₄ for the watercraft-based and shore-based assessment, respectively). This implies sewage effluent BWWTW storm outflow is unlikely to be the major source of phosphate pollution. Elevated freshwater phosphate readings were only recorded for the sampling points F3 and F4 indicating poor status (F3 read 1.06 ppm PO₄ and 0.43 ppm PO₄, whilst F4 read 0.27 PO₄ and 0.33 PO₄ for the watercraft-based and shore-based assessment, respectively). For the shore-based assessment, the marine samples closest to these freshwater outflows (SPRA 1 and SRS 4) gave higher phosphate readings relative to the rest of the marine samples, which could suggest F3 and F4 freshwater inflows are sources of high phosphate levels. Further investigation is required to determine potential sources of phosphate feeding into these freshwater outflows. The available information suggests the gas terminal on the east side of the marine sampling areas is still partly operational in some capacity (Heywood et al., 2020; Čavčić, 2023). Wastewater for gas processing is reported to constitute chemicals containing phosphorous and nitrogen in varying forms, though potentially not in molecules that would be detectable by the nutrient meters used (Pichtel, 2016). The surrounding agricultural fields are potentially a more likely source: fertiliser application and livestock defecation are a recognised contributor to freshwater phosphorous enrichment in the UK (EA, 2019). Another potential source is a large gull colony near the gas terminal reported to be occupying a now unused fenced area and benefiting from the reduced predator exposure (Browning, L., Natural England, personal communications, 2024). Several studies evidence that guano from seabird colonies is a significant source of nutrients (Gould and Fletcher, 1978; de la Peña-Lastra et al., 2021; He et al., 2024).

The nitrogen results present less clear findings. For the watercraft-based assessment, the ammonia results indicate ammonia levels were relatively consistent across the PRA and CB, being slightly higher within RS (with averages of 0.06 ppm NH_3/NH_4 , 0.04 ppm NH_3/NH_4 and 0.19 ppm NH_3/NH_4 ,

respectively). For the shore-based assessment, readings suggest ammonia levels are higher and more variable within the PRA than RS or CB due to two high readings for PRA (0.37 ppm NH₃/NH₄ and 1.29 ppm NH₃/NH₄). The nitrate results presented for the PRA samples indicate higher nitrate concentrations for the shore-based assessment (ranging from 0-0.95 ppm NO₃) than for the watercraft-based assessment (all samples read 0 ppm NO₃). Ammonia toxicity is generally reported in marine animal species around 0·09–3·35 ppm NH₃, which is roughly comparable to freshwater species (Eddy, 2005). The UK Technical Advisory Group (UK TAG) for the WFD proposed a threshold for dissolved inorganic nitrogen (including ammonia and nitrate) in coastal waters (salinity 30-34.5 ppt) for good to moderate status as 13 micromoles per liter (equivalent to 0.221 ppm) (UK TAG WFD, 2008). The positive readings from the watercraft-based assessment are at the lower end of these proposed thresholds (at 0.07 ppm NH₃/NH₄ and 0.09 ppm NH₃/NH₄). Yet, compared to these thresholds some readings from the shore-based assessment appear to be high (at 1.29 ppm NH₃/NH₄, 0.3 ppm NH₃/NH₄, 0.95 ppm NO₃, 0.57 ppm NO₃ and 0.33 ppm NO₃). Hence, the results may indicate elevated levels of nitrate and ammonia, particularly within the PRA based on the shore-based assessment.

Higher ammonia and nitrate readings were found for samples from the shore-based assessment than the watercraft-based assessment. This deviation may reflect the fact the BWWTW storm outflow was discharging for 24 hours previous to the shore-based assessment and not discharging for 24 hours previous to the watercraft-base assessment. However, there is no clear indications from the spatial distribution of the nitrate results. For both assessments, the marine sampling points close to the outflow channels feeding from F3 and F4 appear to exhibit some of the higher ammonia readings. Also, the highest nitrate reading was taken from the shore-based sampling point closet to Cavendish Dock (SPRA 4), with the other high nitrate readings closer to the F3 sampling point and separated by the zero-nitrate reading. Further investigation into the nitrogen status of the freshwater inflows would be necessary to determine if the storm outflow or other freshwater inflows are sources of nitrogen.

Results for temperature, salinity and pH were within the expected ranges for the marine samples (Huthnance, 2010; CEFAS, 2018). For the PRA samples the pH readings were slightly higher than the RS and CB samples, whilst some temperature readings for the PRA were slightly lower. However, overall, the temperature, pH and salinity were relatively consistent across all three sampling areas (only ranging by 0.69, 2°C and 1.5 ppt, respectively). Hence, these readings alone do not present clear indications of pollution affecting the PRA or existing seagrass beds. Given these relatively consistent results and incomplete datasets for comparisons between the two assessments for salinity and temperature, the spatial distribution of the pH, salinity and temperature recordings was not presented for analysis. The high salinity readings increase confidence in the reliability of the nutrient readings: Gawankar and Masten (2023) showed response of the HI-781 Handheld Colorimeter Marine Nitrate LR was most accurate at salinity concentrations approximating the average salinity of seawater. The variation in salinity of freshwater sampling points is likely to reflect the varying extent of seawater influence at each sampling point. None of the freshwater temperature readings gave markedly high or low temperatures so do not provide clear evidence of thermal pollution. Variation in the freshwater temperature readings could relate to environmental factors, such as shading, water depths and current flow at the sampling site (Caissie, 2006).

Overall, the results provide evidence of potentially elevated phosphate levels within the PRA and to a lesser extent in the neighbouring seagrass beds. Although the ammonia and nitrate readings gave less clear indications, these results still evidence high levels of nitrogen pollution in the sampling area, particularly in the PRA. Therefore, further assessment is warranted. Freshwater sampling found elevated phosphate readings for the stream inflows feeding into the east-side of the marine sampling areas. In general, higher nutrient levels were recorded for the shore-based assessment than the

watercraft-based assessment, which is hypothesized to partly reflect the fact the BWWTW storm outflow was discharging for at least 10 hours previous to the shore-based assessment. Also, the higher nutrient readings from the PRA samples could reflect its closer proximity to the storm outflow than the existing seagrass beds. Together, these results highlight some potential pollution sources of concern for further investigation: the BWWTW storm outflow feeding into the PRA and the gas terminal, gull colony and agricultural land on the east shore of the marine sampling area.

5.3 Evaluation of the Method

The results from the watercraft-based and shore-based assessment cannot be directly compared to evaluate if the shore-based samples capture conditions within the PRA and existing beds, since these assessments were undertaken on different days. Given the spatial and temporal variability of water quality, even samples simultaneously from the marine sampling area and adjacent shore may not be directly comparable. Thus, this evaluation is based on the practical feasibility of survey method.

The shore-based method was found to be the most appropriate and is recommended for future assessments. This approach was more cost-effective than the watercraft-based method, requiring less time, personnel and equipment. As a minimum, the watercraft-method requires four surveyors on the water (working in two teams) with two onshore safety cover. The shore-based method only required four surveyors (working in two teams). The watercraft-based approach is also more dependent on sea and weather conditions, which significantly reduced the available survey days. Also, being more efficient, the shore-based method would allow for more samples to be collected in future assessments, therefore improving the robustness of the findings. A shore-based method for collecting samples for assessment follows the approach recommended by other Wildlife Trusts (James, C., Cornwall Wildlife Trust and Jayes, A., Yorkshire Wildlife Trust, personal communications, 2024).

The sampling points used for the shore-based assessment were found to be safe enough to access. Hence, these sampling points are recommended for future surveys. Upon arriving at the first sampling points some were inaccessible at high tide (at a height of 7.77 metres). In future, starting the collection of shore-based samples approximately one hour from high-tide would allow for easier collection.

6. Summary of Recommendations for Future assessments

- The results of this feasibility study indicate potential evidence of pollution of phosphorus and nitrogen. Thus, further water quality assessment to inform seagrass restoration efforts is recommended.
- The shore-based method for collecting samples is more appropriate than the watercraft-based method, and the shore-based assessment is therefore recommended for a future assessment. A trial of collecting shore-based samples from approximately one hour after high tide is recommended for easier accessibility to the sampling points.
- For more reliable results that more accurately capture spatial and temporal variability in water quality, future monitoring should maximise the number of samples, distribute the samples across the three sampling areas and increase the regularity of sampling days.
- The pilot assessments found employing point sampling was resource and time intensive, which limited the number of samples that could be collected. Remote monitoring data loggers (e.g., sondes) are recommended to improve the temporal coverage of sampling (Tuna et al., 2013; Lockridge et al., 2016; Jayes, A., Yorkshire Wildlife Trust, personal communication, 2024), where budgets permit.

- Using standard solutions to test the colorimeter responses should be used to verify the accuracy and precision of the readings at different concentration ranges. This is especially recommended for testing at the lower ranges, which would also allow more reliable LOD to be deciphered (Muir, M., personal communication, 2024).
- Given the results indicate potentially elevated levels of ammonia and nitrate, obtaining meters to test the freshwater samples for ammonia and nitrate is recommended.
- Where the Hanna Instruments colorimeters are used, the samples should be collected and then tested the following day. To determine the effect of storage time on the results, several samples should be tested as soon as possible after sampling and then tested again the following day when all samples are being tested. The difference between the results from the sampling day and the testing day for the same samples should be within the accuracy range of the given colorimeter used to ensure reliable results.
- When testing samples the day after collection, it takes approximately 30 minutes to test one sample for ammonia, nitrate and phosphate using the HI-784 Marine Ammonia Checker, HI-781 Handheld Colorimeter Marine Nitrate LR and H1713 Handheld Colorimeter Phosphate Low Range, respectively. Hence, 12 marine samples (testing for all three nutrients) and 4 freshwater samples (testing for phosphate only) can be tested within 1 working day (7 hours). The limiting factor is the number of colorimeters and the reaction time for each (15 minutes for the HI-784 Marine Ammonia Checker, 8 minutes for the HI-781 Handheld Colorimeter Marine Nitrate LR, 7 minutes for the HI-782 Nitrate High Range Checker and 3 minutes for the H1713 Handheld Colorimeter Phosphate Low Range). Nonetheless, having more than one person for sample preparation and to conduct the tests is recommended to increase efficiency.
- Samples should be filtered as quickly as possible following sampling. Wider literature suggests using > 10 μ m particle retention filter paper may affect the accuracy of the nutrient results (Reed et al., 2023). However, filtering samples with > 10 μ m particle retention filter paper was found to be very time-consuming, and using filter paper of a smaller pore size will likely increase the time spent filtering. Thus, further pilot testing to determine the influence of filtering and storage time on the samples is recommended. For example, during each assessment at least one sample should be filtered and tested immediately and results compared to test results the following day. Again, difference between these results should be within the accuracy range of the given colorimeter used to ensure reliable results.
- It is recommended to use the HI-781 Handheld Colorimeter Marine Nitrate LR rather than the HI-782 Nitrate High Range Checker, given the latter gave zero readings for all samples and readings below the accuracy range of the high range meter (2 ppm) could indicate potentially relevant levels of nitrate.
- For HI-781 Handheld Colorimeter Marine Nitrate LR, methodological difficulties were experienced with the filtering stage within the test procedure (resulting in error messages). To overcome this, the filtering would be done very slowly and all equipment must be thoroughly cleared between each sample.
- Future assessment should conduct sampling as regularly as possible, including days when the BWWTW storm outflow has been recently discharging and when it has not to allow a robust comparison that would provide better insights into whether the storm discharge is a potential source of nutrient pollution on the PRA.

7. Conclusion

This study has investigated the feasibility of carrying out a water quality assessment to inform the NWWT's approach to seagrass restoration. Of the two methods trialed through pilot assessments, the shore-based approach to collecting water samples was found to be more appropriate than the watercraft-based approach. Testing the water samples from the PRA and neighbouring seagrass beds indicates evidence of potentially elevated nutrient levels. The results provide evidence of potentially elevated phosphate levels within the PRA and to a lesser extent in the neighbouring seagrass beds. Although the ammonia and nitrate readings gave less robust findings, these results still indicate high nitrogen levels, particularly in the PRA. The results highlight some potential pollution sources of concern for further investigation: the BWWTW storm outflow feeding into the PRA and the gas terminal, gull colony and agricultural land on the east shore of the PRA. Therefore, further water quality assessments are warranted to inform seagrass restortion. Further recommendations on how these future assessments should be carried out are provided. Key recommendations include increasing the sample size for greater spatial and temporal coverage of the sampling areas, which would be made more possible through utilizing the more efficient shore-based sampling approach.

Overall, these findings provide significant insights that can help inform the NWTWW's approach to seagrass restoration. In turn, this study has also improved the understanding of water quality in the local area, which has relevance for wider environmental and public health concerns. Ultimately, such insights on the feasibility of assessing water quality to determine habitat suitability can help inform practitioners' efforts more broadly, and, thus, contribute to achieving successful coastal habitat restoration further afield.

Acknowledgements

I would like to thank Amber Gould and Beth Churn (Marine Conservation Officers, North West Wildlife Trusts) and Georgia de Jong Cleyndert (Head of Marine, North West Wildlife Trust) for their support with this project. I am grateful to Amber Gould, Beth Churn, Georgia de Jong Cleyndert and Katie Churn (Volunteer, North West Wildlife Trust) for volunteering to help with sample collection. I would also like to the thank those who gave support and advice on the sampling approach and interpretation of results: Laurence Browning and Conor Hinchcliffe (Natural England), Joe Bilous (Environment Agency), Andy Jayes and Ana Cowie (Yorkshire Wildlife Trust), Chloe James (Cornwall Wildlife Trust), Martina Bristow (Durham Wildlife Trust), Dr Cai Ladd (Swansea University), Adam Rounce (Tees River Trust), Dr Michael Muir and Dr Natalie Weldon (University of Glasgow).

This project was made possible through the North West Marine Futures Internship, hosted by the North West Wildlife Trust and supported by the Crown Estate, Natural England and Orsted. I would like to thank everyone involved in my internship.

For more information on the North West Marine Futures Internship visit: <u>https://www.livingseasnw.org.uk/what-we-do/marine-conservation-projects/marine-futures-internship</u>

References

- Alexandre, A., Quinta, R., Hill, P.W., Jones, D.L. and Santos, R. (2020). Ocean warming increases the nitrogen demand and the uptake of organic nitrogen of the globally distributed seagrass Zostera marina. *Functional Ecology*, *34*(7), pp. 1325-1335.
- Aniebone, V.O., Imo, D.O. and Shonde, O.O. (2024). Nutrients Pollution of the Coastal Marine Ecosystems. *International Journal of Innovative Agriculture & Biology Research*, *12*(2), pp.82-95.
- Batley, G.E. and Simpson, S.L. (2009). Development of guidelines for ammonia in estuarine and marine water systems. *Marine Pollution Bulletin*, *58*(10), pp. 1472-1476.
- Bell, T.G., Johnson, M.T., Jickells, T.D. and Liss, P.S. (2007). Ammonia/ammonium dissociation coefficient in seawater: a significant numerical correction. *Environmental Chemistry*, 4(3), pp. 183-186.
- Bower, C.E. and Bidwell, J.P. (1978). Ionization of ammonia in seawater: effects of temperature, pH, and salinity. *Journal of the Fisheries Board of Canada*, *35*(7), pp. 1012-1016.
- Britto, D.T., Siddiqui, M.Y., Glass, A.D.M. and Kronzucher, H.J. (2001). Futile transmembrane NH₄⁺ cycling: a cellular hypothesis to explain ammonium toxicity in plants. *Proceedings of the National Academy of Sciences, 98*, pp. 4255–4258.
- Brun, F.G., Hernández, I., Vergara, J.J., Peralta, G. and Pérez-Lloréns, J.L. (2002). Assessing the toxicity of ammonium pulses to the survival and growth of Zostera noltii. *Marine Ecology Progress Series*, 225, pp. 177-187.
- Brun, F.G., Olivé, I., Malta, E.J., Vergara, J.J., Hernández, I. and Pérez-Lloréns, J.L. (2008). Increased vulnerability of Zostera noltii to stress caused by low light and elevated ammonium levels under phosphate deficiency. *Marine Ecology Progress Series*, 365, pp. 67-75.
- Burkholder, J.M., Glasgow, H.B. Jr and Cooke, J.E. (1994). Comparative effects of water-column nitrate enrichment on eelgrass Zostera marina, shoalgrass Halodule wrightii, and widgeongerass Ruppia maritima. *Marine Ecology Progress Series*, 105, pp. 121–138.
- Burkholder, J.M., Mason, K.M. and Glasgow, H.B. (1992). Water-column nitrate enrichment promotes decline of eelgrass Zostera marina: evidence from seasonal mesocosm experiments. *Marine Ecology Progress Series, 81*, pp. 163–178.
- Caissie, D. (2006). The thermal regime of rivers: a review. Freshwater biology, 51(8), pp. 1389-1406.
- Cale, H. and Churn, B. (2021). *Distribution and Extent of Zostera Beds: Snab Sands.* Cumbria Wildlife Trust.
- Čavčić, M. (2023). Turning depleted natural gas fields into 'one of the biggest carbon storage and hydrogen production clusters'. *Offshore Energy* [online]. Available at: https://www.offshoreenergy.biz/turning-depleted-natural-gas-fields-into-one-of-the-biggest-carbon-storage-andhydrogen-production-clusters/ (Accessed: 10 November 2024).
- Centre for Environment, Fisheries and Aquaculture Science (CEFAS) (2018). Sea surface and water column salinity in 2011-2015. *Marine Online Assessment Tool* [online]. Available at: https://moat.cefas.co.uk/ocean-processes-and-climate/salinity/ (Accessed: 29 November 2024).

- Cereja, R., Brotas, V., Nunes, S., Rodrigues, M., Cruz, J.P. and Brito, A.C. (2022). Tidal influence on water quality indicators in a temperate mesotidal estuary (Tagus Estuary, Portugal). *Ecological Indicators*, 136.
- Christianen, M.J., van Belzen, J., Herman, P.M., van Katwijk, M.M., Lamers, L.P., van Leent, P.J. and Bouma, T.J. (2013). Low-canopy seagrass beds still provide important coastal protection services. *PloS ONE*, 8(5).
- Clifford, D. (2021). Seagrass Restoration Project Report. Seagrass Meadow Recovery in Morecambe Bay: Investigating Restoration Potential and Techniques for a Highly Tidal Environment, Internal Cumbria Wildlife Trust/Natural England Report.
- de la Peña-Lastra, S., Pérez-Alberti, A. and Otero, X.L. (2021). Seabird colonies as the main source of nutrients for the coastal ecosystems in the Atlantic Islands of Galicia National Park (NW Spain). *Chemosphere*, *275*.
- Dhiman, D.B. (2022). Ecosystem degradation and the need for restoration: through the lens of environment and human health. *Environment Pollution and Climate Change*, 6(10), pp.1-4.
- Díaz, S., Settele, J., Brondízio, E.S., Ngo, H.T., Agard, J., Arneth, A., Balvanera, P., Brauman, K.A., Butchart, S.H., Chan, K.M. and Garibaldi, L.A. (2019). Pervasive human-driven decline of life on Earth points to the need for transformative change. *Science*, *366*(6471), pp. 1-11.
- Dunic, J.C., Brown, C.J., Connolly, R.M., Turschwell, M.P. and Côté, I.M. (2021). Long-term declines and recovery of meadow area across the world's seagrass bioregions. *Global Change Biology*, 27(17), pp. 4096-4109.
- Eddy, F.B. (2005). Ammonia in estuaries and effects on fish. *Journal of Fish Biology*, 67(6), pp. 1495-1513.
- Environment Agency (EA) (2019). Phosphorous and Freshwater Eutrophication Pressure Narrative. EA.
- Environment Agency (EA) (2024). Sampling Point: Poaka (Mill) Beck into Cavendish Dock. [online]. Available at: https://environment.data.gov.uk/water-quality/view/sampling-point/NW-88004904 (Accessed: 08 November 2024).
- Environment Protection Agency (EPA) (2016). Water Quality in 2016: An Indicators Report. EPA: Wexford.
- Erzini, K., Parreira, F., Sadat, Z., Castro, M., Bentes, L., Coelho, R., Gonçalves, J.M., Lino, P.G., Martinez-Crego, B., Monteiro, P. and Oliveira, F. (2022). Influence of seagrass meadows on nursery and fish provisioning ecosystem services delivered by Ria Formosa, a coastal lagoon in Portugal. *Ecosystem Services*, 58.
- Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J. and Serrano, O. (2012). Seagrass ecosystems as a globally significant carbon stock. *Nature Geoscience*, *5*(7), pp. 505-509.
- Fox, E., Malcolm-MacKay, A., Lilley, R. and Unsworth, R. (2024). An Assessment of Zostera noltii Water Quality Nutrients in the East of England. Natural England Commissioned Report NECR522. Natural England.

- Gamble C., Debney, A., Glover, A., Bertelli, C., Green, B., Hendy, I., Lilley, R., Nuuttila, H., Potouroglou, M., Ragazzola, F., Unsworth, R. and Preston, J., (eds) (2021). *Seagrass Restoration Handbook*. Zoological Society of London: London
- Garbutt, A., Underwood, G. J. C., Harley, J., Boskova, K., Hardy, M.J., McGarrigle, A., Millington-Drake, M., Gamble, C., Debney A., zu Ermgassen, P.S.E., and Preston, J. (2024). *Seascape Scale Restoration: Restoring Our Coastal Habitats for Nature and People*. Blue Marine Foundation Report: London.
- Gardolinski, P.C., Hanrahan, G., Achterberg, E.P., Gledhill, M., Tappin, A.D., House, W.A. and Worsfold,
 P.J. (2001). Comparison of sample storage protocols for the determination of nutrients in natural waters. *Water Research*, 35(15), pp. 3670-3678.
- Gawankar, S. and Masten, S.J. (2023). Development of an inexpensive, rapid method to measure nitrates in freshwater to enhance student learning. *Journal of Chemical Education*, 100(6), pp. 2141-2149.
- Gould, A. (2022a). Distribution, Extent and Condition of Zostera noltii in the Ravenglass Estuary, Cumbria, UK. Cumbria Wildlife Trust.
- Gould, A. (2022b). Distribution, Extent, Condition and Potential Seed Bank Use of Zostera noltii Beds at Roa Island, Cumbria, UK. Cumbria Wildlife Trust.
- Gould, D.J. and Fletcher, M.R. (1978). Gull droppings and their effects on water quality. *Water Research*, *12*(9), pp. 665-672.
- Goyal, S.S., Lorenz, O.A. and Huffaker, R.C. (1982). Inhibitory effects of ammoniacal nitrogen on the growth of radish plants: I. Characterization of toxic effects of NH₄⁺ on growth and its alleviation by NO₃⁻. Journal of the American Society for Horticultural Science, 107, pp. 125–129.
- Green, A.E., Unsworth, R.K., Chadwick, M.A. and Jones, P.J. (2021). Historical analysis exposes catastrophic seagrass loss for the United Kingdom. *Frontiers in Plant Science*, *12*.
- Griffiths, L.L., Connolly, R.M. and Brown, C.J. (2020). Critical gaps in seagrass protection reveal the need to address multiple pressures and cumulative impacts. *Ocean & Coastal Management*, 183.
- He, J., Owusu-Asumeng, E., Zidar, K., Stolper, J., Attri, S., Price, J.R., Partridge, D., Montalto, F. and Sales,
 C.M. (2024). Impacts of a herring gull colony on runoff water quality from an urban green roof. *Science of The Total Environment*, 946.
- Heywood, M., Uhe, M., Eckardt, T., Greene, M., Racher, R. and Wyatt, R. (2020). North Morecambe onshore terminal adapts to changing feedstock. *Oil & Gas Journal* [online]. Available at: https://www.ogj.com/refining-processing/article/14185058/north-morecambe-onshoreterminal-adapts-to-changing-feedstock (Accessed: 10 November 2024).
- Huthnance, J. (2010). Ocean processes. In: Charting progress 2: the state of UK seas, Chapter 2, 14-26.
 Department for Environment, Food & Rural Affairs (DEFRA), on behalf of the United Kingdom Marine Monitoring and Assessment Strategy (UKMMAS) Community: London.
- Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) (2018). The IPBES Assessment Report on Land Degradation and Restoration. Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services: Bonn.

- Jackson, E.L., Griffiths, C.A. and Durkin, O. (2013). A Guide to Assessing and Managing Anthropogenic Impact on Marine Angiosperm Habitat - Part 1: Literature Review. Natural England Commissioned Report. Natural England.
- Jacob, B., Dolch, T., Wurpts, A. and Staneva, J. (2023). Evaluation of seagrass as a nature-based solution for coastal protection in the German Wadden Sea. *Ocean Dynamics*, 73(11), pp. 699-727.
- Jiang, R., Gao, X., Liu, J., Liu, Z., Qiu, G., Shao, L., He, W., Zhang, J. and He, P. (2024). Effect of temperature on photosynthetic physiology and the kinetics of nutrient absorption in Zostera caespitosa. *Environmental and Experimental Botany*, *219*.
- Jiménez-Ramos, R., Villazán, B., Egea, L.G., Cantero, R., Pérez-Lloréns, J.L., Vergara, J.J. and Brun, F.G. (2022). Differential ecophysiological responses to inorganic nitrogen sources (ammonium versus nitrate) and light levels in the seagrass Zostera noltei. *Marine Ecology Progress Series, 702*, pp. 57-70.
- Johnson, I., Sorokin, N., Atkinson, C., Rule K. and Hope, S. (2007). *Proposed EQS for Water Framework Directive Annex VIII Substances: Ammonia (Un-Ionised)*. Environment Agency: Bristol.
- Jones, B.L. and Unsworth, R.K. (2016). The perilous state of seagrass in the British Isles. *Royal Society Open Science*, *3*(1).
- Kent, F., Lilley, R., Unsworth, R., Cunningham, S., Begg, T., Boulcott, P., Jeorrett, C., Horsburgh, R. and Michelotti, M. (2021). Seagrass Restoration in Scotland: Handbook and Guidance. NatureScot Research Report 1286. NatureScot.
- Kirkby, E.A. (1968). Influence of ammonium and nitrate nutrition on the cation–anion balance and nitrogen and carbohydrate metabolism of white mustard plants grown in dilute nutrient solutions. *Soil Science*, *105*, pp. 133–141.
- Kirkwood, D. (1996). *ICES Techniques in Marine Environmental Sciences No. 17 Nutrients: Practical Notes on their Determination in Sea Water.* International Council for the Exploration of the Sea: Copenhagen.
- Lee, K.S., Park, S.R. and Kim, Y.K. (2007). Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *Journal of Experimental Marine Biology and Ecology*, 350(1-2), pp.144-175.
- Lloyd, C.E., Johnes, P.J., Pemberton, J.A., Yates, C.A., Jones, D. and Evershed, R.P. (2022). Sampling, storage and laboratory approaches for dissolved organic matter characterisation in freshwaters: moving from nutrient fraction to molecular-scale characterisation. *Science of the Total Environment*, 827.
- Marschner, H. (1995). Mineral Nutrition of Higher Plants, 2nd Edition. Academic Press: New York.
- McLeod, E., Chmura, G. L., Bouillon, S., Salm, R., Björk, M., Duarte, C. M., Lovelock, C. E., Schlesinger, W.
 H., & Silliman, B. R. (2011). A blueprint for blue carbon: Toward an improved understanding of the role of vegetated coastal habitats in sequestering CO2. *Frontiers in Ecology and the Environment*, 9(10), pp. 552–560.
- Moreno-Marín, F., Vergara, J.J., Pérez-Llorens, J.L., Pedersen, M.F. and Brun, F.G. (2016). Interaction between ammonium toxicity and green tide development over seagrass meadows: a laboratory study. *PloS ONE*, *11*(4).

- Murray, D.L., W.C. Dennison, and W.M. Kemp. (1992). Nitrogen vs. phosphorous limitation for growth of an estuarine population of eelgrass (Zostera marina, L.). *Aquatic Botany, 44*, pp. 83-100.
- Nejrup, L.B. and Pedersen, M.F. (2008). Effects of salinity and water temperature on the ecological performance of Zostera marina. *Aquatic Botany*, *88*(3), pp. 239-246.
- Nie, J., Feng, H., Witherell, B.B., Alebus, M., Mahajan, M.D., Zhang, W. and Yu, L., 2018. Causes, assessment, and treatment of nutrient (N and P) pollution in rivers, estuaries, and coastal waters. *Current Pollution Reports*, *4*, pp. 154-161.
- Nordlund, L.M., Koch, E.W., Barbier, E.B., Creed, J.C. (2016). Seagrass Ecosystem Services and Their Variability across Genera and Geographical Regions. *PLoS ONE*, *11*(10).
- Ondiviela, B., Losada, I.J., Lara, J.L., Maza, M., Galván, C., Bouma, T.J. and van Belzen, J. (2014). The role of seagrasses in coastal protection in a changing climate. *Coastal Engineering*, *87*, pp. 158-168.
- Peyton-Jones, F. and Rounce, A. (2023). *Seagrass Surveys 2023: Extent, Coverage and Condition*. Cumbria Wildlife Trust.
- Pichtel, J. (2016). Oil and gas production wastewater: Soil contamination and pollution prevention. *Applied and Environmental Soil Science, 2016.*
- Potouroglou, M. and Unsworth, R. (2021). Chapter 1: Seagrass restoration: An introduction. In: Gamble C., Debney, A., Glover, A., Bertelli, C., Green, B., Hendy, I., Lilley, R., Nuuttila, H., Potouroglou, M., Ragazzola, F., Unsworth, R. and Preston, J. (eds) *Seagrass Restoration Handbook*. Zoological Society of London: London, pp. 2-15.
- Potouroglou, M., Whitlock, D., Milatovic, L., MacKinnon, G., Kennedy, H., Diele, K. and Huxham, M. (2021). The sediment carbon stocks of intertidal seagrass meadows in Scotland. *Estuarine, Coastal and Shelf Science*, *258*.
- Preston, J., Debney, A., Bertelli, C., Bull, J., Finger, A., Glover, A., Inman, I., Jackson, E., McCormick, H., Nuuttila, H., O'Neill, L., Unsworth, R., Ward, E. and Gamble, C. (2021). Chapter 2: Getting started: Restoration project design, planning, permitting, licensing and funding. In: Gamble C., Debney, A., Glover, A., Bertelli, C., Green, B., Hendy, I., Lilley, R., Nuuttila, H., Potouroglou, M., Ragazzola, F., Unsworth, R. and Preston, J. (eds) *Seagrass Restoration Handbook*. Zoological Society of London: London, pp. 16-39.
- Reed, M.H., Strope, E.K., Cremona, F., Myers, J.A., Newell, S.E. and McCarthy, M.J. (2023). Effects of filtration timing and pore size on measured nutrient concentrations in environmental water samples. *Limnology and Oceanography: Methods*, 21(1), pp. 1-12.
- Serrano, O., Gómez-López, D.I., Sánchez-Valencia, L., Acosta-Chaparro, A., Navas-Camacho, R., González-Corredor, J., Salinas, C., Masque, P., Bernal, C.A. and Marbà, N. (2021). Seagrass blue carbon stocks and sequestration rates in the Colombian Caribbean. *Scientific Reports*, 11(1), pp. 1-12.
- Short, F.T. (1983). The seagrass Zostera marina L.: plant morphology and bed structure in relation to sediment ammonium in Izembek Lagoon, Alaska. *Aquatic Botany, 16*:, pp. 149-161.
- Tennant, G. (2006). *Experimental effects of ammonium on eelgrass (Zostera marina I.) shoot density in Humboldt Bay, California*. Master Thesis. Humboldt State University: California.

- Thom, R.M., and Albright, R.G. (1990). Dynamics of benthic vegetation standing-stock, irradiance, and water properties in central Puget Sound. *Marine Biology*, *104*, pp. 129-141.
- Touchette, B.W. and Burkholder, J.M. (2000). Review of nitrogen and phosphorus metabolism in seagrasses. *Journal of Experimental Marine Biology and Ecology*, 250(1-2), pp. 133-167.
- UK Technical Advisory Group on the Water Framework Directive (UK TAG WFD) (2008). UK Environmental Standards and Conditions (Phase 2). UK TAG WFD.
- United Nations (UN) (2023). *The Sustainable Development Goals Report 2023: Special Edition*. United Nations: New York.
- United Utilities (2024). *Storm Overlap Map.* [online]. Available at: https://www.unitedutilities.com/better-rivers/storm-overflow-map/ (Accessed: 08 November 2024).
- Unsworth, R.K., Cullen-Unsworth, L.C., Jones, B.L. and Lilley, R.J. (2022). The planetary role of seagrass conservation. *Science*, *377*(6606), pp. 609-613.
- Unsworth, R.K.F., Bertelli, C.M., Esteban, N.E., Rees, S., Nuuttila, H.K. (2018). *Methodological Trials for the Restoration of the Seagrass Zostera Marina in SW Wales. SEACAMS2 Project.* Swansea University: Swansea.
- Unsworth, RKF., McGarrigle, A., Fox, E., Millington-Drake, M., Perrin, E., Swithinbank, H., Harris, F., Thomsen, E., Jones, BLH., Watson, F., Wright, R. (2024). Seagrass vs Water Quality: How the Current Crisis is Damaging Our Ability to Fight Climate Change. Blue Marine Foundation, Project Seagrass and Surfers Against Sewage Collaborative Report. Blue Marine Foundation: London.
- van Katwijk, M.M., Schmitz, G.H.W., Gasseling, A.P. and Van Avesaath, P.H. (1999). Effects of salinity and nutrient load and their interaction on Zostera marina. *Marine Ecology Progress Series*, 190, pp. 155-165.
- van Katwijk, M.M., Vergeer, L.H.T., Schmitz, G.H.W. and Roelofs, J.G.M. (1997). Ammonium toxicity in eelgrass Zostera marina. *Marine Ecology Progress Series*, *157*, pp. 159-173.
- Ward, M., Cullen-Unsworth, L., Geisler, K. G., Lilley, R., Lynch, J., Millington-Drake, M., Pittman, S. J., Smith, A., Taylor, S., Wedding, L. M., Wright, R., Seddon, N. (2023). *Developing a UK Seagrass Carbon Code*. University of Oxford: Oxford.
- Water Framework Directive (WFD) (2000). EU 2000 Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy, Official Journal of European Communities L 327 1 72.
- Waycott, M., Duarte, C., Carruthers, T., Orth, R., Dennison, W., Olyarnik, S., Calladine, A., Fourqurean, J., Heck, K., Hughes, A., Kendrick, G., Kenworthy, W., Short, F. and Williams, S. (2009). Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proceedings of the National Academy of Sciences, 106*(30), pp. 12377-12381.
- World Wildlife Fund (WWF), The Royal Society for the Protection of Birds (RSPB) and The Wildlife Trusts (2024). *Blue Carbon: The Need to Protect Essential Stores of Carbon in Our Seas.* The Wildlife Trusts.
- Zhang, Y., Yu, X., Chen, Z., Wang, Q., Zuo, J., Yu, S. and Guo, R. (2023). A review of seagrass bed pollution. *Water*, 15(21), p.3754.

Appendices

Analyte	Checker	Used for	Accuracy Range of the	LOD Used
	Analysis		Checker	
Nitrate	HI-781	Handheld	\pm 0.25 ppm \pm 2% of	<u>+</u> 0.25 ppm
	Colorimeter	r Marine	reading @ 25°C	
	Nitrate LR			
	HI-782	Handheld	± 2.00 ppm \pm 5% of	<u>+</u> 2.00 ppm
	Colorimeter	r Marine	reading @ 25°C	
	Nitrate HR			
Ammonia	H1784	Handheld	± 0.05 ppm \pm 5% of	<u>+</u> 0.05 ppm
	Colorimeter	r Marine	reading @ 25°C	
	Ammonia			
Phosphate	H1713	Handheld	\pm 0.04 ppm \pm 4% of	<u>+</u> 0.04 ppm
	Colorimeter	^r Phosphate	reading @ 25°C	
	Low Range			

Appendix 1: LOD Use for the Hanna Instrument Nutrient Checkers

Appendix 2: Full Sample Results

Table 4. Full results from the watercraft-based assessment (the samples tested for nitrate readings by the HI-782 Nitrate High Range Checker are highlighted in yellow, all others were tested using the HI-781 Handheld Colorimeter Marine Nitrate LR)

	Coordinates		Nutrient concentration			Salinity		Tomporatura
Sampling points	x	Y	PO₄ (ppm)	NH₄/NH₃ (ppm)	NO₃ (ppm)	(ppt)	рН	(oC)
PRA 1	321976	467497	0.07	0.07	≤LOD	36.2	8.13	12.7
PRA 2	321764	467395	≤LOD	≤LOD	≤LOD	35.1	8.4	12.9
PRA 3	321922	467490	0.05	0.09	≤LOD	34.7	8.48	11.5
PRA 4	321756	467475	≤LOD	0.08	≤LOD	35.3	8.58	10.9
RS 1	322197	466942	≤LOD	0.08	≤LOD	35.5	8	12.7
RS 2	322145	467068	≤LOD	0.32	≤LOD	35.2	8.03	11.9
RS 3	322182	467113	≤LOD	0.28	≤LOD	35.5	8.01	12.5
RS 4	322306	467112	≤LOD	0.07	≤LOD	35.8	7.98	12.3
CB 1	322351	466627	≤LOD	0.08	≤LOD	35.3	7.95	12.3
CB 2	322442	466535	≤LOD	≤LOD	≤LOD	35.6	8.06	12.6
CB 3	322308	466466	≤LOD	0.08	≤LOD	35.8	8.03	12.6
CB 4	322590	466494	≤LOD	≤LOD	≤LOD	35	8.09	12.6

	Coordinates		Nutrient concentration			
Sampling points	x	Y	PO₄ (ppm)	NH₄/NH₃ (ppm)	NO₃ (ppm)	рН
SPRA 1	322446	467475	0.13	≤LOD	0.33	8.15
SPRA 2	322476	467579	≤LOD	0.37	0	8.12
SPRA 3	322421	467695	0.08	0.15	0.57	8.59
SPRA 4	321664	468093	0.2	1.29	0.95	8.55
SRS 1	322406	466916	0.06	0.11	≤LOD	7.95
SRS 2	322481	467018	≤LOD	≤LOD	≤LOD	8.08
SRS 3	322274	466791	≤LOD	0.07	≤LOD	8.09
SRS 4	322548	467144	0.12	0.11	≤LOD	8.03
SCB 1	322652	466567	≤LOD	≤LOD	≤LOD	7.99
SCB 2	322561	466649	≤LOD	0.07	≤LOD	7.9
SCB 3	322326	466727	≤LOD	0.06	≤LOD	8.12
SCB 4	322347	466737	≤LOD	0.07	≤LOD	8.05

Table 5. Full results from the shore-based assessment (the samples tested for nitrate readings by the HI-782 Nitrate High Range Checker are highlighted in yellow, all others were tested using the HI-781 Handheld Colorimeter Marine Nitrate LR)





Published by Cumbria Wildlife Trust, Kendal, Cumbria, UK.