Understanding environmental drivers of dwarf eelgrass (*Zostera noltii*) distribution on Scotland's east coast: implications for habitat restoration

By

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Dissertation

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I hereby certify that this dissertation, which is approximately 7,578 words in length, has been composed by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a degree. This project was conducted by me at the University of St Andrews from January 2024 to August 2024 towards fulfilment of the requirements of the University of St Andrews for the degree of MSc Marine Ecosystem Management under the supervision of Dr Andrew Blight and Professor Len Thomas.

Signed:

Date: 12/08/2024

Table of Contents

Abstract

Eelgrass once thrived in Scotland's coastal waters. However, an estimated 58% of eelgrass meadows have disappeared within the last century, along with the vital functions and services they provide including coastline protection, carbon sequestration and habitat provision for ecologically and commercially important species. Recently, habitat restoration efforts have accelerated and the Scottish Government is committed to reversing this loss. Despite nation-wide support, restoration success is hindered by the limited understanding of optimal environmental conditions for eelgrass establishment. This study investigates water nutrients, hydrodynamics, worm density and sediment properties (particle size, water content, stability and chlorophyll *a* content) as potential drivers of *Zostera noltii* distribution on the east coast of Scotland. These environmental parameters were compared between intertidal areas with and without *Z. noltii* meadows to identify environmental conditions that may support eelgrass growth. Sediment stability and sediment water content were the only variables that showed statistically significant differences between areas with and without *Z. noltii*. Increased stability at *Z. noltii* sites was likely caused by the presence of *Z. noltii* itself therefore was disregarded as a suitable habitat indicator. Sediment water content was the most informative indicator, with metrics remaining similar across all meadows, ranging between 24-32%. Sediment water content is currently not assessed in eelgrass monitoring surveys and its consideration is recommended to understand variability on a local to national scale. Optimal nutrient levels for *Z. noltii* meadows were not documented. However, high concentrations of nutrients (up to 0.45 ppm ammonia and 16.67 ppm nitrate) were detected across *Z. noltii* presence and absence sites, suggesting nutrient enrichment. This raises concerns about the long-term resilience of eelgrass meadows on Scotland's east coast. This study addresses key gaps in knowledge concerning the status of Scotland's eelgrass meadows and variability of environmental conditions between meadows.

Keywords: seagrass, eelgrass, *Zostera noltii,* seagrass restoration, habitat suitability modelling.

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1 Introduction

Historically, eelgrass abundance was so prolific in Scotland, it was harvested for roof thatching, building insulation, bedding and fertiliser before its decline in the 19th century (Unsworth et al., 2022; Wyllie-Echeverria et al., 1999). Today, Scotland has lost an estimated 58% of its eelgrass meadows within the last century (Green et al., 2021), leaving remaining meadows fragmented and in poor status (Jones and Unsworth, 2016). The vital ecosystem functions and services eelgrass provides is gaining recognition across Scotland, and efforts to restore meadows are accelerating as a result. In response, the Scottish Government Biodiversity Strategy is committed to "Publish a plan for marine and coastal ecosystem restoration, including prioritising habitats and locations suitable for restoration by 2025." (Scottish Government, 2023). Although scientific recognition of eelgrass loss in British waters is longstanding (Ranwell, 1974), data is considerably deficient in Scotland due to the lack of monitoring effort (Green et al., 2021; Strachan et al., 2022). Many meadows have not been monitored for over 20 years (Green et al., 2021), and the Tay estuary was surveyed for the first time in 2008 (Wilkie, 2012). Without baseline data, knowledge gaps on the environmental conditions of eelgrass meadows limit our ability to identify and prioritise suitable locations for restoration.

1.1 Eelgrass Functions and Services

Eelgrass are a genus of the seagrasses (Phylum Angiospermophyta), the only marine flowering plants (Hemminga and Duarte, 2000). Two species of seagrass grow around the British Isles: *Zostera marina* (known as common eelgrass) grows in the intertidal and subtidal zones, and *Zostera noltii* (known as dwarf eelgrass due to its thinner and shorter leaves (Figure 1)) is an intertidal species. A seagrass plant consists of leaves above the sediment and a basal meristem below the sediment from which vertical and horizontal rhizomes extend a root network (Hogarth, 2015). With the ability to regulate physical and biogeochemical cycles, seagrass is often referred to as a foundation species and an ecosystem engineer (Koch, 2001).

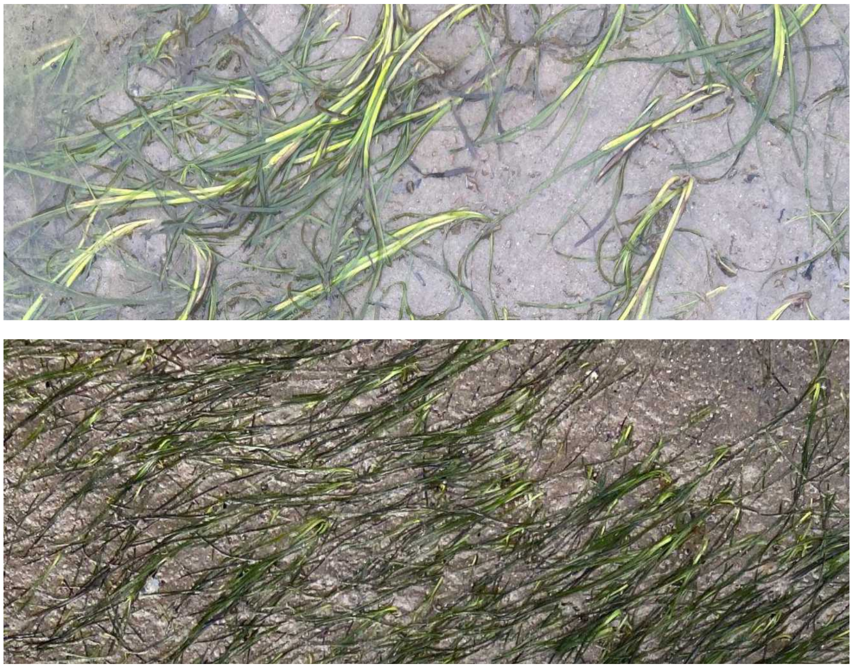


Figure 1. Zostera marina (top) have larger leaves than Zostera noltii (bottom) (image by author).

Seagrass form extensive, dense meadows that support every trophic level from microorganisms and detritivores to predators (Hogarth, 2015; Ugarelli et al., 2017). Notably, seagrass is a nursery ground for commercially important species including plaice, pollock and herring which rely on the structural complexity of meadows for refuge and resources (Bertelli and Unsworth, 2014; Lilley and Unsworth, 2014). Seagrass rhizomes stabilise the sediment while the leaves above dampen current velocity and wave energy (Duarte et al., 2013; Potouroglou, 2017; Koch, 2001). This protects the coastline from storms and erosion, and can improve water quality through reduced turbidity and increased sediment nutrient retention (De Boer, 2007; Koch, 2001; Ward et al., 1984). Seagrass absorbs vast quantities of carbon dioxide from the water and stores the carbon in the seabed, bound by rhizomes. Despite seagrass covering less than 0.2% of the seabed, it is responsible for 15% of global carbon storage in the ocean; approximately 27-40 Tg C y^{-1} which is considerably greater than the carbon storage of terrestrial habitats (Fourqurean et al., 2012; Laffoley and Grimsditch, 2009).

1.2 Eelgrass Distribution in Scotland

Eelgrass grows across much of Scotland's coastline due to extensive sheltered sea lochs and embayments on the West Coast, and estuaries on the East. *Z. marina* is widely distributed around the Western Isles as well as the Orkney and Shetland archipelagos. However, it is less abundant on the East Coast where it grows alongside *Z. noltii,* which is predominantly distributed from the Dornoch Firth to the Firth of Forth (Figure 2). Environmental and anthropogenic stressors have caused Scotland's eelgrass distribution to fragment, with patches ranging from 0.03 ha in Gruinard Bay (Moore et al., 2011) to meadows of 1,200 ha in the Cromarty Firth (Green et al., 2021).

1.3 Threats to Scottish Eelgrass

Around the British Isles, an estimated 8% of eelgrass remains from its historic coverage (Green et al., 2021). Much of this decline has occurred in Scottish waters, from a conservative estimate of 8,312 to 2,164 ha over the last century (Green et al., 2021).
In the 1930s, a 'wasting disease' caused by an endophytic slime mould, *Labyrinthula zosterae*, reduced *Z. marina* distribution by 90% across the Atlantic (Muehlstein, 1989). Although no epidemic has resurfaced, the disease remains prevalent, causing mortality where eelgrass is under stress due to reduced light availability or increased nutrients or sea temperatures (Bockelmann et al., 2012; Brakel et al., 2014; Den Hartog, 1989; Hughes et al., 2018). There is concern that the virulence of *L. zosterae* will increase with climate change (Brakel et al., 2019).
At the turn of the 19th century, the industrialisation of Scotland's cities, including Edinburgh and Dundee on the east coast, led to the uncontrolled release of factory effluents into the environment which severely impacted the water quality in rivers, estuaries and coastal seas (Marsden and Mackay, 2001). In the 20th century in Inverness, vast reclamation of intertidal areas was documented after the construction of numerous oil refineries and oil platform construction yards (Rae, 1979).

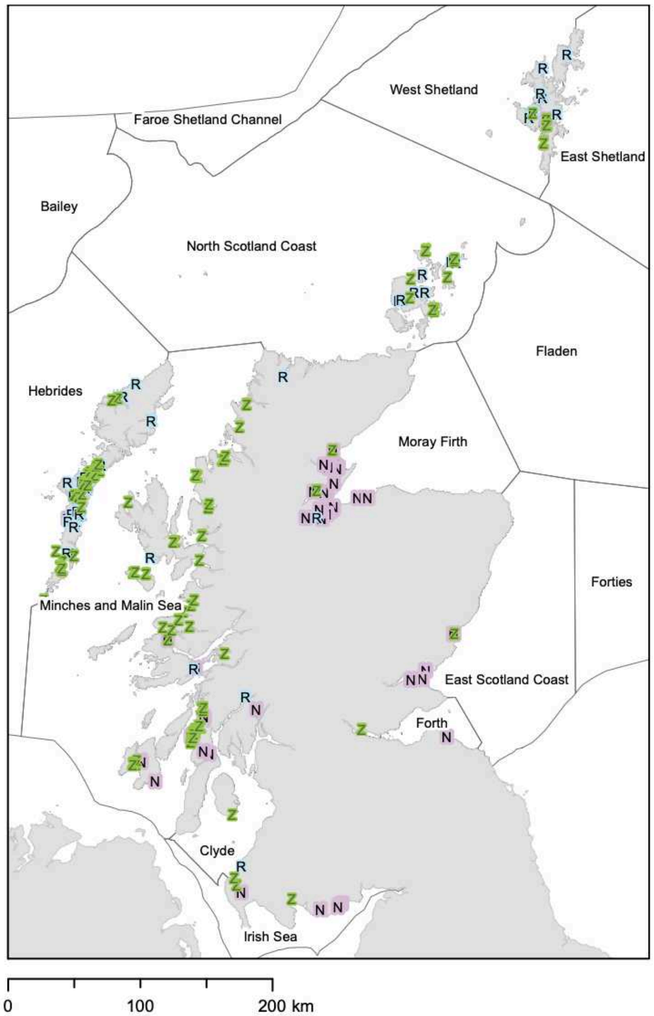


Figure 2. Zostera marina ('Z') and Zostera noltii ('N') and Ruppia maritima ('R' - an aquatic plant commonly known as tasselweed) distribution around Scotland. Note Z. noltii grows around Orkney (Porter et al., 2021) and maps remain outdated (image by Burrows et al., 2014).

Agricultural intensity accelerated in the mid-to-late 20th century with amplified use of pesticides and fertilisers (Whelan et al., 2022). Agricultural runoff remains highly prevalent, leaching high concentrations of nutrients into Scotland's rivers (SEPA, 2007). This can shift an eelgrass ecosystem to a eutrophic state whereby excess nutrients surpass the growth requirements and storage capacity of the eelgrass, and is utilised instead by fast growing epiphytic algae, macroalgae and phytoplankton. This algal proliferation can increase turbidity and shade eelgrass from sunlight (Burkholder et al., 2007). In extreme circumstances, hypoxia or anoxia can result, affecting all trophic levels (Duarte, 1995).

Estuaries and bays are particularly exposed to growing anthropogenic pressures; less exposed to ocean conditions, these areas are favourable for recreation and the establishment of ports and industry. Coastal construction, boat anchorages and moorings disturb sediment and rip out eelgrass plants, causing significant habitat loss (Collins et al., 2010; d'Avack et al., 2014; Larkum et al., 2006). Such damage has occurred in the bays of Barra and Eriskay due to the construction of a causeway (Kent et al., 2021).

1.4 Eelgrass Restoration

Alleviating anthropogenic pressures on existing meadows should be prioritised to mitigate further decline and biodiversity loss (Kent et al., 2021). Active restoration to aid eelgrass reestablishment is appropriate where the environment is not under stress, and has suitable conditions for eelgrass growth.

Eelgrass restoration involves transplanting seeds or seedlings collected from a donor meadow to a site with little to no eelgrass. For decades, this practice has taken place across the globe (Fonesca, 2011) but only recently gained momentum in Scotland, driven by non-governmental organisations (NGOs) and local communities. Through several trials, restoration practitioners have learnt that poor site selection is the main reason for failed eelgrass reestablishment (Govers et al., 2022; Kent et al., 2021; Unsworth et al., 2019). Historic presence of eelgrass does not determine a site's suitability as its subsequent absence is likely to have physically and biologically transformed the environment through disruption of ecological feedback mechanisms (Maxwell et al., 2017; Moksnes et al., 2021; Suding et al., 2004). Therefore, restoration handbooks state the importance of understanding long-term spatial and temporal environmental conditions, and using habitat suitability models (HSM) as a decision-making tool, to ensure site suitability before restoration can commence (Gamble et al., 2021; Kent et al., 2021; Moksnes et al., 2021). This fundamental stage of planning demands attention, to maximise success in this costly and labour intensive practice.

1.4.1 Habitat Suitability Models

HSMs are statistical models used to predict a habitat's distribution based on the statistical associations between the existing distribution of the habitat and the environmental conditions which control or affect its growth (Guisan and Zimmermann, 2000). It is a well-established conservation management tool in Scotland (Elsäßer et al., 2013; Millar et al., 2019) however, its application for Scottish eelgrass and restoration is in its infancy (Govers et al., 2022; Oreska et al., 2021).

HSM accuracy is dependent on three aspects: (1) accurate mapping of existing habitat distribution in the form of presence and absence data, (2) using the most relevant environmental variables which control habitat growth, and (3) high data resolution. Currently, data on eelgrass distribution is deficient (Green et al., 2021) and knowledge gaps on environmental drivers of eelgrass growth and natural variability of meadows (Kent et al., 2021) is inhibiting the accuracy and progression of HSMs in Scotland.

1.5 Environmental Variables

Eelgrass distribution is restricted to shallow, sheltered bays and estuaries, and their growth is controlled by abiotic and biotic factors. As estuarine plants, eelgrass tolerate wide salinity and temperature ranges and require mud or sand sediment, low hydrodynamic activity, water depths which permit adequate light penetration and prevent desiccation, and a low nutrient environment (Hemminga and Duarte, 2000; Hogarth, 2015). Organisms can inhibit eelgrass growth through sediment destabilisation and seed consumption (Reise, 2002), and can promote its growth through consumption of competitive algae (Kitting et al., 1984). Despite this, typically only abiotic variables are considered in HSMs. Bertelli et al. (2022) reviewed seagrass HSMs across the globe and found the most utilised variables to be seawater temperature, bathymetry, light availability, salinity, wave action, substrate and seabed slope. Nevertheless, recent literature stresses the importance of incorporating both biotic and abiotic variables (Gräfnings et al., 2023; Hu et al. 2021) as macrofaunal density and anthropogenic pressures influence seagrass distribution and restoration success (Crow et al., 2023; Philippart, 1994; Suykerbuyk et al., 2012; Valdemarson et al., 2011).

Currently, no HSMs exist specifically for *Z. noltii* in Scotland. Despite the spatial overlap of eelgrass species, *Z. marina* HSMs are unsuitable for *Z. noltii* due to the different conditions in the intertidal and subtidal zones. Therefore, this study aims to investigate the relevance of abiotic and biotic factors as potential indicators of suitable *Z. noltii* habitat. Eight variables will be investigated: hydrodynamic energy, sediment particle size, sediment chlorophyll *a* content, sediment water content, sediment stability, worm density, and surface water ammonia and nitrate concentrations.

Hydrodynamics is typically considered as a driver of eelgrass distribution as it is well known that its establishment requires low wave and current energy environments (Bertelli et al., 2023; De Boer, 2007; Hogarth, 2015; Stevens and Lacy, 2011). The substrate is a key factor in determining benthic community composition and is overlooked in HSMs. Although classifying sediment type by particle size is frequently considered, many other sediment properties influence nutrient availability (De Boer, 2007), and microbial and macrofaunal community composition (Wyness et al., 2021). Chlorophyll content in the water column has been applied to HSMs, used as a proxy for nutrient enrichment (Effrosynidis et al., 2018; Gräfnings et al., 2023; Hu et al., 2021). The chlorophyll content of the substrate however, can indicate the density of microphytobenthos which are photosynthesising microbial communities that create biofilm through the biological cohesion of sediment particles (Kelly et al., 2001). Biofilm increases sediment stability which indirectly benefits seagrass by reducing sediment erosion and turbidity in the water column (Reidenbach and Timmerman, 2019). Investigating sediment water content is particularly relevant in the intertidal zone where changes in air exposure and temperature at low tide affects the rate of photosynthesis in eelgrass (Leuschner et al., 1998). Sufficient water retention in meadows is essential to prevent desiccation at low tide. The water retention abilities of different sediment types and the variation of water content across ridges and runnels may influence horizontal as well as vertical *Z. noltii* distribution.

Arenicola marina (commonly known as lugworm) and *Hediste diversicolor* (ragworm) are ecosystem engineering polychaete worms. They modify the environment structurally and influence biogeochemical cycles through two behavioural mechanisms: 'bioturbation' and 'bioirrigation'. Bioturbation occurs where worms destabilise the sediment through burrowing and particle transport. Worms ventilate their burrows with bioirrigation, by flushing pore water through the sediment, thus influencing sediment stability, redox, water content and nutrient availability (De Backer et al., 2011; Wyness et al., 2021). Nutrients are regularly considered for HSMs, yet the nutrient levels and its impacts on Scottish eelgrass has not been investigated (Kent et al., 2021).

1.6 Aims & Objectives

Aims:

1. Investigate the relevance of environmental variables as potential indicators of suitable *Z. noltii* habitat.
2. Investigate inter-meadow variation in environmental conditions across the east coast of Scotland.

Objectives:

1. Collect fine-scale environmental data from sites with and without *Z. noltii* from the Beauly, Tay, Eden and Forth estuaries on hydrodynamics; sediment particle size, stability, water content and chlorophyll *a* content; surface water ammonia and nitrate concentrations; and worm density.
2. Compare environmental variables between sites with and without *Z. noltii.*
3. Identify conditions that support *Z. noltii* growth by assessing the relationship between *Z. noltii* and each environmental variable.

2 Materials and methods

2.1 Study Area

The study area encompassed 14 sites across the Beauly, Tay, Eden and Forth estuaries: 7 with *Z. noltii* absent and 7 with *Z. noltii* present (Figure 3; Appendix Table 1). To maximise the practicality of this project, the selected *Z. noltii* absence sites are of restoration interest to organisations: Mossy Earth, Oxygen Conservation and Project Seagrass in the Beauly, Tay and Forth estuaries respectively. The *Z. noltii* presence sites were sourced from SeagrassSpotter, an open-access seagrass locator map operated by Project Seagrass, and based on the expertise of the aforementioned organisations. Sites were geographically clustered within estuaries except for the Forth estuary where sites were separated into Limekilns in the west and Drum Sands in the east (Figure 3). An attempt was made to have both absence and presence sites within each cluster, but this was not achieved in the Eden estuary where both sampled sites had *Z. noltii* present.

Sites were sampled between 30th May and 19th June 2024. Environmental data and sediment samples were collected in the field and later analysed in the laboratory at the Scottish Oceans Institute, University of St Andrews. Observations of site characteristics (e.g. species abundance and distribution, and perceived environmental stressors) were also documented to aid data interpretation.

2.2 Environmental Variable Selection

Sediment stability, sediment water content, sediment chlorophyll *a* content, sediment particle size, surface water ammonia and nitrate, hydrodynamics and worm density were selected as variables. Variables were selected based on their potential importance as drivers of *Z. noltii* distribution after the consideration of previous studies on seagrass ecology, biology and HSM applications. Reasoning behind the selection of each variable is outlined in Introduction - Environmental Variables.

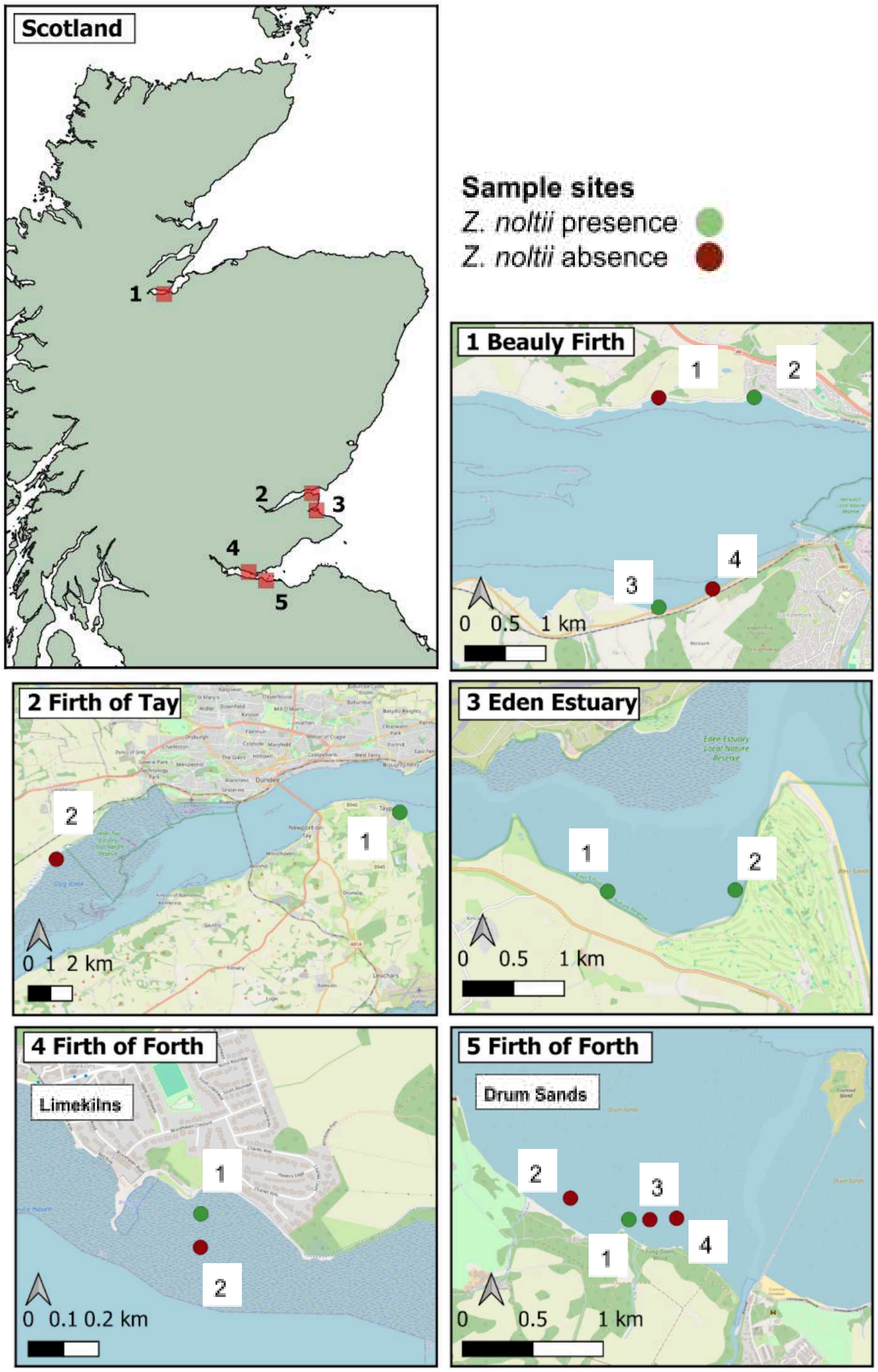


Figure 3. The study areas across five clusters of sites at four estuaries on the east coast of Scotland (top left). Z. noltii presence and absence sites are identified within each study area and numbered for referral. [CRS: EPSG: 3857 / WGS 84 Pseudo-Mercator; Map of Scotland scale: 5171830 (QGIS (3.32 Lima); Wessel, 2017)].

2.3 Data Collection

At each site, 20 samples were collected at random locations within a 50 m^2 area during low tide. This was conducted for all variables except for hydrodynamics of which three randomly placed samples were collected per site.

Sediment properties – Sediment stability at 7 cm was recorded using a shear vane which measures the sediment's shear strength i.e., resistance to erosion (Figure 4a). The shear vane was pushed into the sediment and turned with gradual force until the device clicked and the resulting shear strength (units in kPa) was indicated on the dial. A contact corer (44 mm diameter x 2 mm deep) was used to collect surface sediment samples to later analyse particle size, chlorophyll *a* and water content (Figure 4b). The contact corer was gently pushed 2 mm onto the sediment surface and 50 ml liquid nitrogen was poured into the well of the corer to flash freeze the sample. After the liquid nitrogen evaporated, the sample was removed from the corer with a knife, wrapped in tin foil and stored in a flask of liquid nitrogen before being transferred and stored in a -80℃ fridge upon return to the laboratory.

Worm density – A 0.5 x 0.5 m quadrat was placed on the sediment surface and a photograph was taken to determine *A. marina* and *H. diversicolor* cast density within the quadrat (Figure 4c).

Water nutrients – An eppendorf was filled with 10 ml of surface water and analysed for ammonia and nitrate concentration in the laboratory.

Hydrodynamics – The erosion of substrate due to tidal and wave energy was replicated by deploying Plaster of Paris blocks. The weight of plaster eroded was used as a proxy to measure the rate of erosion due to hydrodynamics in the environment. First, 600 g of Plaster of Paris and 500 ml of water was mixed until smooth and poured into silicone ice cube trays to make 48 blocks. The blocks were left to set for 24 hours before being transferred into a drying oven (Grenlab e3) at 50℃ for 72 hours. The blocks were sewn into mesh bags and their weights recorded before being zip-tied onto steel stakes. In situ the stakes were inserted into the sediment so that the blocks were just above the sediment surface and left for two days before collection (Figure 4d).

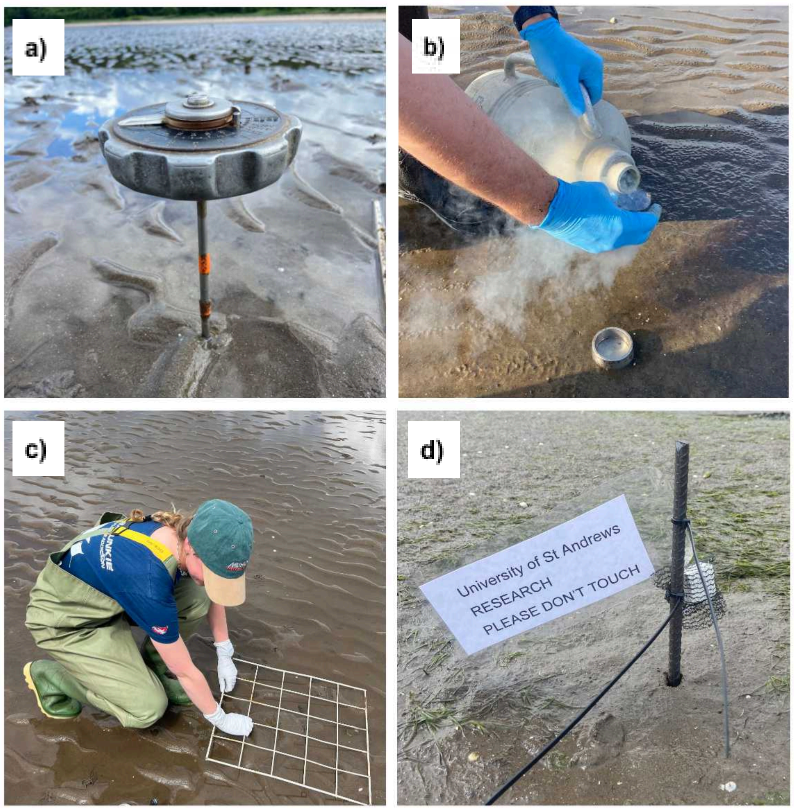


Figure 4. Data collection of a) sediment stability using a shear vane; b) sediment sample using a contact corer; c) worm density using a quadrat; and d) hydrodynamics using the erosion of plaster as a proxy.

2.4 Sample Processing

Sediment samples were handled in dim light and stored on ice in a cool box during processing to prevent the breakdown of chlorophyll pigments.

Sediment water content — The samples were weighed as frozen discs before being freeze-dried (Edwards freeze dryer Modulyo) for 24 hours to dehydrate the sample. The samples were then broken-up to homogenise the sediment and weighed again. The percentage water content in the sediment was calculated as following:

$$\% \, water\ content \ = \ \frac{(wet\ weight - dry\ weight)}{wet\ weight} \ \times \ 100$$

Sediment chlorophyll a content – Sediment subsamples of 0.2-0.5 g were put into 3 ml eppendorfs followed by 1.5 ml of 90% acetone before being vortexed and placed in the freezer (-20℃) for 24 hours. The samples were vortexed again and returned to the freezer for a further 24 hours. After 48 hours, the chlorophyll biomass was determined with a spectrophotometer (Cecil CE 3021 3000 series). The samples were centrifuged for 3 minutes at 1300 rpm and the spectrophotometer was blanked using 90% acetone. Once centrifuged, the supernatants from the samples were extracted with a syringe and needle, and transferred to a 1 ml cuvette using a syringe filter to prevent sediment particle transmission. The cuvette went in the spectrophotometer and the absorbances were read at 630, 647, 664 and 750 nm. Between samples, 90% acetone was used to clean the cuvette and syringe, and syringe filters were replaced. Chlorophyll *a* was then calculated as follows:

$$chlorophyll\ a \ = \frac{[11.85(E_{664} - E_{750}) - 1.54(E_{664} - E_{750}) - 0.08(E_{630} - E_{750})]\ x\ V_e}{Weight\ of\ sample\ (g)}$$

E_{630}, E_{664} and E_{750} are the spectrophotometric readings which are subtracted to correct the wavelength readings for background scattering effects. This is multiplied with the absorbance correction factors, 11.85, 1.54 and 0.08, to isolate chlorophyll *a* from 90% acetone. Multiplying with V_e accounts for the volume of acetone combined with the sample, and division by the initial sediment weight provides the chlorophyll *a* concentration per gram of sediment.

Sediment particle size – The remaining dried sediment samples were combined by site, weighed and subsequently sieved through a tower of sieve fractions: 1000, 710, 500, 355, 250, 180, 125, 90, 63 and <63 µm. The residual sediment from each fraction was separately weighed. All weights were entered into the GRADISTAT programme which calculated sediment statistics including the particle sizes and their distribution, textural group and sediment name. The modal particle size was used to represent the dominant particle size, and the textural group and sediment name were documented to understand the prevailing sediment type in each site

Nutrients – From each site, six water samples were tested for each ammonia and nitrate using the API Saltwater Master Test Kit. For ammonia analysis, eight drops of ammonia test solution ‘bottle #1’ followed by eight drops of ‘bottle #2’ were added to a 5 ml water sample, shaken vigorously for 5 seconds and left for 5 minutes. A classification chart with concentrations (ppm) and corresponding colours was used to classify the sample’s ammonia concentration based on the water's colour after 5 minutes. For nitrate analysis, 10 drops of nitrate test solution ‘bottle #1’ was added to a 5 ml water sample and inverted; ‘bottle #2’ was shaken vigorously for 30 seconds before 10 drops were added to the water sample. The sample was vigorously shaken for 1 minute and left for 5 minutes. Similarly, a classification chart was used to determine the nitrate concentration after 5 minutes.

Hydrodynamics – The Plaster of Paris blocks were dried in the oven at 50℃ for 72 hours and subsequently weighed. Weight loss due to erosion was calculated as follows:

$$\%\, erosion = \frac{(pre\ \ weight - post\ weight)}{pre\ weight} \times 100$$

2.5 Statistical Analysis

R statistical software v 4.1.1 (R Core Team, 2024) was used to perform all statistical analyses. The mean values of environmental variables from 20 samples per site were used for statistical analysis, except for the ammonia and nitrate means which were taken from six samples per site, and hydrodynamic means, taken from three samples per site. The package ‘ggplot2’ was used for data visualisation (Wickham, 2016).

Environmental variables – The primary analysis objective was to determine whether each environmental variable differed on average between sites with and without *Z. noltii*. If presence and absence sites had been chosen independently then a *t*-test (or nonparametric equivalent) would have been appropriate, but here the sampling design involved first selecting five estuary clusters and then selecting sites within clusters (Figure 3). Therefore, to control for potential variability in environmental variables between clusters, a linear mixed-effects model (LMM) was used, with estuary cluster as a random effect and the presence or absence of *Z. noltii* as a fixed effect (a factor covariate). The model formula used was:

$$y_{ij} = (\beta_0 + u_j) + \beta_1 \chi_{ij} + \epsilon_{ij} \ ,$$

where y_{ij} is the response variable, representing the environmental variable for observation *i* at estuary *j*; β_0 is the fixed intercept, representing the baseline level of the environmental variable when *Z. noltii* is absent; u_j is the random effect of estuary cluster *j*, representing variability across estuary clusters; β_1 is the fixed effect coefficient, representing the effect of *Z. noltii* presence on the environmental variable; χ_{ij} is the indicator variable for *Z. noltii* presence and absence for observation *i* at estuary cluster *j;* and ϵ_{ij} is the residual error for observation *i* at estuary cluster *j*, assumed to be normally distributed with a mean of zero and constant (but unknown) variance.

LMMs were fitted using the 'lmer' function from the 'lme4' package (Bates et al., 2015). Models were fitted using maximum likelihood (ML) instead of the default restricted maximum likelihood (REML) to allow for the comparison of models with different fixed effects (Bolker, 2015). For each environmental variable, two models were fitted: one with and one without the effect of *Z. noltii*. The null hypothesis of no effect of *Z. noltii* on each environmental variable was tested by comparing the two models using the 'anova' function in R, which performed a chi-squared test with one degree of freedom. A *p*-value at the significance level of 0.05 was used to assess whether there was a statistically significant difference on average for that variable between presence and absence sites.

Meadow variability – A second objective was to assess the variability between estuaries in the environmental variables. Therefore, using the fitted LMMs, the proportion of variability within environmental variables attributable to differences between estuary cluster was assessed by calculating the intra-class correlation coefficient (ICC), i.e., the proportion of total variance explained by the random effect.

3 Results

3.1 Environmental Variables

Average sediment water content, sediment chlorophyll *a* concentration, sediment particle size, water nitrate concentration and hydrodynamics were lower in *Z. noltii* meadows compared to absence sites. While average sediment stability, water ammonia concentration and worm density were higher in *Z. noltii* meadows compared to absence sites.

The mean average (±SD) of sediment water content was 27.9 ± 3.15% in presence sites and 37.6 ± 14.45% in absence sites (Figure 5a). Sediment stability was 10.85 ± 2.58 pKa in presence sites and 8.59 ± 2.40 pKa in absence sites (Figure 5b). Sediment chlorophyll *a* content was 21.8 ± 8.36 µg g in presence sites and 32.4 ± 30.63 µg g absence sites (Figure 5c). However, an outlier of 99.1 µg g in the Tay estuary absence site (Tay 2) influenced this result (Figure 7a). Mean modal sediment particle size was 242 ± 114.45 µm in presence sites and 306 ± 170.01 µm in absence sites (Figure 5d). Water ammonia concentrations were 0.29 ± 0.105 ppm in presence sites and 0.23 ± 0.074 ppm in absence sites (Figure 5e). Water nitrate concentrations were 5.86 ± 3.68 ppm in presence sites and 8.15 ± 6.05 ppm in absence sites (Figure 5f). Hydrodynamic measurements, represented by mean erosion of plaster blocks, was 19.3 ± 9.28 % in presence sites and 28.1 ± 11.39 % in absence sites (Figure 5g). Worm density was 2.59 ± 2.34 individuals in presence sites and 2.12 ± 2.20 individuals in absence sites per 0.25 m^2 (Figure 5h).

Sediment water content and chlorophyll *a* content were positively correlated (correlation = 0.740) (Figure 6). From the LMM, sediment stability was significantly higher in presence sites (p=0.031, chisq = 4.633) (Table 1), with sediment 23.2% more stable than absence sites (Appendix Table 2). Sediment water content was significantly lower in presence sites (p=0.039, chisq = 4.269) (Table 1), with 29.6% less water content than absence sites (Appendix Table 2). The remaining variables did not significantly differ between presence and absence sites (Figure 5; Table 1).

3.2 Meadow Variability

High variance in meadow conditions was attributed to estuary differences. Estuaries explained 68.2% variance in sediment stability. Mean average sediment stability ranged from 14.9 (Limekilns 1) to 7.05 pKa (Beauly 4) (Figure 7b). Estuaries explained 51.6% variance in sediment particle size which ranged from a mean modal of 427.5 (Beauly 1) to 76.5 µm (Drum Sands 1). See Appendix Table 3 for sediment statistics in each site. Estuaries explained 42.5% variance in hydrodynamics which ranged from 36.1% of plaster blocks eroded (Drum Sands 1) to 12.7% (Beauly 1) (Figure 7d). Estuaries explained 41.7% variance in sediment chlorophyll *a* content which ranged from 8.24 (Beauly 1) to 25.8 µg g (Eden 2) (Figure 7a). Estuaries explained 36.7% variance in surface water ammonia concentration which ranged from 0.08 (Eden 1) to 0.45 ppm (Beauly 3) (Figure 7e). Sediment water content which was relatively similar between meadows; estuaries explained 27.2% the variance which ranged from 23.8 (Eden 2) to 32.2% (Limekilns 1) (Figure 7f). Surface water nitrate concentration was variable however, estuaries only explained 25.5% variance which ranged from 2 (Beauly 1) to 12.5 ppm (Eden 2) (Figure 7g). Worm density was variable between meadows however, estuaries only explained 22.8% variance which ranged from 0.37 (Tay 1) to 7.1 individuals/0.25 m^2 (Beauly 3) (Figure 7f).

3.3 Field Observations

In the Beauly estuary, thick mats of macroalgae were extensive in both presence sites, predominantly Beauly 1 (Appendix Fig. 1a,c). All sites were particularly muddy and retained considerable surface water at low tide (Appendix Figure 1).

In the Tay estuary presence site, spatial distribution of *Z. noltii* and worms generally did not overlap and topography generally controlled their distribution, with worms more prevalent in runnels and *Z. noltii* more prevalent on ridges. Runnels through the sandflat fragmented *Z. noltii* patches (Appendix Fig. 2a). The Tay estuary absence site was particularly muddy and uniform in topography (Appendix Figure 2b,c).

In the Eden estuary, there was little spatial overlap between worms and *Z. marina* in runnels and *Z. noltii* on crests. *Z. noltii* patches were considerably fragmented (Appendix Figure 3).

The *Z. noltii* meadow in Limekilns was dense, relatively extensive, and a high abundance of snails were found on top (Appendix Figure 4a). Rocky reefs situated between the meadow and sea acted as a barrier, retaining considerable surface water in the meadow at low tide (Appendix Figure 4a,b). The Limekilns absence site, located on the seaward side of the rocky reefs, was less protected by rocks.

In Drum Sands, high water energy and different directions of flow (perhaps due to the undulating surface of the mudlfat) was evident as *Z. noltii* was spread on the sediment surface in various directions (Appendix Figure 5a). There was a high abundance of worms although there was a distinct separation between worms and *Z. noltii* patches (Appendix Figure 5b). The sediment was increasingly muddy with distance from the *Z. noltii* meadow.

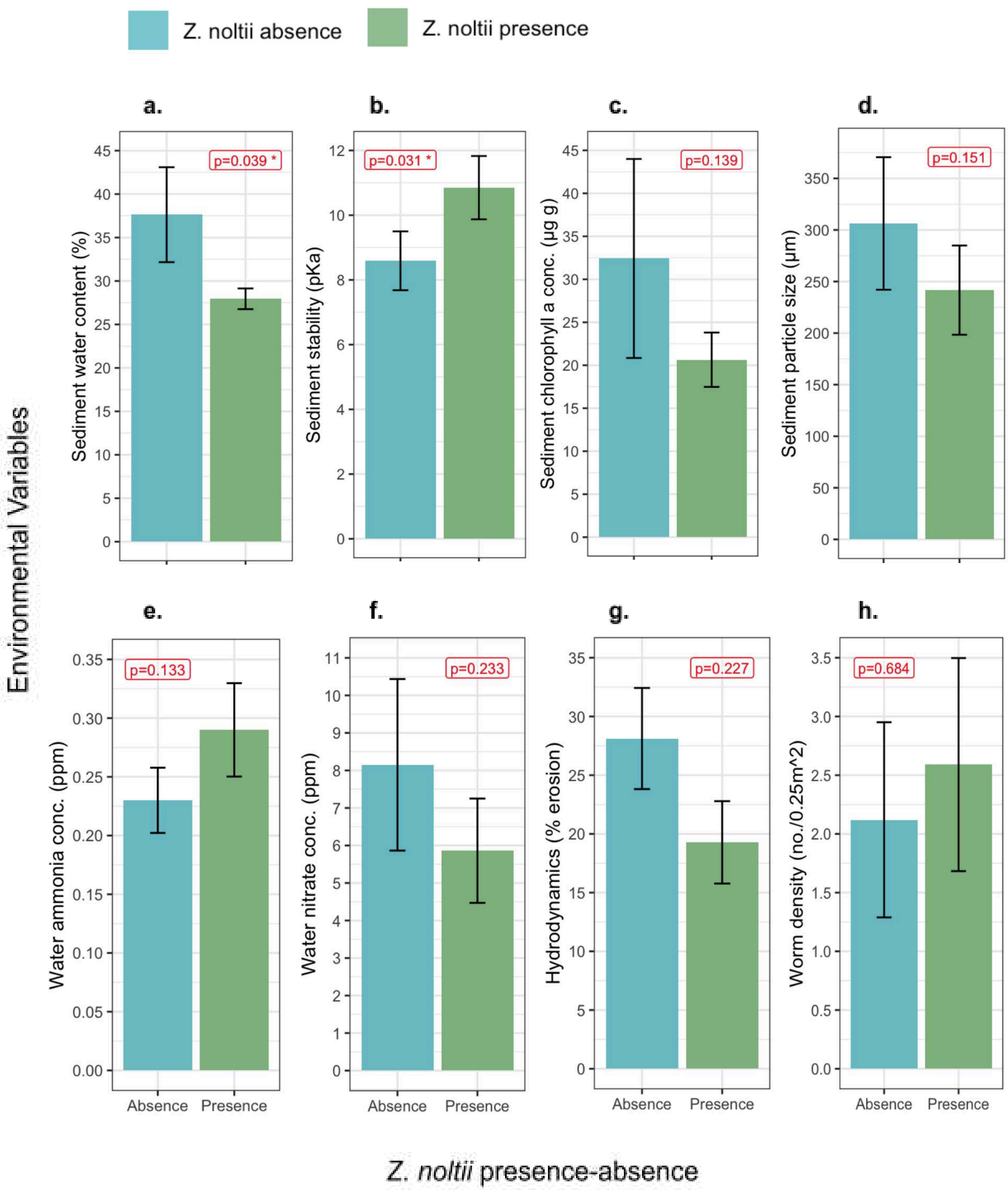


*Figure 5. Mean values of environmental variables from seven sites with Z. noltii and seven sites without Z. noltii. Error bars represent standard error. Significance is indicated by * = p<0.05.*

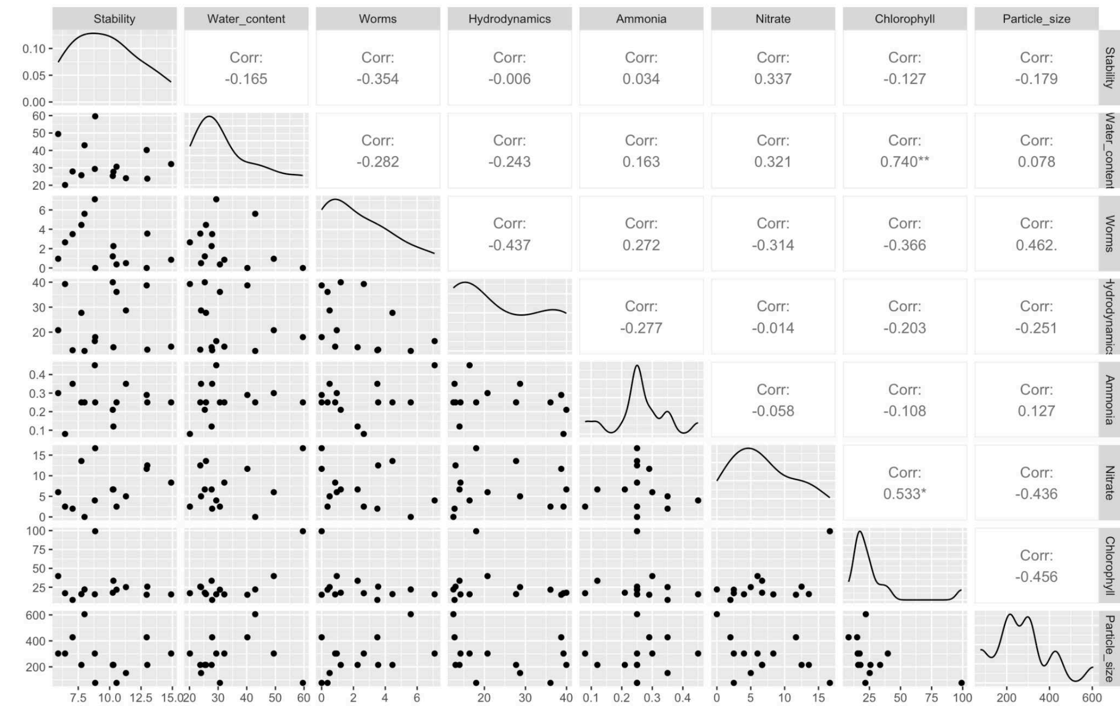
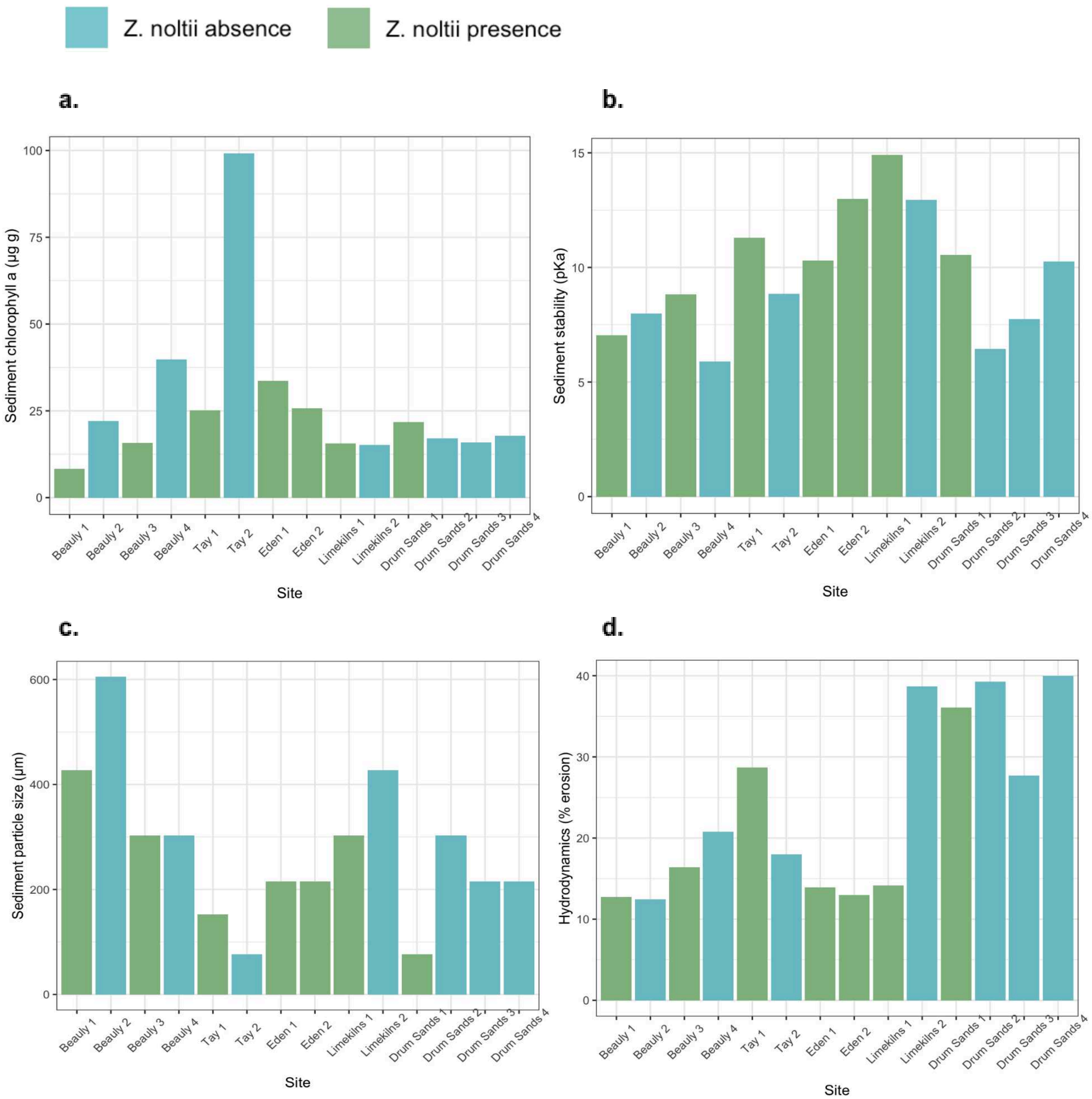


Figure 6. Pairwise relationships among environmental variables across all sites.The scatter plots show the relationship between two variables, indicating any linear trends. The histograms show the distribution of each variable, indicating the spread and central tendency of the data. The correlation coefficients are represented by the “Corr” values, indicating the strength and direction of the relationships between variables.



Z. noltii absence
Z. noltii presence
a.
Sediment chlorophyll a (µg g)
100
75
50
25
0
Beauly 1
Beauly 2
Beauly 3
Beauly 4
Tay 1
Tay 2
Eden 1
Eden 2
Limekilns 1
Limekilns 2
Drum Sands 1
Drum Sands 2
Drum Sands 3
Drum Sands 4
Site
b.
Sediment stability (pKa)
15
10
5
0
Site
c.
Sediment particle size (µm)
600
400
200
0
Site
d.
Hydrodynamics (% erosion)
40
30
20
10
0
Site

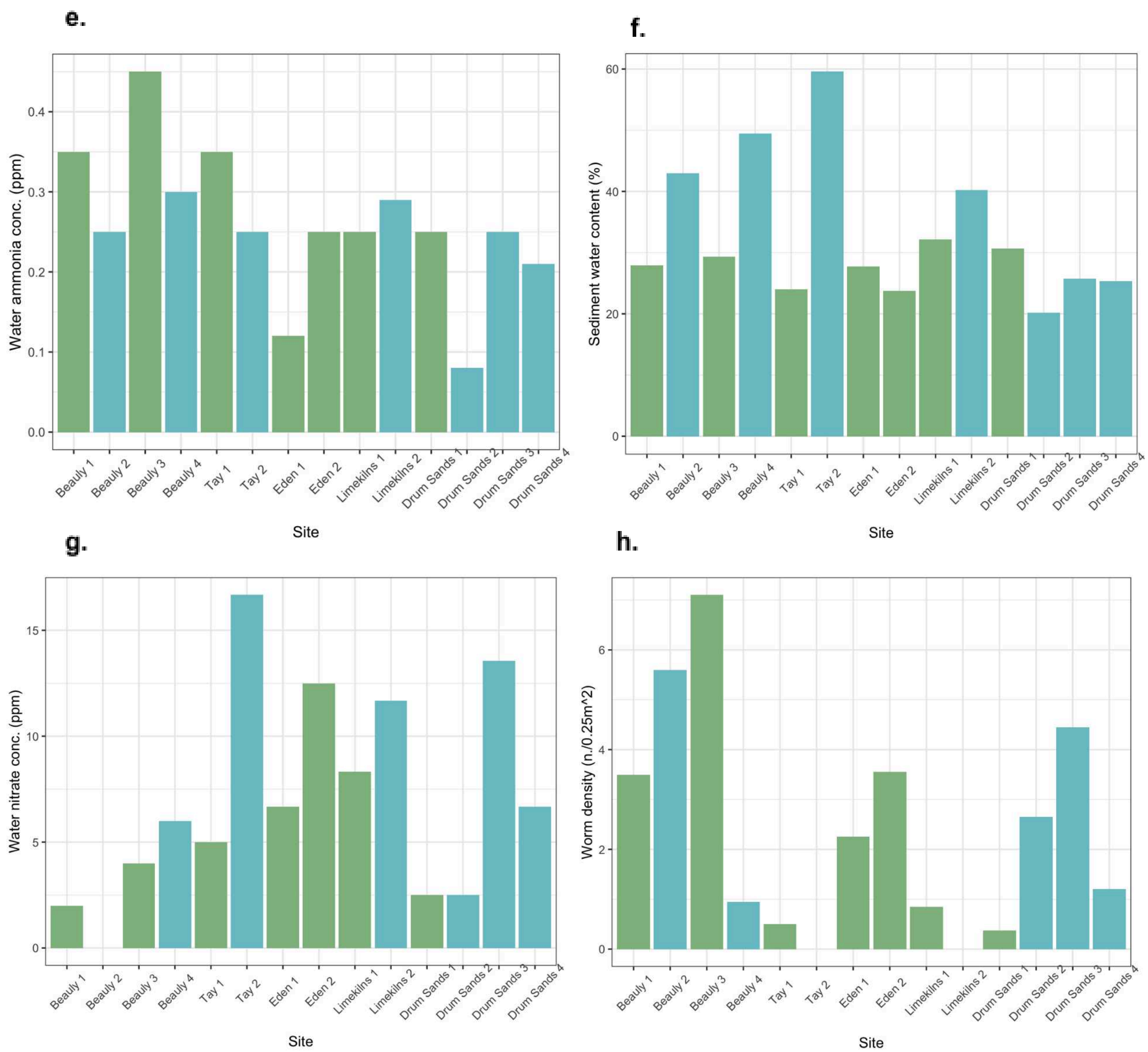


Figure 7. The mean values of environmental variables (a-h) from 20 samples per site, with exception to ammonia and nitrate with means are taken from six samples, and the hydrodynamic means taken from three samples. Sites constitute Z. noltii presence (green) and absence (blue) sites from five geographical locations across four estuaries: Beauly, Tay, Eden, Limekilns in the Forth, and Drum Sands in the Forth

Table 1. Mixed effects model results for each environmental variable. The fixed effect coefficient shows the response of the variable to the presence or absence of Z. noltii. The random effect variance shows the variance attributable to differences between estuary clusters (random effect). The residual variance is the unexplained variance. Intra-class correlation coefficient (ICC) calculates the proportion of total variance explained by the random effect, with values closer to 1 indicating higher variance attributable to the random effect.

Environmental Variable	**Fixed Effect Coefficient**	**Fixed Effect Std. Error**	**Chisq**	***p*-value**	**Random Effect Variance**	**Residual Variance**	**ICC**
Sediment water content	-11.280	4.634	4.269	0.039 *	25.10	67.25	0.272
Sediment stability	1.885	0.789	4.633	0.031 *	3.829	1.783	0.682
Sediment chlorophyll *a* conc.	-16.220	9.449	2.191	0.139	192.8	269.2	0.417
Sediment particle size	-82.30	53.80	2.058	0.151	9107	8544	0.516
Water ammonia conc.	0.063	0.038	2.261	0.133	0.00258	0.00445	0.367
Water nitrate conc.	-3.039	2.264	1.421	0.233	5.513	16.133	0.255
Hydrodynamics	-5.228	4.131	1.459	0.227	38.00	51.37	0.425
Worm density	0.429	1.051	0.165	0.684	1.037	3.506	0.228

4 Discussion

4.1 Environmental Variables

4.1.1 Sediment stability

Sediment stability was consistently higher in *Z. noltii* meadows than absence sites (mean average difference of 23.2%). Higher stability in *Z. noltii* meadows was expected as eelgrass increases the cohesion of sediment by binding particles with its rhizomes (Koch, 2001). These findings support previous studies which found sediment stability to be higher in *Z. noltii* beds than bare sediment (Friend et al. 2003). Stability in *Z. noltii* sites was highly variable between estuaries (maximum of 14.9 pKa in Limekilns and minimum of 7.05 pKa in Beauly) which suggests that additional factors influence stability on a local scale. No correlations were found between sediment stability, water content, chlorophyll *a* content and particle size contrary to numerous studies (Friend et al., 2003; Paterson et al., 2000; Widdows et al., 2008). It is possible that more dense meadows enhanced stability due to thicker rhizome mats (Widdows et al., 2008) and it is recommended that meadow density and coverage be documented in future field studies. The significance of stability in this study was likely due to *Z. noltii* presence, rather than it being a habitat feature which supports *Z. noltii* establishment. Therefore, stability is not the most informative metric to aid the identification of restoration sites as bare mud- and sand flats are not likely to have comparable stability without vegetation.

4.1.2 Sediment water content

The second significant result in this study was the considerably lower water content observed in *Z. noltii* meadows compared to absence sites (mean average difference of 29.6%). This was the most consistent parameter between meadows with a range between 23.8% (Eden 2) to 32.2% (Limekilns 1). These results are within the scope of a *Z. noltii* field study by Azevedo et al. (2016) which found ~20% water content in medium sand substrate and ~25-55% in fine sand/mud. Friend et al. (2003) reported a slightly higher water content of ~40% compared to this study; however, it remains below the metrics for absence sites in Beauly, Tay and Limekilns (40.2-59.6%). These results in conjunction with Friend et al.'s (2003) study suggests a sediment water content over 40% could be a factor hindering *Z. noltii* growth on Scotland's east coast. In contrast, the absence sites in Drum Sands had a sediment water content comparable to *Z. noltii* meadows (20-25%). This suggests that water content at these sites could be suitable for *Z. noltii* growth, particularly Drum Sands 2 which had a medium sand substrate (Appendix Table 3) and 20% sediment water content which is comparable to the *Z. noltii* conditions reported in Azevedo et al.'s (2016) study. Sediment water content appears to be an informative indicator of suitable sediment for *Z. noltii* and further research is warranted to establish an optimal sediment water content range for *Z. noltii* on Scotland's east coast.

4.1.3 Sediment chlorophyll a content

There was no statistical difference in sediment chlorophyll *a* content between sites with and without *Z. noltii*. In the absence sites, chlorophyll *a* was positively correlated with sediment water content. In contrast, in *Z. noltii* sites chlorophyll *a* tended to be lower where sediment water content was higher. The high worm density may explain the low chlorophyll *a* content in Beauly as bioturbating worms disrupt the biofilm layer formed by microphytobenthos (Engel et al., 2012; Volkenborn et al., 2007). However, this opinion conflicts with a study by Chennu et al. (2015) which reported that worms enhanced microphytobenthos abundance through delivery of porewater nutrients to the sediment surface. Saggar (2024) similarly found no significant difference in chlorophyll *a* content inside and outside eelgrass beds, and the present study suggests that chlorophyll *a* is not the most informative indicator of suitable *Z. noltii* conditions. Rather, detecting chlorophyll *a* can be helpful for identifying eutrophication as recognised in Gräfnings et al. 's (2023) study.

4.1.4 Sediment particle size

Particle size was generally smaller in *Z. noltii* sites (mean average difference of 23.6%) although this was nonsignificant. Sediment particle size was variable between meadows which was expected as eelgrass is known to thrive in both sand- and mudflats

(Hemminga and Duarte, 2000). The smaller particles in Tay 1 and Drum Sands sites were unexpected as the high hydrodynamics observed at these sites suggest particle size would be larger as water with higher velocity has greater energy to deposit larger particles. Similarly, the larger particles in Beauly were also unexpected due to the low hydrodynamics and high water content at these sites. These results conflict with previous studies which found definitively smaller particle size in *Z. noltii* meadows (Azevedo et al., 2016; Kohlmeier et al., 2014; Valle et al., 2011; Widdows et al., 2008). Both Valle et al. (2011) and Azevedo et al. (2016) found particle size a highly effective indicator of *Z. noltii* presence, however it is not the most informative variable for this study area. Inconsistency in parameter importance between studies suggests that restoration should take a tailored approach to different regions.

4.1.5 Worm density

Unexpectedly, no relationship was found between worm density and *Z. noltii* presence-absence. Worms were observed in *Z. noltii* sites in Beauly, Eden and Drum Sands which could have been influenced by the larger sediment particle size (215-605 µm) at these sites as worms prefer sand substrate over mud (Eklöf et al., 2015; Volkenborn et al., 2007). In Drum Sands, worm density was lower in the presence site than absence sites as expected based on field observations (Appendix Figure 5b,c). This finding along with field observations suggests high worm density and *Z. noltii* can co-exist but not overlap. This may have implications for restoration at Drum Sands as small transplant patches may get excluded by dominating worms. Previous studies report correlations between worm density and sediment water content, hydrodynamics, sediment chlorophyll *a* and water nutrients (Asmus and Asmus, 1998; Eklöf et al., 2015; Engel et al., 2012; Govers et al., 2014; Paterson et al., 2000; Philippart, 1994; Volkenborn et al., 2007) however, no relationships were found in this study, most likely due to fairly low worm density across sites. Despite this variable not being informative in this study area, monitoring worm density is recommended as high worm density is known to negatively affect eelgrass (Gräfnings et al., 2023; Philippart, 1994). A worm density of 30 individuals m^{-2} has been documented to prevent eelgrass growth (Oncken et al., 2022) but no metrics currently exist for Scotland's meadows.

4.1.6 Water nutrients

No difference in ammonia and nitrate concentrations were found between presence and absence sites; high and low concentrations were found across sites. The markedly high ammonia concentrations in the Beauly estuary (0.25-0.45 ppm) was potentially influenced by the high sediment water content and low hydrodynamics at these sites. Reduced sediment permeability traps nutrients, while low hydrodynamics reduces sediment resuspension, aiding nutrient retention (De Boer, 2007; Koch, 2001; Kohlmeier et al., 2014). In the Beauly estuary, the high ammonia concentrations in conjunction with the comparably low nitrate concentrations (0-2 ppm) suggests the sediment at these sites may be anoxic thereby limiting nitrification (Hemminga, 1998). Eutrophication can create anoxic conditions and is likely the case for Beauly sites, where extensive and thick algal mats were observed over the sediment and eelgrass (Appendix Figure 1a,b). The high ammonia concentration for the Tay presence site, and the high ammonia and nitrate concentrations in the Tay absence site was likely explained by the small particle size, low hydrodynamics and high sediment water content. Relationships for the remaining sites were not as definitive. Proximity of sites to agricultural land and point sources would help explain these results however, it was outwith the scope of this study.

The global median concentration of ammonia in the water column and porewater of seagrass meadows is 0.03 and 1.02 ppm respectively (Hemminga, 1998). For nitrate, median water column and porewater concentrations are both around 0.17 ppm (Hemminga, 1998). All sites in this study exceeded these water column nutrient concentrations (except for one absence site, Beauly 2, which had a nitrate concentration of 0 ppm). These findings build upon a previous (and first) assessment of nutrients in British eelgrass meadows which reported nitrogen concentrations in eelgrass tissue to be 75% higher than the global average (Jones and Unsworth, 2016). Jones and Unsworth (2016) did not investigate Scottish eelgrass therefore, this study contributes crucial information regarding the state of Britain's eelgrass meadows.
In literature, the concentration of nutrients which causes eelgrass decline is not definitive. Experimental studies report necrosis and mortality in *Z. noltii* after exposure to 1.7 ppm of ammonia (Brun et al., 2002), and 0.22 and 0.62 ppm of nitrate (Burkholder

et al., 1992, 1994). However, it should be noted that Burkholder et al. 's (1992, 1994) studies were mesocosm experiments, and are arguably incomparable to in situ studies. Nutrient tolerance is likely to vary between meadows depending on meadow density and coverage (Maxwell et al., 2017), environmental conditions, and cumulative stressors in the environment which would explain nutrient variability observed across sites. Although nutrient levels were not informative here for identifying suitable *Z. notlii* conditions, the results strongly suggest the importance of monitoring nutrients in Scotland's meadows. This will allow detection of vulnerable meadows, and suitable restoration sites with low nutrient levels.

4.1.7 Hydrodynamics

There was no significant difference in hydrodynamic activity between presence and absence sites contrary to extensive literature which recognise hydrodynamics as a primary driver of seagrass distribution (Bertelli et al., 2023; De Boer, 2007; Hogarth, 2015; Stevens and Lacy, 2011). Rather, differences were more definitive between *Z. noltii* sites as exposure to wind, waves and tidal flows depends on a meadow's location in an estuary. Hydrodynamics in *Z. noltii* meadows was lowest in the Beauly and Eden estuaries (12.7 and 13.9% plaster block erosion respectively) and highest in Drum Sands (36.1%). This was predicted as Beauly and Eden are small estuaries with a lower tidal volume. In contrast, Tay 1 and the Drum Sands sites are far more exposed, situated at the mouth of their respective estuaries, exposed to prevailing winds and a high tidal volume. In this study, hydrodynamics was comparable between presence and absence sites therefore, no optimal level of hydrodynamic energy for *Z. noltii* was found.

4.2 Implications for Eelgrass Restoration

Assessment of novel parameters are required to help explain drivers of eelgrass distribution and decline, and the variability in meadow conditions across Scotland. This will highlight important parameters which should be routinely monitored and in doing so, capture region-specific tolerance thresholds. Relevant variables and high resolution data improve HSM accuracy. Accuracy is particularly crucial for selecting restoration sites as restoration trials occur on a small scale, often 0.2-1 ha and as small as 100 m^2

(Govers et al., 2022; Oreska et al., 2021). In this study, the high variability of parameter measurements within and between sites highlights the dynamic nature of coastal areas and stresses the importance of collecting fine-scale data to detect ecological processes.

4.3 Limitations

Fieldwork

Data had to be collected over a three week duration and the tidal cycle and weather events were predicted to influence results. However, data was collected on a spring tide in the Beauly, Tay and Eden estuaries, and halfway between a spring and neap tide in the Forth therefore the tidal energy was relatively similar. Documenting *Z. noltii* meadow density and size was without the scope of this study, but its consideration is recommended to improve interpretability of results. Meadow density and size affects ecological feedback mechanisms, and tolerance levels to external pressures (Maxwell et al., 2017).

Statistical analysis

Sample size limited the statistical power of this study. Prior to field studies, it is recommended to conduct a power analysis to decide the most appropriate sample size to improve interpretability and robustness of results (Thomas, 1997). The LMMs used assumed that the environmental variables were normally distributed. With only 14 sites, it was challenging to robustly test this assumption; however, with a larger sample size, normality should be tested. An Anderson-Darling test is recommended due to its sensitivity to deviations from normality (Shin et al., 2011). Similarly, the LMMs assumed the variation between estuary clusters was normally distributed; with only five estuary clusters, there was little scope for testing this.

Testing multiple variables in the model increased the chance of detecting false positive results i.e., incorrectly rejecting the null hypothesis when it is true (a Type I error). The sequential Bonferonni correction method is recommended to address this problem (Abdi, 2010).

5 Conclusion

To ensure eelgrass can recover in Scottish waters, it is essential to protect existing meadows and assist its reestablishment with active restoration. This study investigated the environmental conditions in *Z. noltii* presence and absence sites to understand which environmental variables could be used to help identify suitable sites for restoration. Sediment water content appeared to be the most important variable that distinguished *Z. noltii* habitat conditions. Further research of sediment water content across more meadows on the east coast of Scotland is urged to investigate if it is a viable variable for use in HSMs and identifying restoration sites in this region. Whilst sediment stability was indicative of *Z. noltii* habitat, this was likely caused by the presence of *Z. noltii* itself. Investigation of water nutrients did not inform optimal nutrient levels in *Z. noltii* habitat however, the results highlighted meadows exposed to nutrient enrichment, and absence sites which may not be suitable for restoration as a result. The remaining variables did not allude to specific *Z. noltii* habitat conditions, likely due to this study's low statistical power. Environmental conditions showed high variability between meadows in different estuaries which reinforces the importance of understanding local conditions prior to restoration efforts. Future studies should do a power analysis to ensure an appropriate sample size to detect biologically significant effects, and document eelgrass coverage and density in conjunction with parameters.

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Appendix

Table 1. GPS coordinates of all sites across all estuaries.

Site	Z. noltii presence (p) - absence (a)	Estuary	Latitude	Longitude
Beauly 1	p	Beauly	57.504727	-4.271572
Beauly 2	a	Beauly	57.504698	-4.291254
Beauly 3	p	Beauly	57.481467	-4.291254
Beauly 4	a	Beauly	57.4835	-4.280157
Tay 1	p	Tay	56.447345	-2.873595
Tay 2	a	Tay	56.428373	-3.124772
Eden 1	p	Eden	56.356956	-2.848785
Eden 2	p	Eden	56.357072	-2.828374
Limekilns 1	p	Forth	56.030632	-3.47497
Limekilns 2	a	Forth	56.029778	-3.474994
Drum Sands 1	p	Forth	55.984735	-3.314497
Drum Sands 2	a	Forth	55.986094	-3.321157
Drum Sands 3	a	Forth	55.984725	-3.312107
Drum Sands 4	a	Forth	55.984813	-3.309049

*Table 2. Mean average measurements for all environmental variables in Z. noltii presence and absence sites. [SD = Standard deviation; Significance indicated by * = p<0.05].*

Parameter	***Z. noltii*** **presence (mean)**	**SD**	***Z. noltii*** **absence (mean)**	**SD**	**% Difference**	***p*-value**
Stability (kPa)	10.85	2.58	8.59	2.40	23.25	0.031 *
Hydrodynamics (% erosion)	19.28	9.28	28.12	11.39	37.30	0.227
Ammonia (ppm)	0.29	0.105	0.23	0.704	14.81	0.133
Water content (%)	27.95	3.15	37.63	14.45	29.63	0.039 *
Particle size (µm)	241.64	114.45	306.29	170.01	23.60	0.151
Nitrate (ppm)	5.86	3.68	8.15	6.05	32.70	0.233
Chlorophyll *a* (µg g)	20.84	8.36	32.42	30.63	43.48	0.139
Worm density	2.59	2.34	2.12	2.20	19.96	0.684

Table 3. Sediment characteristics for Z. noltii presence and absence sites across all estuaries. Each site constitutes 20 sediment samples combined for analysis. Metrics are given by GRADISTAT.

Site	Mode (µm)	Arithmetic mean (µm)	Geometric mean (µm)	Textural group	Sediment name
Beauly 1	427.5	305.1	182.2	Muddy Sand	Very Coarse Silty Medium Sand
Beauly 2	605	222.2	62.5	Muddy Sand	Coarse Silty Fine Sand
Beauly 3	302.5	255.7	156.2	Muddy Sand	Very Coarse Silty Medium Sand
Beauly 4	302.5	162.1	48.54	Muddy Sand	Very Fine Silty Medium Sand
Tay 1	152.5	163.3	100.3	Muddy Sand	Very Coarse Silt Fine Sand
Tay 2	76.5	57.78	17.95	Sandy Mud	Very Fine Sand Very Coarse Silt
Eden 1	215	188	150.1	Sand	Moderately Sorted Fine Sand
Eden 2	215	252.7	216.2	Sand	Well Sorted Fine Sand
Limekilns 1	302.5	362.1	229.4	Sand	Poorly Sorted Medium Sand
Limekilns 2	427.5	376.5	203.9	Muddy Sand	Very Coarse Silty Medium Sand
Drum Sands 1	76.5	93.49	42.79	Muddy Sand	Coarse Silty Very Fine Sand
Drum Sands 2	302.5	240.9	183.5	Sand	Moderately Sorted Medium Sand
Drum Sands 3	215	197.2	155	Sand	Moderately Sorted Fine Sand
Drum Sands 4	215	175.8	148	Sand	Moderately Sorted Fine Sand

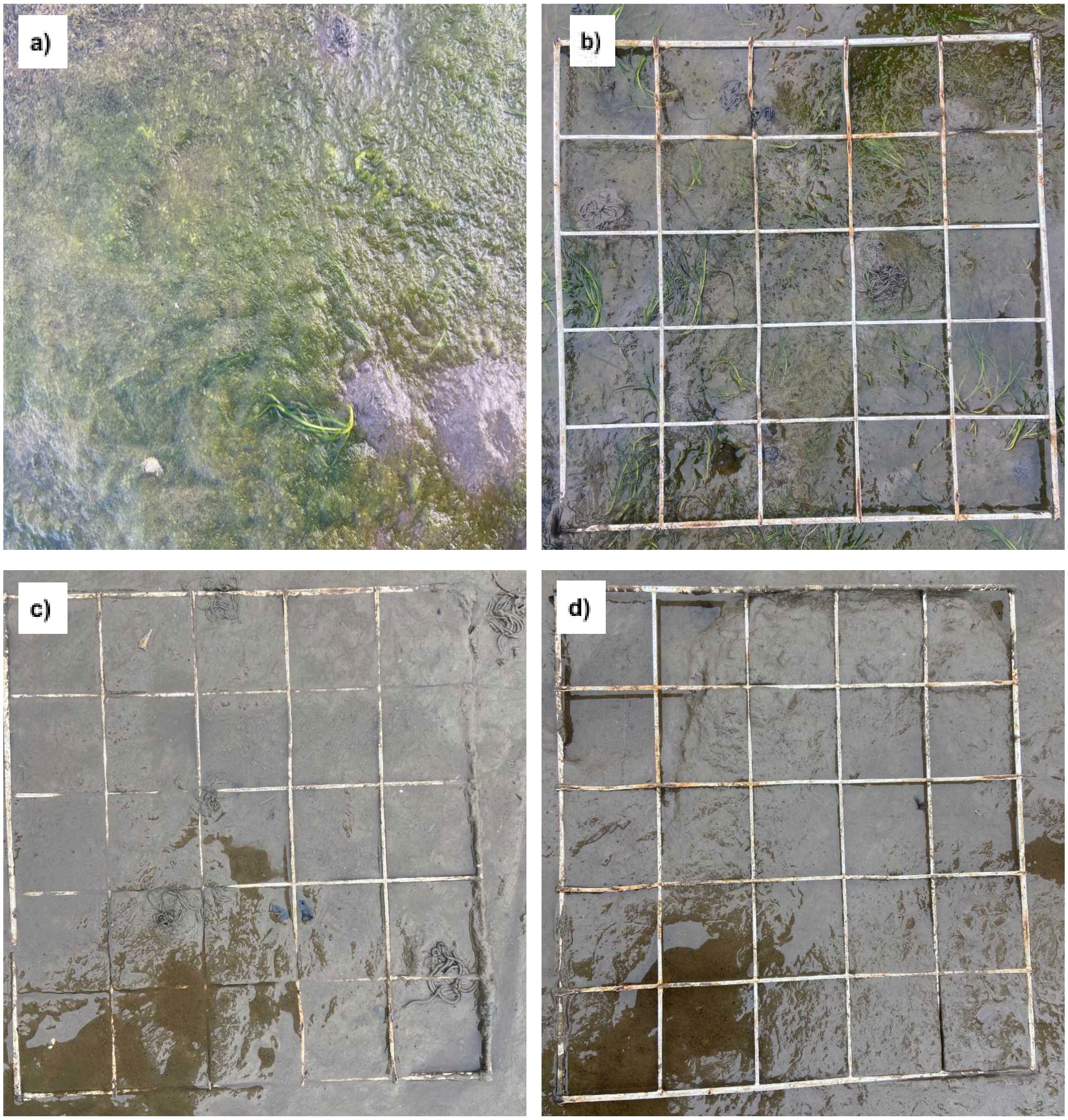

Figure 1. Beauly estuary. Significant macroalgae growth at Beauly 1 (a) and Beauly 3 (b), and high surface water at Beauly 2 (c) and Beauly 4 (d).

Figure 2. The Tay estuary. Spatial separation observed between worms and Z. noltii at Tay 1 (a); muddy and uniform topography at Tay 2 (b) and (c).

Figure 3. The Eden estuary. Z. marina and worms overlapped spatially in runnels. While Z. noltii was restricted to ridges (not shown in photo).

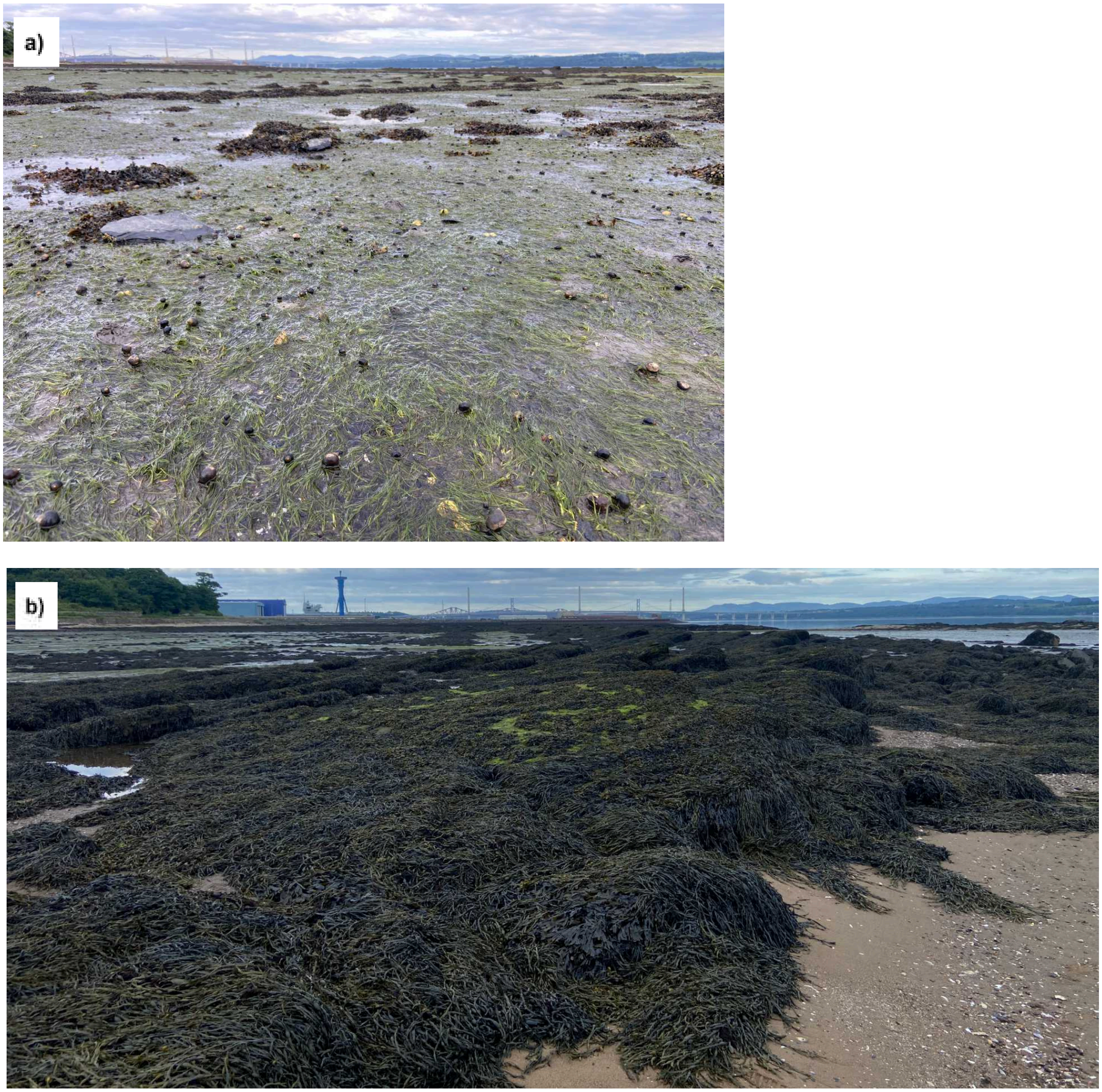

Figure 4. Limekilns in the Forth estuary. Z. noltii is adjacent to a rocky reef and a high abundance of snails on the meadow (a); rocky reef acts as a barrier between the Z. noltii meadow (left of photo) and the sea (b).

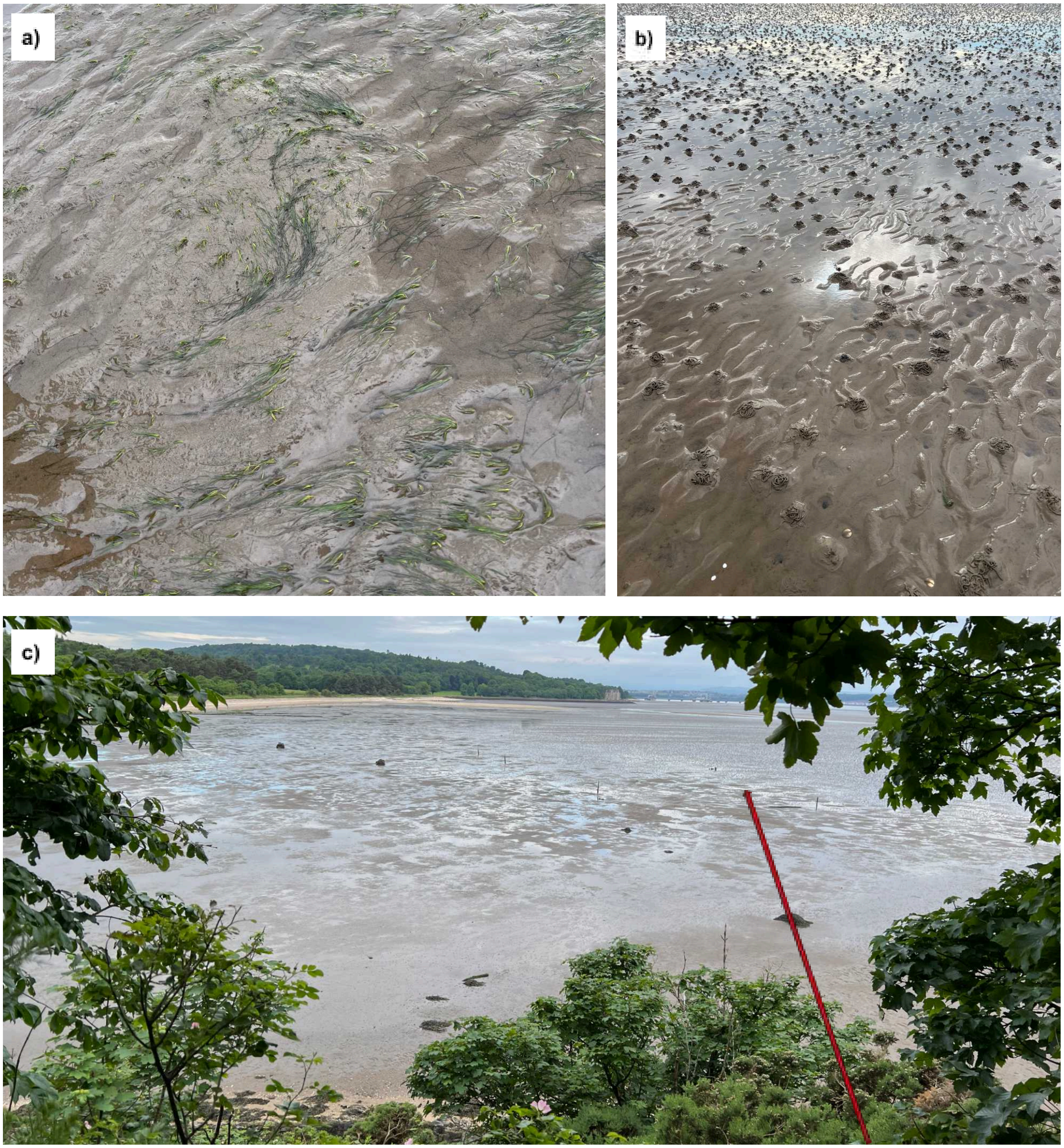

Figure 5. Drum Sands in the Forth estuary. High and turbulent water flow shapes the position of eelgrass on the sediment surface in Drum Sands 1 (a); a high abundance of worm casts outside the meadow (b); a distinct spatial separation of worm abundance (shown by the red arrow), with lower density inside the Z. noltii meadow (left of photo) and significantly higher density outside the Z. noltii meadow (right of photo) (c).