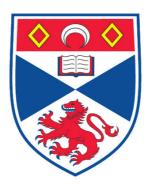
# THE ROLE OF INTERTIDAL SEAGRASS ZOSTERA SPP. IN SEDIMENT DEPOSITION AND COASTAL STABILITY IN THE TAY ESTUARY, SCOTLAND

#### **Lorna Wilkie**

# A Thesis Submitted for the Degree of PhD at the University of St. Andrews



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# The role of intertidal seagrass Zostera spp. in sediment deposition and coastal stability in the Tay Estuary, Scotland

# Lorna Wilkie



A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of St Andrews

School of Biology University of St Andrews December 2011

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# **Abstract**

The Tay estuary is situated on the east coast of Scotland. The estuary is dominated by sediment biotopes, including mudflats which support sparse beds of two nationally scarce seagrass species, *Zostera marina* var. *angustfolia* (Hornem.) and *Z. noltii* (Hornem.). Seagrasses have been described as ecosystem engineers, shaping their sediment environment, and this may increase sediment deposition and stability. In this thesis the ecosystem engineering characteristics of seagrass habitats are explored.

In 2008, the distribution of *Zostera* spp. in the Tay estuary was surveyed and mapped for the first time. Sediments within beds of *Z. marina* and *Z. noltii* were compared to investigate the influence of seagrasses on sediment characteristics. To explore the role of seagrass in sediment deposition and erosion, and coastal stability, sediment depth measurements were made in patches of *Z. noltii*, *Z. marina* and bare sediment over one year. The role of the root/rhizome system on sediment retention over winter was also considered. Sediment deposition in *Z. noltii* beds, and the influence of the plants on nearbed flow dynamics was further explored in the laboratory, using an 8 m seawater flume. In the field the retention of particles over 2 and 14 tides was measured, and the results of this experiment led to a study of the influence of leaf and sediment biofilms on particle retention, using the novel method of magnetic particle induction (MagPI).

The efficacy of artificial seagrass beds and *Z. noltii* transplantation as habitat restoration techniques were compared over one year. During the trial, sediment deposition and changes in sediment chacteristics were determined, and the protection given to saltmarsh cliffs fringing the study plots was assessed.

Mechanisms underlying the results are suggested and the findings discussed.

This study provides an insight into the ecology of seagrass in the Tay estuary and its role as an ecosystem manager. It may offer valuable data which could be utilised for future conservation policies, habitat restoration schemes and management planning of the area.

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6.3	One -way ANOVA of the differences in sediment grain sizes fractions (%)	180
	before and after the restoration trial, with Tukey's post hoc tests. Means	
	that do not share a letter are significantly different ( $p < 0.05$ ).	
6.4	Repeated measurements MANOVAs of the mean sediment deposition or	182
	erosion between measurements for the full duration of the experiment, for	
	each plot type; and of the mean sediment levels relative to the baseline	
	measurement, up to 1 <sup>st</sup> February 2010.	
6.5	Tukey's post hoc tests and coefficient of variation (CV) (%) of the	183
	difference in sediment height between test plots and measurement dates,	
	and of the mean sediment levels relative to the baseline measurement.	
	Means that do not share a letter are significantly different ( $p < 0.05$ ).	

# **Chapter 1**

# **General Introduction**

# 1.1 The River Tay and the Tay estuary

# 1.1.1 The physical environment

The River Tay, at 193 km, is the longest river in Scotland (SEPA 2008) and is the largest river in Britain in terms of flow (Maitland & Smith 1987). The Tay, its tributaries and the River Earn drain an area of approximately 6500 km² (Pontin & Reid 1975; McManus 1998; Bates *et al.* 2003), the largest drainage area in Scotland (SEPA 2008). Although the Tay catchment is just over half the catchment area of the River Thames at Kingston (9948 km²) (CEH 2008b), the observed mean discharge at Ballathie Gauging Station, 15 km north of Perth, is 167.94 m³s⁻¹ (CEH 2008a), more than double that of the Thames (65.52 m³s⁻¹) (CEH 2008b). Discharges often exceed 1500 m³s⁻¹ in winter, but may fall to less than 20 m³s⁻¹ during prolonged periods of drought (McManus 2005). The River Earn discharges an additional 31 m³s⁻¹ into the estuary (Maitland *et al.* 1994; McManus 2005; Williams & West 1975). At its lower reaches the Tay is an eighth order river, one of only two in Britain; the other being the Humber (Smith & Lyle 1994).

Hydrological characteristics are largely dictated by rainfall, which varies greatly throughout the Tay basin. The mean annual precipitation ranges from 1344 mm at Ardtalnaig, near the source of the river at Loch Tay, to 654 mm at Leuchars, near the mouth of the estuary (Met Office 2008). Rainfall is mainly cyclonic, approaching from the west (Jones 1968) and the rainfall regime is markedly seasonal with winter maxima in December and January and minima in June (Met Office 2008). Winter flow peaks are lowered and the summer discharge of freshwater is enhanced by the regulation of flows by the Breadalbane and Tummel Valley hydro-electric schemes (Jones 1968;

Pontin & Reid 1975). Winter run-off may be inhibited and delayed during hard winters where snow is preserved. However, throughout the year the rainfall-controlled pattern of run-off is "flashy" due to frequent short duration but high intensity cyclonic storms (Jones 1968).

The Tay estuary is situated on the east coast of Scotland, between the coastal towns of St Andrews and Carnoustie (Figure 1.1). The tidal limit of the estuary extends to Scone, 3 km upstream of Perth and to 4 km above Bridge of Earn for the rivers Tay and Earn respectively (West 1972; Pontin & Reid 1975). Typically salt water intrusion reaches Newburgh (West 1972; Williams & West 1975), and surveys by Alexander *et al.* (1935) and Maitland and Smith (1987) found no freshwater animals or plants downstream of Newburgh. At high tide, salinity commonly reaches over 32 psu in the seaward reaches of the estuary (Maitland *et al.* 1994; McManus 2005).

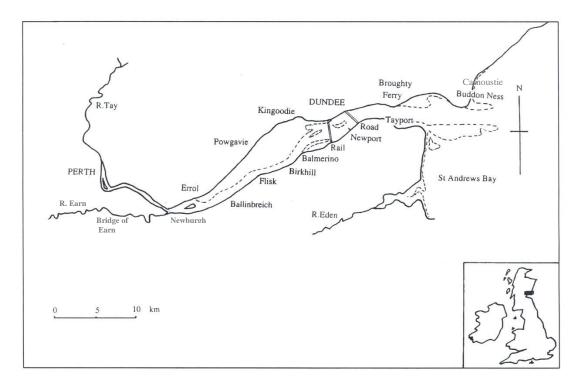


Figure 1.1: The Tay estuary (from McManus 2005).

The estuary is subject to strong tidal currents (Bates *et al.* 2003). The tides are associated with the northernmost of three Kelvin Waves, which has its amphidromic centre ~60 km off the southern coast of Norway. This Kelvin Wave controls the North

Sea circulation and influences the water levels of the east coast of Scotland. The rising tide is carried southwards by its anticlockwise movement, across the mouth of the estuary. Ordinary spring tidal range at Dundee and Buddon Ness, towards the entrance is 5 m, whereas neap tide range is ~4 m, and equinoctial ranges can reach 6 m. Tidal ranges are less at Newburgh and Perth upstream (West 1972; Charlton *et al.* 1975; Maitland *et al.* 1994; McManus 2005). The wind can significantly modify tidal levels: a wind of 25 ms<sup>-1</sup> (Force 10-11 on Beaufort scale) along the estuary can result in a water level difference of 1.9 m between Buddon and Newburgh (Charlton *et al.* 1975).

Thorough mixing of the water occurs on the rising tide but, during the high water slack period, mixed waters are frequently replaced by salinity layering in as little as half an hour (McManus 2005). The runoff of freshwater from the rivers Tay and Earn, and the exchange of water with the North Sea strongly influence the physical and chemical dynamics and the ecology of the estuary (Maitland & Smith 1987; McManus 2005), and the well-mixed shallow water column results in the tight coupling between benthic and pelagic processes. The input of freshwater influences estuarine hydrography by transporting silt, inorganic nutrients and organic material into the estuary, and by creating salinity gradients and stratification. Tidal and wind-generated water exchange from the marine areas impose a sizeable pulse of physical and chemical forcing on the estuarine ecosystem, also introducing nutrients and organic material which support primary and secondary production (Flindt *et al.* 1999).

The Tay estuary has a high suspended sediment load (Bates *et al.* 2003). During sustained periods of calm sea conditions and moderate river discharge, little suspended material enters the estuarine circulation from external sources, and the waters of the landward and seaward ends of the estuary contain relatively low concentrations of suspended matter (Buller 1975; McManus 2005). Within the estuary itself the concentration of suspended matter rises towards the head of salt water intrusion, creating a weak turbidity maximum in which waters with increased suspension move up and down the estuary with the tide. Within the turbidity maximum zone, concentrations are often an order of magnitude greater than to landward or seaward (McManus 2005).

The estuary is dominated by sediment biotopes (Bates *et al.* 2003). The subtidal sediments of the tidal freshwater region are composed of poorly-sorted fine sands or pebble and boulder patches, bordered by narrow intertidal mudflats and reedbeds (Buller & McManus 1975). These have a relatively impoverished fauna compared to areas with more stable substrata or in the main estuary (Maitland *et al.* 1994). Intertidal areas within the estuary are generally muddy, whilst the sediments of more exposed shores in the outer part of the estuary tend to be better drained (Bates *et al.* 2003). The estuary's beaches, sand bars and channels comprise a dynamic and variable shoreline (SNH 2006).

# 1.1.2 Habitats, flora and fauna of the Tay estuary

The plants and animals present in the Tay estuary are representative of communities associated with a high quality estuarine habitat. The sediments contain plant and animal communities that reflect the gradients of exposure and salinity, and the abundance, distribution and composition of these biological communities are ecologically representative of northern North Sea estuaries (Bates *et al.* 2003).

The intertidal areas consist of extensive sediment flats, over 3 km wide in places, composed of fine silty sands and mud with marshes at or above high water level. These are colonised by typical estuarine mud-dwelling invertebrates (SNH 2006), although in places, due to the unstable nature of the course sediments, the fauna is naturally impoverished (Buck 1993). The muddy sediment of the inner and middle estuary are commonly dominated by species such as ragworm (*Hediste diversicolor*). Mudflats in the outer estuary are mosaics of lugworm-dominated (*Arenicola marina*) muddy sediments, mussel beds (*Mytilus edulis*), fucoid algae (*Fucus vesiculosus*) and transient mats of the green macroalgae *Ulva* sp. These mudflats support sparse beds of two nationally scarce eelgrass species, *Zostera marina* var. *angustifolia* (Hornem.) (Riechb.) and *Z. noltii* (Hornem.) (Bates *et al.* 2003; SNH 2006). The sandflats of the outer estuary and mouth of the Firth are more exposed. They tend to be cleaner and better drained, and are commonly dominated by amphipods, such as *Bathyporeia pelagica* and

*Pontocrates altamarinus* (Bates *et al.* 2003). The sandflats and sand dunes at Barry Links and Tentsmuir are of geomorphological interest, as sediment deposited at the mouth of the estuary is actively building extensive bar and spit systems (Buck 1993).

Along the north shore of the inner estuary are the most extensive reedbeds (*Phragmites australis*) in Britain, extending for 15 km over the mudflats. The reeds were introduced during the 18<sup>th</sup> century for coastal protection, and to encourage sediment deposition prior to land reclamation. In addition to protecting against erosion and stimulating accretion, the reedbeds provide materials for the thatching industry (Alizai & McManus 1980; Bates *et al.* 2003). The Tay reedbeds support nationally important breeding populations of marsh harrier (*Circus aeruginosus*), water rail (*Rallus aquaticus*) and bearded tit (*Panurus biarmicus*) (SNH, 1999).

The reedbeds are bordered by one of the largest areas of saltmarsh on the east coast of Scotland. There are also smaller, more isolated areas of saltmarsh at Balmerino, Tayport, Monifieth and Carnoustie (Buck 1993). The saltmarsh communities are representative of the various stages of a typical saltmarsh succession within an east coast estuary. They are mainly comprised of saltmarsh rush (*Juncus gerardii*), and club-rushes (*Scirpus* spp., and *Schoenoplectus* spp.) (Bates *et al.* 2003).

In the middle and outer estuary there are dense reefs of mussels (*Mytilus edulis*) in subtidal beds and on intertidal banks. The reefs support stands of fucoid algae in the intertidal and common starfish (*Asterias rubens*) in subtidal areas (Khayrallah & Jones, 1975; SNH 2006). Rocky reefs, comprised of areas of boulders, bedrock and manmade structures are also found in the outer parts of the estuary. In parts of the outer Tay there is also an unusually abundant sponge fauna (Bates *et al.* 2003).

The invertebrate-rich mudflats provide important feeding and roosting areas for waterfowl (Bates *et al.* 2003; SNH 2006). In winter, wading birds such as sanderling (*Calidris alba*), and internationally important populations of bar-tailed godwit (*Limosa lapponica*) and redshank (*Tringa totanus*) feed on invertebrates such as shrimps, shellfish, lugworms and ragworms. In the waters of the estuary up to 11,500 eider

(Somateria mollissima) feed on shellfish and mussels, while average counts of over 4200 roosting pink-footed geese (Anser brachyrhyncus) have been recorded in winter. Also in winter months, high numbers of red-breasted merganser (Mergus serrator) and goosander (Mergus merganser) feed on fish. Throughout the summer, sandwich terns (Sterna sandvicensis) can be see diving for sandeels, sprats and whiting (Holden & Cleeves 2002; Musgrove et al. 2007).

Upstream of Dundee, the water depth varies from 2–6 m, but near Broughty Ferry it reaches a maximum depth of 30 m. The channel in the upper estuary is defined by shifting mud and sandbanks, which are covered by seawater at all times. In the central section of the estuary between Balmerino and Broughty Ferry the shores are narrow, and the channel meanders around a number of mobile sandbanks (SNH 2006).

A population of the nationally rare fish the smelt or sparling (*Osmerus eperlanus*) occurs in the Firth of Tay (Bates *et al.* 2003; SNH 2006). The eggs and planktonic larval stages of demersal fish such as haddock, cod, whiting and lemon sole are carried into the estuary by drift on the south-going current. Plaice spawn close to the coast and spend their first year in sandy bays, at depths of 1.5-3.5 m, and adults also prefer sandy substrata and shallow water. Nearby St Andrews Bay is the most important plaice nursery in Scottish waters, and is vital to the health of the Scottish North Sea plaice fishery. There are also suitable areas in the Tay for juvenile cod. Plaice and cod are therefore likely to be vulnerable to any adverse changes in the estuarine environment. Pelagic fish play a relatively minor economic role in the fisheries of the Tay estuary compared to other Scottish coastal areas (Thomas & Saville 1972). However, the Tay is world renowned for its salmon fishing beats and for trout fishing. Both salmon and sea trout use the estuary on their descent to sea as smolts, and on their return to spawn in their native rivers (Thomas & Saville 1972).

# 1.1.3 Human influences on the physical and chemical environment

As well as naturally occurring changes to the estuary bed, humans have had a

significant impact on the physical environment of the Firth of Tay. In the early 19<sup>th</sup> century the upper reaches of the Tay estuary underwent considerable engineering modifications in order improve navigation. A consequence of the dredging operations between Newburgh and Perth was the facilitation of river water outflow. Also during the 19<sup>th</sup> century, major dredging operations to provide access for deep draft boats to Dundee, and the steady progression of land reclamation are likely to have permitted saline waters to penetrate further upstream than previously (McManus 2005). Today capital and maintenance dredging are still carried out in the estuary. These operations have the potential to cause deterioration of habitats and communities through direct loss and disturbance (SNH 2006).

Flood mitigation measures have involved flood embankment construction, channel steepening and the removal of meanders in the river Tay. These have resulted in increased flood peaks downstream, due to the loss of floodplain storage, and an increase in the velocity of these flood flows (Maitland *et al.* 1994). Conversely, a by-product of the hydro-electric schemes upstream is the creation of storage capacity for flood water. The dams postpone flood discharges, allowing high discharges from non-controlled tributaries to pass through lower reaches before the stored water is discharged into the main stream. However, the storage capacity available during the storm event dictates the effectiveness of this system. The schemes also reduce the release of suspended sediments from the river into the estuary (Jones 1968; Pontin & Reid 1975). Construction of any structures (e.g. jetties, piers, harbours, sea defences) has the potential to cause deterioration or direct loss of habitat, to interfere with deposition and erosion processes, with subsequent effects on seabed community structure (SNH 2006).

By the mid 19<sup>th</sup> century many rivers throughout Britain and western Europe had become heavily polluted by the discharge of industrial and domestic effluents to watercourses. The Rivers Pollution Commission, set up by the British Government in 1868 investigated the pollution of rivers by sewage and industrial wastes, and recommended methods of treatment. The Commission found the Tay river basin to be virtually unpolluted; furthermore that sewage from Perth (then population 26,356) was lost in the huge volume of water discharged by the river (Maitland *et al.* 1994). Alexander *et al.* 

(1935) carried out a detailed survey of the Tay, one of the earliest studies of estuarine science in Britain. In their investigation into pollution in the Tees estuary, the Tay was used as an "unpolluted" control area for purposes of comparison. A later study found no evidence of above-background contamination of organochlorines such as dieldrine, dichloro-diphenyl-trichloroethane (DDT) or (polychlorinated biphenyls) PCBs in fish or shellfish (Holden and Topping 1972). Despite Dundee being Scotland's second industrial city, pollution from industry was considered to be minimal compared to waste products derived from agriculture and domestic effluent. In contrast to the heavy industries of the River Forth, the traditional Tayside industries of jam, jute and journalism did not produce large amounts of toxic waste, and the engineering works that replaced them also produced little effluent (Stewart 1972). Khayrallah and Jones (1975) found neither the rivers Earn and Tay, nor the estuary to be heavily polluted, although some localised effects of domestic sewage and industrial effluent were evident near Dundee. The Tay is still considered to be a non-polluted estuary with good ecological status (SEPA 2010).

Sewage treatment facilities have recently been improved through the Tay Wastewater Scheme, which provides wastewater treatment and disposal facilities for the City of Dundee, Broughty Ferry, Monifieth, Carnoustie and Arbroath. The Scheme intercepts foul flows from 41 outfalls previously discharging into the Tay Estuary and lower Angus coast. The scheme increases storm storage facilities, and provides extended outfalls to ensure discharge below low water spring tide level (LWS). The final effluent from the Hatton water treatment plant, west of Carnoustie, flows through a long-sea outfall with its discharge point located 1.6 km offshore (Scottish Executive 2000).

The Tay is quantitatively the most important riverine source of nutrients to Scottish North Sea coastal waters (Balls 1994). Nutrient concentrations in individual rivers and estuaries are related to land use within the catchment area (Herbert 1999), and the Tay region is a particularly rich agricultural area (Stewart 1972). The main diffuse sources of nutrients in the Tay catchment are runoff from agricultural land and forestry plantations (Maitland *et al.* 1994).

The river is a valuable resource with regards to the generation of hydro-electric power, water supply, fisheries, tourism and recreation (Maitland & Smith 1987). Numerous leisure activities take place in the Firth of Tay, and aquatic-based sports are centred around the outer estuary and Broughty Ferry (Buck 1993).

# 1.1.4 Conservation status of the Tay Estuary

The Firth of Tay & Eden Estuary was designated as a Special Area of Conservation (SAC) on 17th March 2005 for the habitats 'Estuaries', 'Mudflats and sandflats' and 'Sandbanks which are slightly covered by sea water all the time', which are listed on Annex I of the Habitats Directive, as well as for the Annex II species 'Common seal *Phoca vitulina*'. This site is also referred to as a 'European marine site' or marine SAC (SNH 2006). Other designations within or adjacent to the Tay Estuary are: SSSI sites (Balmerino Wormit-Shore, Earlshall Muir, Eden Estuary, Flisk Wood, Inner Tay, Barry Links, Tayport and Tentsmuir Coast, Tentsmuir Point National Nature Reserve; Barry Links terrestrial SAC; Firth of Tay & Eden Estuary Special Protection Area; Firth of Tay & Eden Estuary Ramsar site (SNH 2008).

# 1.2 Seagrass

# 1.2.1 Ecological and economic significance of seagrass

Seagrasses are found throughout the world in marine or estuarine habitats with around 50 species adapted to marine waters (den Hartog & Kuo 2006). They typically occur as dense stands on sands and muds in the intertidal and shallow subtidal areas, to a maximum depth of around 10 m (Thayer *et al.* 1984; Stevenson 1988; James 2004). Seagrass communities are widely regarded as critical habitats influencing the structure and function of coastal ecosystems (Hemminga & Duarte 2000) and the communities are an important biological feature of coastal waters. The architectural complexity of the plants, and the physical and chemical effects of the community on water movement

and sediment characteristics has numerous impacts on the local environment (Cleator 1993). Until the latter half of the 20<sup>th</sup> century, seagrass meadows were both abundant and economically important in the UK (Tubbs 1995).

In both tropical and temperate zones, seagrass beds provide habitat, food and protection for an abundant marine flora and fauna, including several exploitable species of fish, crustaceans and molluscs (Kwak & Klumpp 2004; Tubbs 1995) and there are also a number of rare species which are associated with seagrass habitats (Cleator 1993). Seagrasses store nutrients in the leaves and roots, stabilising the environment and ensuring relatively high species diversity within the intertidal habitat (Flindt *et al.* 1999). The plants are rooted in the sediment and aerate its upper horizons, improving the mud as habitat for benthic infauna (Tubbs 1995).

Due to high prey densities estuaries are important feeding areas for a variety of marine organisms. Although direct grazing pressure on temperate seagrasses by herbivores is generally low (Cebrian & Duarte 2001) – except for seasonal grazing by wildfowl (Charman 1977; Tubbs & Tubbs 1983) - seagrasses support populations of gastropods that graze on the epiphyton, reducing epiphytic cover, preventing seagrass decline through light deprivation and enhancing growth (Hily *et al.* 2004; Stevenson 1988). The foliage is rich with epiphytic epifauna such as amphipods, isopods and tanaids, and free-swimming crustaceans are provided with shelter as well as food (Kwak & Klump 2004; Vichkovitten & Holmer 2005).

Before the decline, the seagrass beds in the estuaries of the Solent, southeast England were important for prawns and shrimps, and produced a harvest of several fish species, particularly eels (*Anguilla anguilla*), grey mullet (*Liz spp.*) and bass (*Dicentrarchus labrax*) (Tubbs 1995). Although the Tay estuary supports the eggs, juvenile and adult stages of several fish species, the role of *Zostera* in the Tay estuary in the life cycle of fish species is uncertain (*pers. comms.*) Estuarine fish species each have different requirements within the estuarine habitat. The fish may use components of the habitat year round or seasonally, for part or for their whole life cycle (Elliot *et al.* 1990). Dependence on the estuary may be physiological, behavioural, or related to food preference.

Seagrass beds are also important for several bird species. In Britain Zostera beds are an important and preferred autumn and early winter food source for wigeon (Anas penelope L.), brent geese (Branta bernicla L.), mute (Cygnus olor L.) and whooper swans (Cygnus cygnus L.) which consume the leaves and rhizomes, and Teal (Anas crecca L.) which eat the seeds (Charman 1977; Tubbs & Tubbs 1983). Estuary dependent marine bird species are exposed to threats such as the loss of habitat and feeding areas through dredging, land reclamation and the building of barrages, or through poor water quality (Elliot et al. 1990).

In shallow coastal waters, tide- and wind-induced currents can have a significant impact on water turbidity (Gabrielson & Lukatelich 1985; Ward *et al.* 1984). Within seagrass colonies the vegetation attenuates wave energy, consequently suppressing resuspension and increasing deposition of suspended material. During periods of high winds, at normal water levels, wave induced resuspension occurs in unvegetated areas while the concentration of suspended particulate matter inside the bed can remain unchanged (Ward *et al.* 1984). Seagrasses provide physical protection against water turbulence, and the rhizomes form branching mats which stabilise the substratum, thus dense seagrass meadows stabilise the sediment and decrease the amount of sediment resuspension (Fonseca *et al.* 1982; Ward *et al.* 1984).

In addition to stabilisation of sediments seagrasses trap fine grained suspended particles by altering water movement within the meadow (Agawin & Duarte 2002; Fonseca *et al.* 1982; Gacia & Duarte 2001; Ward *et al.* 1984). Ward *et al.* (1984) observed that suspended particulate material concentrations were significantly lower in seagrass beds compared to adjacent unvegetated areas in Chesapeake Bay. Although sedimentation rates are substantially higher in seagrass communities than in unvegetated areas, Gacia and Duarte (2001) found the decrease in resuspension to be significantly higher than primary deposition in *Posidonia oceanica* beds in the Mediterranean. Lower resuspension contributes to increased sediment retention, reducing erosion in the coastal zone due to, for example, rising sea levels (Gacia & Duarte 2001).

Seagrass leaves, and their epiphytic communities, have the ability to remove nutrients and contaminants from the water column, maintaining water quality for neighbouring

benthic communities and acting as a buffer to eutrophication (Cornelisen & Thomas 2002; de Wit *et al.* 2001; Stapel *et al.* 1996; Thomas *et al.* 2000). In addition to causing the deposition of suspended organic material and slowing down the flow through of nutrients from riverine inputs to coastal waters, this capability may also be important for decreasing nutrient losses from the system. Nutrients diffusing from the sediment to the overlying water column, or released from decomposing organic matter produced by seagrass leaves and by other primary producers, such as epiphytes and benthic algae, are recaptured (Hemminga *et al.* 1991; Pedersen & Borum 1993).

Compared to macroalgae and other substrates for bacterial communities, organic matter derived from the decompostion of eelgrass is of minor importance in some temperate waters in north western Europe (Boschker *et al.* 2000). However, seagrass also supplies organic matter to the sediment pore water directly through root excretion (Blaabjerg & Finster 1998; Hansen *et al.* 2000). The inputs of organic matter from and within seagrass meadows, and the export of detrital material to nearby habitats, provide substrate to benthic bacterial communities which promote carbon and nitrogen mineralisation in aquatic ecosystems (Boschker *et al.* 2000).

As the build up of greenhouse gases leads to increased atmospheric and ocean temperatures, ways are being sought to sequestrate carbon and to reduce CO<sub>2</sub> emissions. Although vegetated wetlands, including seagrass beds, occupy only 2% of the total seabed area worldwide, they are responsible for 50% of the carbon transfer from oceans to sediments (Crooks *et al.* 2011). Buried organic carbon can remain stored in sediments for months (Mateo *et al.* 2006) to millennia, in the case of *Posidonia oceanica* (Romero *et al.* 2004; Mateo *et al.* 2006). While the longterm rate of soil/sediment C accumulation in tropical and temperate forests is 2.3-2.5 gC m<sup>-2</sup> y<sup>-1</sup> and 1.4-12.0 gC m<sup>-2</sup> y<sup>-1</sup> respectively, in seagrass meadows it is as high as 83 gC m<sup>-2</sup> y<sup>-1</sup> (Duarte *et al.* 2005; Pidgeon 2009; Table 1.1). The loss of coastal wetlands and marine ecosystems such as salt marshes, mangroves and seagrass beds decreases the capacity for carbon sequestration, and can also cause the emission of large amounts of CO<sub>2</sub> back into the atmosphere (Crooks *et al.* 2011).

**Table 1.1**: Comparison of carbon stocks and longterm accumulation of carbon in soils in key terrestrial and coastal marine ecosystems. (From Pidgeon 2009).

	Standing carbon stock		Total global area	Global carbon stocks		Longterm rate of carbon accumulation in sediment
Ecosystem type	(gC m <sup>-2</sup> )		$(*10^{12} \text{ m}^2)$	(*10 <sup>15</sup> gC)		(gC m <sup>-2</sup> y <sup>-1</sup> )
	Plants	Soil		Plants	Soil	
Tropical forests	12045	12273	17.6	212	216	2.3 - 2.5
Temperate forests	5673	9615	10.4	59	100	1.4 - 12.0
Boreal forests	6423	34380	13.7	88	471	0.8 - 2.2
Tropical savannas and grasslands	2933	11733	22.5	66	264	
Temperate grasslands and shrublands	720	23600	12.5	9	295	2.2
Deserts and semi-deserts	176	4198	45.5	8	191	1.8
Tundra	632	12737	9.5	6	121	0.2 - 5.7
Croplands	188	8000	16	3	127	
Wetlands	4286	72857	3.5	15	225	20
Tidal salt marshes			Unknown (0.22 reported)			210
Mangroves	7990		0.152	1.2		139
Seagrass meadows	184	7000	0.3	0.06	2.1	83
Kelp forests	120 - 720	na	0.02 - 0.4	0.009 - 0.02	na	na

Seagrass has had other economic uses. Until the early 19<sup>th</sup> century it was a component in the construction of sea-dykes in the Netherlands, where it was used to bind the clay used in the embankments (den Hartog 1970). In the 20<sup>th</sup> century dried *Zostera* straw was used as packing material, for stuffing mattresses and pillows, and it is said that the walls of the early BBC studios were sound-proofed with *Zostera* (Tubbs 1995).

Seagrasses are often heralded as indicator species because they are long-lived and integrate biological, physical and chemical parameters (Kaldy 2005). Because of this, they are an indicator of biological quality in coastal zones, within the context of the European Water Framework Directive (EU Directive 2000/60/CE).

# 1.2.2 Seagrass species in Scotland

The *Zostera* spp. seagrasses, or eelgrasses, are a group of submerged flowering plants of the Zosteraceae family. These are glabrous, perennial herbs with creeping rhizomal roots, and submerged stems which may be exposed at low tide. Each stem has several alternate, distichous, linear leaves. Flowering stems are lateral or terminal and the inflorescences are enclosed in a leaf-sheath or spathe (Clapham *et al.* 1987; Stace 2001).

Two species of *Zostera* occur in the UK: marine eelgrass (*Zostera marina* L.) and its intertidal morphotype, narrow-leaved eelgrass (*Zostera marina* var. *angustifolia* (Hornem.) Reichb.); and dwarf eelgrass (*Zostera noltii* Hornem.), and are considered to be scarce (JNCC 1995). These species are found in Scottish waters, and form two kinds of *Zostera* community: sublittoral meadows of *Z. marina* (Figure 1.2a.) are principally found on the west coast of Scotland, while the intertidal mixed *Z. marina* and *Z. noltii* (Figures 1.2b. and 1.2c.) community predominantly occurs in the east coast firths (Cleator 1993).

Z. marina (Figure 1.3) is the largest British species. Non-flowering shoots are 20-50 cm long and 4-10 mm wide. The apex of the leaves is rounded or rounded-mucronate (Figure 1.3). The flowering shoots are shorter and narrower. However, flowering stems are up to 60 cm in length and are much branched. The stigma is twice as long as the style. Z. marina is found in the northern Atlantic and Pacific, and is the most dominant seagrass in northern temperate oceans. In the Atlantic its range extends from the Arctic Circle to Gibraltar. In Britain Z. marina is regarded as a fully marine species (Cleator 1993). It is typically found growing on sea-coasts on sheltered gravel, sand or mud below LWS to 4 m (Stace 2001).

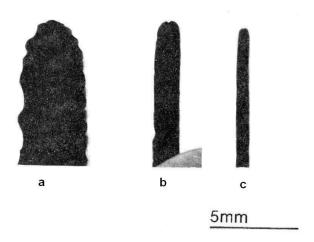
The intertidal *Z. marina* var. *angustifolia* (Figures 1.3 & 1.4) is more slender than marine *Z. marina* with non-flowering shoots 15-30 cm long and 2-3 mm wide. The apex of the leaves is rounded when young, often becoming emarginated when mature. The leaves of the flowering shoots are shorter but of a similar width. The flowering stems are up to 10-30 cm in length, are only 1mm wide and branched. The style and stigma are of the same length. Outside of the UK the species has been identified and recorded as *Z. angustifolia* only in Denmark and Sweden. In British waters it is found on mud banks, creeks and estuaries from half-tide to LWS, or rarely to 4 m below. The apparently restricted distribution of this species may be a consequence of taxonomic problems, i.e., identification as either *Z. angustifolia* or *Z. marina* (Cleator 1993; Stace 2001).

Z. noltii (Figures 1.3 &1.4) is the smallest British seagrass. Non-flowering shoots are 6-



Figure 1.2: Distribution of a) Zostera marina, b) Z. marina var. angustifolia, and c) Z. noltii, in Scotland: all records to 1993. (from Cleator 1993)

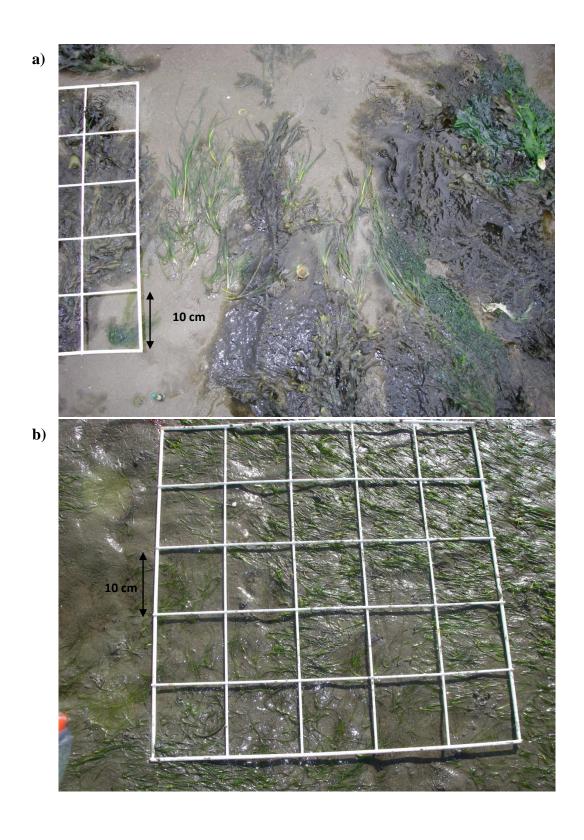
22 cm long and 0.5-1.5 mm wide. The apex of the leaves is emarginated. The flowering stems are un-divided or rarely divided at the base. In Britain *Z. noltii* is found in similar habitats and has a similar distribution to intertidal *Z. marina*. It has a more southerly distribution than *Z. marina* and is restricted to the Atlantic, extending from southern Norway to the tropic of Cancer (Cleator 1993; Stace 2001). Although the status of *Z. noltii* as a true species is accepted (Cleator 1993), like *Z. marina* it is morphologically variable, though characters such as leaf length and width are typically constant within individual populations (Tubbs 1995).



**Figure 1.3**: Leaf-apices of a) *Zostera marina,* b) *Z. marina* var. *angustifolia,* and c) *Z. noltii* (from Stace 2001).

Eelgrasses can be ecologically plastic, and populations of *Z. marina* and *Z. noltii* can be found living across a range of habitat variations. Where eelgrass meadows comprise both *Z. marina* and *Z. noltii*, the two species form a mosaic as *Z. noltii* out-competes *Z. marina* on firmer sediments towards the upshore limits of the beds and on patches of firmer mud. However, on firm, organic sand, *Z. noltii* may occur at a lower shore level than *Z. marina*, extending downshore to LWS (Tubbs 1995).

As it is for land plants, the substantial plasticity of growth programme and architecture in eelgrass species is an important component of their capacity to adapt to stress and heterogeneity in the environment (Hemminga & Duarte 2000; Tubbs 1995).



**Figure 1.4:** The intertidal seagrasses a) *Zostera marina* var. *angustifolia* and b) *Z. noltii* found growing at Tayport in the Tay estuary, Scotland. Each quadrat measures  $0.5 \text{ m} \times 0.5 \text{ m}$ , thus each grid square measures  $0.1 \text{ m} \times 0.1 \text{ m}$ .

For example, horizontal rhizomes have an exploratory function, allowing the extension of plant into new areas, while vertical rhizomes have the ability to deploy leaves and roots to utilise resources. Consequently, changes in architecture and growth may influence both the exploratory and the resource use potential of the plant (Hemminga & Duarte 2000). Seagrass species often have a root-rhizome:shoot ratio (RSR) >1. This morphology is efficient as physical forces caused by waves can remove above-sediment biomass. The roots of aquatic vascular plants are functional in terms of nutrient uptake, therefore a large root biomass and surface area increases nutrition from the sediments. High rhizomal growth provides storage for nutrients and photosynthetic products, and protection against loss from wave action and grazers, conserving energy and nutrients within the beds (Stevenson 1988).

There has been an ongoing general decline of *Zostera* populations since the 1960s (den Hartog & Polderman 1975). Scottish populations may be threatened by a number of factors including eutrophication and coastal development (Cleator 1993).

# 1.2.3 Seagrass in the Tay estuary

The Tay estuary contains a number of important biological features including mixedbeds of the interidal seagrasses *Zostera marina* and *Z. noltii* priority habitat (SNH 2006). The range of eelgrass growing in the Tay estuary has remained unchanged in the last century. As of 2002, the western-most eelgrass beds were at Tayport Beach on the south shore of the outer estuary, and at Broughty Ferry on the north shore. A comparison of data from surveys by Alexander *et al.* in the 1930s (Alexander at al. 1935), Khayrallah and Jones in 1973 (Khayrallah & Jones 1975), a broad scale habitat mapping survey undertaken in the summer of 2002 for Scottish Natural Heritage (Bates *et al.* 2003), and Botanical Society of the British Isles (BSBI) records from 1835 to 1987 show that *Zostera* has never been recorded further into the estuary. Historical texts about the surrounding area (e.g. Wilson, 1910) and the anecdotal evidence of local people (*pers. comms.*) suggest that the species have declined within their former ranges,

with beds becoming fragmented and less dense. However, no dedicated survey or mapping of the species distribution has ever been carried out.

# 1.2.4 Causes of decline in seagrass species

Globally, seagrass beds cover an estimated 177,000 km² (Green & Short 2003); however, this area has declined by 29% since the 19<sup>th</sup> century, and in the last 40 years the rate of loss is estimated to have increased by an order of magnitude (Waycott *et al.* 2009). *Zostera* species in the UK are considered to be scarce (JNCC 1995), and Scottish seagrass populations are threatened by several factors. Populations may be affected by pollution and eutrophication, fishing activity and coastal development. These pressures are likely to pose a more serious threat to the east coast, mixed communities of *Z. marina* and *Z. noltii* due to high population densities around estuaries. On the west coast, the sublittoral populations of *Z. marina* have declined since the 1930's when a wasting disease devastated *Z. marina* populations throughout the Atlantic (Cleator 1993).

#### Natural declines

In the early 1930s an almost simultaneous breakdown of *Z. marina* populations occurred on both sides of the North Atlantic due to the "wasting disease" (den Hartog 1987; Tubbs 1995). Wasting disease was first reported in 1930, on the east coast of North America where beds of sublittoral *Z. marina* were rapidly lost from Virginia to the Gulf of St. Lawrence. In 1932 a similar disease was reported at Roscoff, Brittany, in The Netherlands and in Britain, and by the mid 1940s the decline of *Z. marina* was widespread around the coast of Britain (Tubbs 1995).

The symptoms of the disease are quite characteristic. Small brown spots develop on the leaves; the spots darken in colour as they spread longitudinally, to cover the entire leaf after a few weeks; the leaves ultimately become detached from the rhizome leading to defoliation (den Hartog 1987; Hemminga & Duarte 2000). Discoloration can also be

seen in the rhizome, although this part of the plant can survive for up to a year before repeated denudation weakens the plant and leads finally to death (den Hartog 1987).

The cause of wasting disease has never conclusively been determined, however, the slime mold *Labyrinthula zosterae*, an infectious protist present in eelgrass meadows as a secondary decomposer of aged planet material, is consistently associated with the disease (den Hartog 1987; Muehlstein 1989, 1992; Muehlstein et al. 1988, 1991; Tubbs 1995; Vergeer & den Hartog 1991). Under normal conditions L. zosterae is inconspicuous in eelgrass meadows; therefore wasting disease seems to be a widespread endemic phenomenon (den Hartog, 1987). It is now widely believed that the pathogenic function of the slime mold may be induced under conditions of environmental stress, either natural or anthropogenic, when plants are more vulnerable to infection (den Hartog 1987; Short et al. 1988; Tubbs 1995; Vergeer & den Hartog 1991). Adverse conditions such as increased salinity, raised water temperatures during the growing season, heavy cloud cover or increased turbidity cause deterioration of the plants and make them susceptible to pathogens and secondary decomposers (Burdick et al. 1993; Short et al. 1988). For example, increased water turbidity and the resulting low irradiance levels in the Dutch Wadden Sea during 1931 and 1932, after the completion of the Afsluitdijk and the closure of the Zuyder Zee, may have made Z. marina vulnerable to the disease, and the region's subtidal meadows were lost (den Hartog & Polderman 1975). Burdick et al. (1993) have suggested the quantity of lesions caused by Labyrinthula can be used as an indicator of environmental stress.

Many scientists rejected slime mold as the primary cause, attributing the decline to localised causes such as human-induced pollution or siltation; climatic factors, citing historic declines correlating with periods of both drought and flooding; and with high summer water temperatures coupled with mild winters (den Hartog 1987; Hemminga & Duarte 2000). However, none of these has been able to explain all the situations suggested. Prior to the 1930s little knowledge had been collated in the form of distribution maps or reports on the status of *Z. marina* communities, and this may be why there were so many hypotheses for the sudden dramatic decline (den Hartog 1987).

den Hartog and Polderman (1975) noted that in 1932 when wasting disease reached the Netherlands, only the sublittoral *Z. marina* populations were destroyed, while eulittoral populations of *Z. marina* and *Z. noltii* were unaffected. Vergeer and den Hartog (1991) have since found evidence of *Labyrinthula zosterae* in herbarium specimens dating from the mid-nineteenth century onwards, and in samples collected from locations along the coast of western Europe in 1989 and 1990. They observed that it can cause similar damage to the leaves of *Z. noltii*, although a similar large-scale deterioration of *Z. noltii* has not yet been recorded. Durako and Kuss (1994) have also observed symptoms of wasting disease in *Thalassia testudinum* in tropical waters, caused by a species of *Labyrinthula*. A strain of the disease has been linked to a decline of seagrass in Florida Bay in the late 1980s (Hemminga & Duarte 2000).

After 1935 seagrass beds began to slowly recover (den Hartog & Polderman 1975). Since the main outbreak, other recurrences of the disease, although more restricted spatially and temporally, have been observed in eelgrass meadows along the eastern coast of the USA (Short *et al.* 1988), and in the Exe estuary and Solent Harbour of England (Tubbs 1995).

The decline of seagrass beds could also be part of a natural cycle, as the formation and breakdown of seagrass beds recurs over years or decades (den Hartog 1987). Along the coasts of northwest Europe, intertidal *Z. marina* beds typically display an annual cycle. After the growing season vegetation is lost due to grazing by waterfowl, mortality through low temperatures and frost damage, or the scouring and erosion of beds by floating ice (Hemminga & Duarte 2000). The annual growth cycle corresponds with seasonal cycles of sedimentation, when leaves are present and sediment erosion when the surface is exposed during the winter. The balance of sedimentation and erosion throughout the year maintain the constant elevation of the beds within the tidal range (Koch 1999).

# Declines due to human activity

Seagrass researchers have noted that since the 1960s there has been an ongoing general decline of Zostera populations, and some consider these declines to be a consequence of the increasing pollution and nutrient loading (e.g. den Hartog & Polderman 1975; McGlathery et al. 2007; Ducrotoy 1999). In the last few decades massive nutrient loading and diffusive runoff from urbanised areas and extensively cultivated land have led to the increased eutrophication of the world's estuaries (Flindt et al. 1999; McGlathery 2001; Short & Burdick 1995). Water exchange is limited in estuaries and coastal lagoons (Herbert 1999) so in comparison to other aquatic systems, estuaries are among the most eutrophic ecosystems (Flindt et al. 1999). Increased nutrient loading has serious consequences for the ecology of estuaries as primary production is shifted from the dominance of rooted macrophytes to ephemeral macroalgae and phytoplankton (Sand-Jensen & Borum 1991; Duarte 1995). Seagrass shoot density and biomass, and the depth to which is can grow is light-limited (Duarte 1991; Hemminga & Duarte 2000; Zimmerman 2006) and the algae reduce the light available for seagrass photosynthesis (Hemminga & Duarte 2000; Brun et al. 2003). Changes in plant community composition consequently impact on heterotrophic organisms that are specialised to live on certain primary producers, or in the environment they provide (Flindt et al. 1999; Sand-Jensen & Borum 1991).

Increased suspended sediment concentrations also raise water turbidity, attenuating light within the water column. Human activities within the catchment, such as deforestation to provide land for agriculture and living space, can cause high rates of soil erosion, increasing riverine sediment transport (Hemminga & Duarte 2000). Dredging, an operation carried out in the Tay to maintain shipping channels, can also increase water turbidity (Onuf 1994; Erftemeijer & Lewis 2006). High sediment loads may also increase deposition, causing shoot mortality. Large short-term accretion/depositional events in seagrass beds can result in relatively sizeable sediment depth fluctuations, with too high levels of sediment deposition having negative effects on seagrasses (Mills & Fonseca 2003; Cabaço & Santos 2007). Smaller seagrass species, such as *Z. noltii*, are unable to withstand even low levels of burial (Cabaço *et al.* 2008).

Dredging (Onuf 1994; Erftemeijer & Lewis 2006), boat moorings and propellers (Burdick & Short 1999), and fishing techniques such as bottom trawling (Gillanders 2006), that disturb bottom sediments all have the potential to damage seagrass shoots and rhizomes, leading to the reduction of seagrass cover. The direct loss of seagrass habitat may also be caused by the construction of coastal structures such as jetties, piers, harbours or sea defences (Hemminga & Duarte 2000; Walker *et al.* 2006; SNH 2006).

# 1.3 Aims of the thesis

Seagrass research is carried out in coastal waters throughout the world. In temperate waters much of this work is done in France, Denmark, the Netherlands, and the east coast of the USA. However, very little seagrass research has been carried out in the UK or Scotland. In addition, most studies of *Zostera* have focussed on aspects of sub-tidal *Z. marina* ecology, possibly because it is the most abundant and widespread seagrass in northern temperate oceans. Relatively few studies have been carried out on *Z. noltii* or intertidal *Z. marina*, the two species present in the Tay estuary. Studies in the estuary have generally focussed on physical processes such as salinity and currents, and the main bodies of this work were published in the 1970s and 1980s by the Royal Society of Edinburgh.

This study provides an insight into the ecology of seagrass in the Tay estuary and its role as an ecosystem manager. It may offer valuable data which could be utilised for future conservation policies, habitat restoration schemes and management planning of the area.

#### Chapter 2

Previous biological surveys in the estuary have investigated the types and distribution of all animal and plant life (Alexander *et al.* 1935; Khayrallah & Jones 1975), and a recent SNH survey of sediment biotopes (Bates *et al.* 2003) was based on satellite imagery,

with limited ground-truthing. The Botanical Society of the British Isles have no records for *Zostera* in the estuary after 1987, no terrestrial-based macrophyte survey has been carried out since 1973 and no dedicated *Zostera* survey has ever been carried out in the Tay estuary. In Chapter 2 the distribution of *Zostera* spp. in the Tay estuary will be determined, and the physical characteristics of its sediment environment will be explored.

# Chapter 3

Sedimentation in estuaries is a continuous process regulated by sediment inputs from both internal and external sources (McManus 1998; Little 2000), and controlled by prevailing atmospheric and hydrodynamic forces (Dyer 1997; Quaresma *et al.* 2007). Short-term studies of annual cycles of sedimentation have demonstrated that on tidal flats there are phases of accretion, mainly during spring and summer, but that surface levels may also erode during periods of high winds and in winter (e.g. McManus & Alizai 1987; Andersen & Pejrup 2001; Marion *et al.* 2009). Seagrasses stabilise their sediment environment by altering the hydrodynamic conditions near the sediment surface, dissipating flow energy (Gambi *et al.* 1990; Widdows *et al.* 2008) and increasing sediment deposition and retention (Terrados & Duarte 2000; Bouma *et al.* 2008). In Chapter 3 the role of the intertidal seagrasses *Z. noltii* and *Z. marina* var. *angustifolia* in sediment accretion in the Tay Estuary will be explored.

# Chapter 4

In Chapter 4 the relative particle trapping ability of *Z. noltii* will be examined in an 8 m seawater flume in the laboratory, and *in situ* in the Tay estuary. The influence of *Z. noltii* on water flow will be explored in the seawater flume. The physical conditions causing the deposition and retention of sediment particles will be considered for conditions present in *Z. noltii* beds in the Tay estuary.

# Chapter 5

Sediment stabilisation is influenced at a microscopic scale by microphytobenthos, in the form of biofilms and microbial mats (Holland *et al.* 1974; Dade *et al.* 1990; Paterson 1997). The microorganisms, such as diatoms and cyanobacteria, that comprise sediment biofilms, secrete mucilaginous "Extracellular Polymeric Substances" (EPS) (Underwood *et al.* 1995; de Winder *et al.* 1999). EPS forms a cohesive matrix surrounding the particles of intertidal sediments or a coating over plant surfaces to which sediment particles may adhere (Decho 2000). In Chapter 5 the adhesive properties of sediment and leaf biofilms present in the Tay Estuary will be explored using magnetic particle induction (MagPI) (Larson *et al.* 2009).

# Chapter 6

In Great Britain 25% of our natural intertidal areas, including saltmarsh, have been lost in the last century (Doody 2008) and over 67% of the eastern coastline has shown a landward retreat of the low-water mark (Taylor *et al.* 2004). These losses are due to both human activities (Hughes 2001), and to erosion by high tides and increased wave height (Hughes & Paramor 2004; Wolters *et al.* 2005). As the requirement for sustainable coastal defence increases, coastal managers are seeking cost-effective and relatively fast-acting ways to restore marshes where losses have taken place, or reduce erosion in front of marshes. In Chapter 6 the role of *Z. noltii* as an ecosystem engineer, increasing deposition in front of saltmarsh habitat, will be investigated. The efficacy of artificial seagrass beds and transplantation of *Z. noltii* as habitat restoration techniques will be compared in the Tay Estuary.

# 1.4 Study site

After carrying out an initial survey of the distribution of *Zostera* spp. in the Tay estuary, sampling and experimental work was carried out on the shore between Tayport (56°26'N 02°52'W) and Tentsmuir forest (56°26'N 02°51'W), on the southern shore of

the estuary mouth. The shore comprises wide intertidal muddy sand flats. Towards Tentsmuir, on the eastern side of the site, the upper shore is fringed by saltmarsh. At Tayport however, a seawall was built circa 1950 (Tayport Town Council 1957), "reclaiming" a large piece of saltmarsh, which is now playing fields and a caravan park, and causing the loss of a large area of intertidal habitat. The sea defences are now failing in some places (Figure 1.5) and Fife Council is considering remediation to prevent further seaward erosion (Fife Council 2011).



**Figure 1.5**: Eroded sea defences behind a sparse meadow of *Z. noltii* at Tayport on the southern shore the Tay estuary, Scotland.

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#### **Additional data**

Botanical Society of the British Isles

# **Chapter 2**

# The status of *Zostera* Spp. in the Tay estuary

# **Abstract**

The Tay Estuary contains a number of important biological features, including beds of the nationally scarce intertidal seagrasses *Zostera marina* var. *angustifolia* (Hornem.) and *Z. noltii* (Hornem.). However, no dedicated survey mapping of the species has previously been carried out in the Tay to determine the distribution of these species. Therefore, in the summer of 2008, a survey of intertidal seagrasses was carried out and the influence that seagrasses in the Tay have on their sediment environment was explored.

Around 3 ha of *Z. noltii* was recorded growing high up on the shoreline at Tayport. It was found at sites with median grain size (151  $\mu$ m, IQR: 129  $\mu$ m, 177  $\mu$ m) with a high silt fraction. Around 1 ha of *Z. marina* was found growing on poorly drained fine sand (median grain size 222  $\mu$ m, IQR: 189  $\mu$ m, 280  $\mu$ m) containing a high medium sand content. Substratum type and tidal inundation/exposure time were the primary factors influencing the distribution of these two species on the shoreline. The sorting of sediment grains appeared to be governed by the hydrodynamic conditions within the estuary, rather than the presence of seagrass shoots.

The presence of *Z. noltii* increased sediment organic content of the sediment, leading to a decrease in redox potential. This effect was not evident in *Z. marina* beds, however the low sample size may have led to results unrepresentative of the community. Carbohydrate concentration was higher in vegetated *Z. noltii* plots and bare sediment within *Z. noltii* patches, than in bare sediment outside patches, suggesting the increased pool of organic matter leads to higher microbial decomposition.

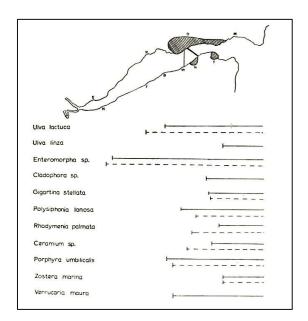
Seagrasses can be ecologically plastic and populations of *Z. marina* and *Z. noltii* can be found living across a range of habitats, as demonstrated by physical differences between sites containing the same species. Although some habitat requirements are satisfied by hydrodynamic and topographical conditions of the site, seagrasses also modify their physical environment to some degree.

This study has provided a basic overview of the environmental variables the grasses prefer in the Tay – to what extent are these conditions are present already, or whether they are partially due to ecosystem engineering by the seagrass.

#### 2.1 Introduction

# 2.1.1 The distribution of seagrass in the Tay estuary

The Tay estuary is dominated by sediment biotopes, and contains a number of important biological features including sparse beds of the nationally scarce intertidal seagrasses *Zostera marina* var. *angustifolia* (Hornem.) and *Z. noltii* (Hornem.) - a priority habitat (Bates *et al.* 2003; SNH 2006). *Z. noltii* abundance has been positively related to sediment clay content (Phillippart & Dijkema 1995) and it typically grows on the upper shore on firm, organic sediments (Tubbs 1995), and often fringing saltmarsh communities (Davison & Hughes 1998). Intertidal *Z. marina* is found on the mid to lower shore on poorly draining muddy fine sand (Davison & Hughes 1998) that retains water at low tide (Wyer *et al.* 1977).



**Figure 2.1**: Penetration of the estuary by Chlorophyceae, Rhodophyceae, Angiosperms and Lichens in 1973 (——) and 1935 (- - - -) (from Khayrallah and Jones, 1975).

Zostera spp. occur on shores sheltered from strong current and tides (Davison & Hughes 1998). The range of seagrass growing in the Tay estuary has remained unchanged in the last century (Figure 2.1), occurring only at Tayport beach on the south shore of the outer estuary. A comparison of data from habitat surveys by Alexander *et* 

al. in the 1930s (Alexander at al. 1935), Khayrallah and Jones in 1973 (Khayrallah & Jones 1975), a broad scale habitat mapping survey undertaken in the summer of 2002 for Scottish Natural Heritage (Bates et al. 2003), and the Botanical Society of the British Isles (BSBI) records from 1835 to 1987 show that Zostera has never been recorded further into the estuary. Historical texts about the surrounding area (e.g. Wilson, 1910) and the anecdotal evidence of local people (pers. comms.) suggest that the species have declined within their former ranges, with beds becoming fragmented and less dense. However, no dedicated survey or mapping of seagrass distribution has ever been carried out in the Tay.

# 2.1.2 Physical effects of intertidal seagrass on sediments

Seagrass communities are widely regarded as key habitats, influencing the structure and function of coastal ecosystems. Seagrasses have been described as ecosystem engineers (Hemminga *et al.* 1991; Terrados & Duarte 2000; Gacia & Duarte 2001; Bouma *et al.* 2008), modifying their environment to render it more suitable to their requirements (Jones *et al.* 1997). The physical and chemical effects of the community on water movement and sediment characteristics have a number of impacts on the local environment.

Seagrasses trap fine grained suspended particles by altering water movement within the meadow (Agawin & Duarte 2002; Fonseca *et al.* 1982; Gacia & Duarte 2001; Ward *et al.* 1984), and the sediment environment is stabilised by increased sediment deposition and retention (Hemminga *et al.* 1991; Terrados & Duarte 2000; Gacia & Duarte 2001; Bouma *et al.* 2008). The deposition and accumulation of organic matter and seagrass detritus within the meadow increases the pool of organic substrate available for microbial decomposition compared to unvegetated areas (Gacia & Duarte 2001; Marbà *et al.* 2006). In dense meadows a large proportion of detrital matter is retained within the canopy, providing a source of organic particles (Hemminga & Duarte 2000; Mateo *et al.* 2006). Compared to macroalgae detritus and other substrates for bacterial communities, organic matter derived from the decomposition of eelgrass is of minor importance in some temperate waters in north western Europe (Boschker *et al.* 2000);

however, seagrass also supplies organic matter to the sediment pore water directly through root excretion (Blaabjerg & Finster 1998; Hansen *et al.* 2000). The inputs of organic matter from and within seagrass meadows, and the export of detrital material, such as dead leaf particles, to nearby habitats, provide a substrate to benthic bacterial communities which promote carbon and nitrogen mineralisation in aquatic ecosystems (Boschker *et al.* 2000).

Where the oxic layer is only a few millimetres thick, aerobic mineralisation of organic matter is restricted to the sediment surface (Vichkovitten & Holmer 2005). The plants are rooted in the sediment and aerate its upper horizons, improving the mud as habitat for benthic infauna (Tubbs 1995). Oxygen produced by eelgrass during photosynthesis is translocated, through the lacunar system, to the below-ground tissues, where it can diffuse into the surrounding sediments. This allows oxygen to penetrate deeper into the sediment, increasing the thickness of the oxic layer and the depth of the oxic/anoxic boundary layer (Vichkovitten & Holmer 2005). Conversely, the presence of seagrass can also lead to a lower sediment redox potential in comparison to non-vegetated sediments, due to respiration in the sediments driven by organic inputs (Neubauer *et al.* 2004; Vichkovitten & Holmer 2005).

Seagrass leaves, and their epiphytic communities, have the ability to remove nutrients and contaminants from the water column, maintaining water quality for neighbouring benthic communities and acting as a buffer to eutrophication (Cornelisen & Thomas 2002; de Wit et al. 2001; Stapel et al. 1996; Thomas et al. 2000). In addition to promoting the deposition of suspended organic material, the slowing of flow may also be important for decreasing nutrient losses from the system: nutrients diffusing from the sediment to the overlying water column, or released from decomposing organic matter produced by seagrass leaves and by other primary producers, such as epiphytes and benthic algae, are recaptured through a combination of decreased advection and the physical canopy acting as a barrier (Hemminga et al. 1991; Pedersen & Borum 1993; Burkholder et al. 2007).

While predictions may be made about effects of seagrasses on their sediment environment, it must be noted that species occupy different ecological niches, e.g.

positions on the shoreline. These areas are subject to different physical stressors. Thus, differences between study sites may be due to their position on the shoreline, even within species.

#### 2.1.3 Aims

The aims of this work were to ascertain where *Zostera* spp. occurs within the Tay Estuary, and to explore the influence that the seagrass has on its sediment environment. Comparisons were made between sediments where *Zostera* is growing at Tayport, and sediments where it is not. This study will provide an insight into the ecology of *Zostera* in the Tay estuary. Furthermore, the data will be used in the design of experiments within the scope of this thesis.

The following hypothesis will be tested:

H<sub>1</sub> Intertidal seagrasses significantly modify their physical sediment environment.

#### 2.2 Materials and Methods

# 2.2.1 Full survey of intertidal Zostera spp. and mapping of Zostera beds

In June 2008 a survey of the distribution of *Zostera marina* and *Z. noltii* meadow priority habitat was carried out in the intertidal zones of the outer Tay estuary from Barry Sands in the north, to Dundee in the West and to Tentsmuir Sands in the south. Visits were also made to Mugdrum Island and Kingoodie in the inner estuary, where sediment habitats are present. Survey areas were divided into 500 x 500 m squares. Two 500 m transects of each square were walked, and presence or absence of *Zostera* was recorded. The OSPAR Convention stipulates that a habitat must extend over an area of at least 25 m<sup>2</sup> for it to be recorded at a site; however, as *Zostera* occurs in

patches, plant densities providing at least 5% cover qualify as *Zostera* beds (OSPAR 2008). Thus, in addition to recording *Zostera* beds, individual patches of less than 25 m<sup>2</sup> were also noted.

Between 16<sup>th</sup> June and 25<sup>th</sup> July 2008 the density of *Zostera* within beds was recorded at intervals of 5 m, along transects 5 m apart. The number of shoots was counted using a 0.25 m<sup>2</sup> quadrat separated into 0.1 m divisions (25 squares). The location of each quadrat was recorded using GPS. Data were mapped using ArcMap. The distribution maps were compared to the records of *Z. marina* penetration into the estuary from 1935 and 1973 (Khayrallah & Jones 1975) and to the SNH 2002 broad-scale biotope mapping exercise (Bates *et al.* 2003). As well as providing important biological monitoring data, this survey and mapping exercise will be used to plan future work within the scope of this thesis.

# 2.2.2 Sediment sampling

The study sites were at Tayport in the Tay Estuary, Scotland. Sampling took place at six *Zostera* beds, two of *Z. marina* and four of *Z. noltii*, on Tayport sands between 11<sup>th</sup> and 25<sup>th</sup> August 2008 (Figure 2.3). Sampling at each site commenced as the tide went off and continued for no longer than two hours. At each bed, three replicates of three conditions were selected: sediment where *Zostera* is growing; bare sediment within the bed, with an area of at least 1m<sup>2</sup>; and bare sediment control plots 2 m outside the bed. Using the survey data, *Zostera* treatments were chosen at random from 0.25 m<sup>2</sup> quadrats with shoot densities of ~600 shoots m<sup>-2</sup> for *Z. noltii* and ~100 shoots m<sup>-2</sup> for *Z. marina*, the mean shoot densities for these species (see section 2.3.1).

At each plot the upper 2 mm of sediment was sampled using a contact core, as described below. Three contact cores were taken at each sample plot. Samples were analysed for water concentration, wet bulk density, organic concentration, colloidal-S carbohydrate concentration, chlorophyll *a* concentration, and grain size distribution.

# Contact coring

The contact corer (Ford & Honeywill 2002; Consalvey & van Leeuwe 2005) consists of a metal dish of 44 mm diameter comprising a hollow metal dish (15 mm deep) which is filled with liquid nitrogen. The base of the dish has a 2 mm skirt which penetrates the sediment (Figure 2.2). The contact corer was placed on the sediment, and gently pushed downwards until the base of the dish touched the sediment surface. Any *Zostera* leaves were carefully moved out the way beforehand using forceps, and taking care not to disturb the underlying sediment. Liquid nitrogen was poured into the dish and left for 15 to 30 s (depending on the sediment water content) until the surface of the sediment under the dish (the sample) became frozen.



**Figure 2.2**: Two contact corers. The left hand contact corer is upside down showing the sample skirt area. The right hand core is upright and shows the well for liquid nitrogen.

The contact corer was then removed from the sediment complete with the underlying frozen sediment. A knife was used to scrape across the base of the core to leave a flat disc of sediment as defined by the depth of the skirt (2 mm), although thicker samples were sometimes taken where the freezing was more rapid than expected. Additional sediment frozen around the edge of the corer was also removed. The frozen disc of sediment was released from the contact core using the tip of the knife and was wrapped

in labelled foil and stored immediately in liquid nitrogen, before transfer to a -80  $^{\circ}$ C freezer. The contact core had an area of 15.2 cm<sup>2</sup>, and sediment disc depths varied between 2.3 mm and 5.5 mm. Measurements of sediment properties (water content, organic content, bulk density, carbohydrate content and chlorophyll a) were taken from the cores. Samples to undergo chlorophyll a analysis were kept in the dark at all stages.

#### Water content

The weights of the frozen sediment disc, in a pre-weighed and labelled plastic bag, were recorded before and after freeze-drying (lyophilisation) until the samples were completely dry (approx. 24 h). Water content was determined as a percentage of the wet sediment weight (Equation 2.1) (Consalvey 2005). Sediments were lyophilised, rather than oven-dried, to avoid the breakdown of thermosensitive pigments (Rowan 1989). Dried samples were sealed and stored in the dark at -80 °C.

% water content = 
$$\left(\frac{Wtwet-Wtdry}{Wtwet}\right) * 100$$
 $Wt_{wet}$  = wet sediment mass (g)

 $Wt_{dry}$  = dry sediment mass (g)

# Wet bulk density

The wet bulk density was determined as the weight of wet sample per cm<sup>3</sup> (Equation 2.2) (Consalvey 2005).

bulk density (gcm<sup>3</sup>) = 
$$\frac{\text{wet sediment (g)}}{\text{volume of sediment (cm}^3)}$$
 (Equation 2.2)

# Organic content

Organic content of sediment was determined using the loss on ignition method (Consalvey 2005). Lyophilised samples of a known weight (1.86 g  $\pm$  0.04) were ground to a fine powder and placed in pre-weighed crucibles. The samples were combusted in a muffle furnace for 4 h at 450 °C, after which they were placed in a desiccator and allowed to cool to room temperature. The samples were reweighed (minimising exposure to atmospheric humidity) and the organic content was determined (Equation 2.3).

(Equation 2.3)

% organic content = 
$$\left( \left( \frac{\text{wt before combustion-wt after combusttion (g)}}{\text{wt before combustion (g)}} \right) * 100 \right)$$

### Pigment analysis (Chlorophyll a)

To extract chlorophyll a, 1 ml of 90% acetone was added to a known quantity ( $\sim 0.1$  g) of lyophilised sediment. Extraction was carried out in the dark, in a -80 °C freezer for 48 h. Sediment was separated from the solvent prior to HPLC analysis, by filtration through a 0.2  $\mu$ m pore syringe filter (Whatman<sup>TM</sup>).

Chlorophyll a content was analysed using an HPLC. Sample extractions were injected into a tertiary solvent gradient at a flow rate of 1.0 ml min<sup>-1</sup> (Wiltshire  $et\ al.\ 2000$ ). Each sample produced a chromatogram at an excitation wavelength of 430 nm. The retention time of the pigment on the column and the amount of pigment leaving the column determines the intensity of the signal (peak) produced by the detector. Four chlorophyll a standards were run with each set of samples: 0.5, 1, 2, and 4 mg l<sup>-1</sup>. These four concentrations were used to make a standard curve, from which chlorophyll a concentrations of sample extracts were calculated using regression analysis (Equations 2.4 to 2.6).

(Equation 2.4)

Chlorophyll a (mg  $l^{-1}$ ) = (<u>peak area – intercept</u>) \* dilution factor gradient

(Equation 2.5)

Chlorophyll a (µg chlorophyll a / g dry sediment) =

((Equation 2.4 \* (<u>dilution factor</u>) / sample weight) \* 1000 1000

(Equation 2.6)

Chlorophyll a (µg cm<sup>-2</sup>) = ((Equation 2.5 \* (<u>sample dry weight</u>) \* 1000 surface area

#### Sediment grain size

Grain size analysis was carried out using a Coulter Laser Particle Sizer (LS230). This method determines the particle size distribution through laser diffraction. The sample (~5 g) was added to the sampling chamber and sonicated for 30 s before the measurement was taken. Each sample was measured three times. Classification of the sediment samples was done after the Wentworth Scale (1922).

# Redox potential

Root density was highest in the 1 to 4 cm layer (Isaksen & Finster 1996) and the mean rhizome depths in the Tay species were determined as 23 mm  $\pm$  0.1 SE (n = 30) for *Z. noltii* and 23 mm  $\pm$  0.2 SE (n = 30) for *Z. marina* (pers. comms.), thus measurements were focused at this depth. At plots 1, and 3 to 6, three measurements of salinity and redox were taken at ~ 2cm depth using a Thermo Scientific Orion 5 Star meter: these variables could not be measured at site 2 due to equipment failure.

# 2.2.3 Statistical Analysis

The statistical software used was GenStat 12.1 and Minitab 16.

The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. Data for redox potential, wet bulk density, organic content and colloidal carbohydrate content were log transformed, and data for water content were reciprocally transformed to normalise these data. Statistical analysis was carried out using a two-way ANOVA (Analysis of an unbalanced design using GenStat regression), where the factors were species and plot type. Further differences between groups were explored using a one-way ANOVA, where the factor was site, and *post hoc* two-sample t-tests. Results were considered significant at p = 0.05.

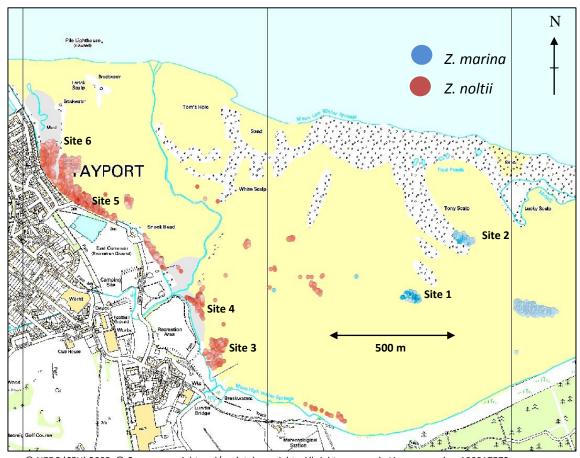
Sediment grain size data were not normally distributed and could not be transformed, and so were analysed using a non-parametric Kruskal-Wallis test. Further differences between groups were explored using *post hoc* Mann-Whitney tests. Results were considered significant at p = 0.05.

#### 2.3 Results

# 2.3.1 Full survey of intertidal Zostera spp. and mapping of Zostera beds

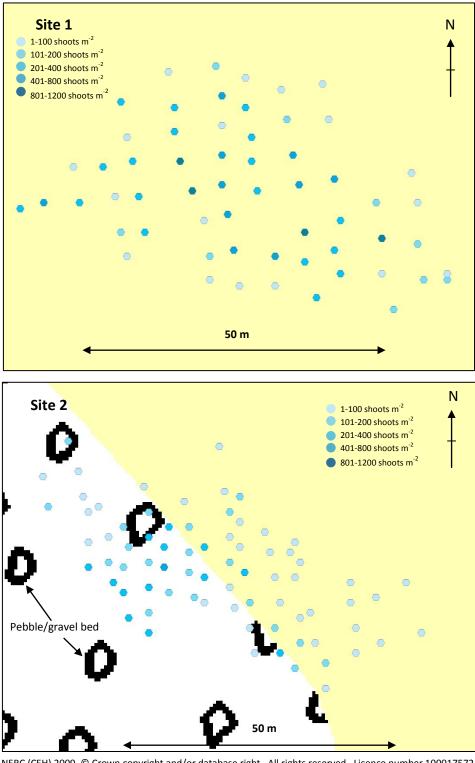
Beds and patches of Z. marina and Z. noltii were found only on mudflats near Tayport (Figure 2.3). Around 1 ha of Z. marina and  $\sim 3$  ha of Z. noltii were recorded. The densest beds of Z. marina were found on the mid- to lower shore, between 250 m to 700 m from the shoreline. Z. noltii was present from the upper shore to 150 m from the shoreline, although some isolated small patches occurred up to 600 m from the shoreline. Z. marina may have been under-recorded, as many single fronds were present at low densities on the shore north of Tentsmuir forest. Shoots were counted in 1478 0.25 m<sup>2</sup> quadrats, 411 containing Z. marina and 1079 containing Z. noltii (only 12)

quadrats contained both species). Where *Z. marina* meadows occurred, shoot densities were lowest around the margins of the meadow, and higher towards the centre (Figure 2.4). *Z. marina* shoot densities ranged from 1 to 296 shoots per quadrat (4 to 1184 shoots m<sup>-2</sup>: mean 98 shoots m<sup>-2</sup>  $\pm$  9 SE: median 40 shoots m<sup>-2</sup>, IQR: 16, 94). *Z. noltii* mainly grew in discrete patches, ranging in size from 0.25 m<sup>2</sup> to 25 m<sup>2</sup>, with shoot densities of 1 to 758 shoots per quadrat (4 to 3032 shoots m<sup>-2</sup>: mean 644 shoots  $\pm$  14 SE: median 572 shoots, IQR: 256, 921) (Figure 2.5).



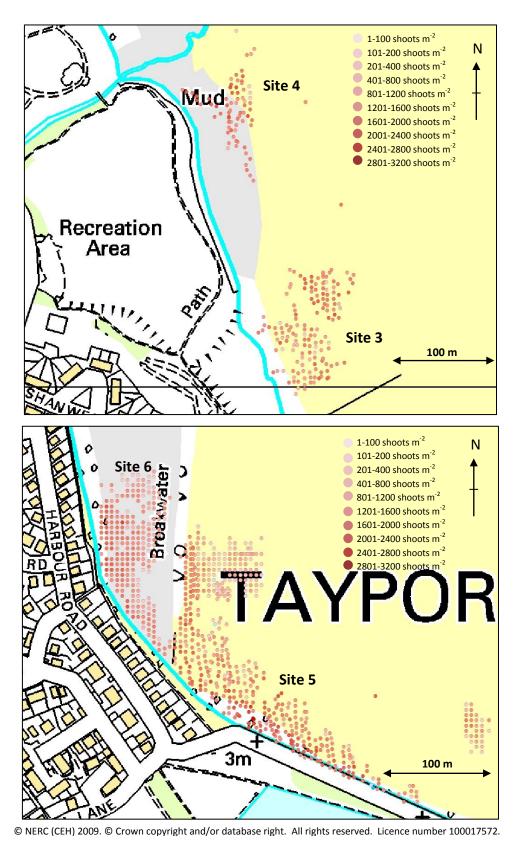
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**Figure 2.3**: Map of the distribution and density of *Zostera marina* and *Z. noltii* in the Tay Estuary, Scotland in 2008. Sampling sites are indicated: *Z. marina* site 1 (central grid reference NT 47800 28525) and site 2 (NT 47580 28290), *Z. noltii* site 3 (NT 46800 28050), site 4 (NT 46710 28260), site 5 (NT 46250 28690) and site 6 (NT 46100 28850).



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**Figure 2.4**: Map of the density of *Zostera marina* at sampling site 1 (central grid reference NT 47800 28525) and site 2 (NT 47580 28290).



**Figure 2.5**: Map of the density of *Zostera noltii* at sampling site 3 (central grid reference (NT 46800 28050), site 4 (NT 46710 28260), site 5 (NT 46250 28690) and site 6 (NT 46100 28850).

The current distribution and western-most limits of *Zostera* spp. habitat was unchanged from the surveys of Alexander *et al.* (1935) and Khayrallah and Jones in (1975). The broad scale habitat mapping survey undertaken in the summer of 2002 for Scottish Natural Heritage (Bates *et al.* 2003) found less coverage of *Z. noltii* at Tayport than current levels, although the distribution was similar. Although Bates *et al.* (2003) recorded *Zostera* growing at Broughty Ferry on the north side of the estuary, there was none present there in 2008.

# 2.3.2 Comparison between the sediments of *Z. marina* and *Z. noltii* beds within the Tay estuary.

Comparisons between plots of the two seagrass species showed a significant difference (two-way ANOVA,  $F_{2,49} = 5.00$ , p = 0.03, n = 53; Table 2.1) in sediment mean wet bulk density, and a highly significant (p < 0.01) difference in mean organic content, water content, and redox potential, and median sediment grain size (Kruskal-Wallis, H = 22.76, p < 0.001, n = 54; Table 2.2). Organic content and water content were lower in the Z. marina sites compared to Z. noltii sites (Figure 2.6). Redox potential was also lower in the Z. marina sites in comparison to Z. noltii, although readings were positive at all sites. The median grain size in Z. marina sites was 222 µm (IQR: 189 µm, 280 μm), while in Z. noltii sites the median grain size was 151 μm (IQR: 129 μm, 177 μm). Higher fractions of medium sand (250-500 µm) occurred in Z. marina plots, with 49.6% (IQR: 46.2%, 52.5%) at site 2 compared to 7.0 % (IQR: 6.6%, 9.2%) at site 3. The highest fractions of silt (20-62.5 µm) were found in Z. noltii sites with 22.9 % (IQR: 18.9%, 26.0%) at site 3 compared to 4.7 % (IQR: 4.2%, 5.1%) at site 2. There was a negative correlation between organic content and grain size ( $r^2 = -0.5644$ ; Figure 2.7), but there were no correlations between other combinations of organic content, redox potential, grain size, wet bulk density, carbohydrate or chlorophyll a content.

When data were separated by species, further analysis showed that vegetated plots of Z. *noltii* had significantly lower redox potential than bare plots both within (two-sample t-test, t = 2.13, p = 0.02, n = 21; Figure 2.6) and outside the patch (two-sample t-test, t = 2.13) and outside the patch (two-sample t-test).

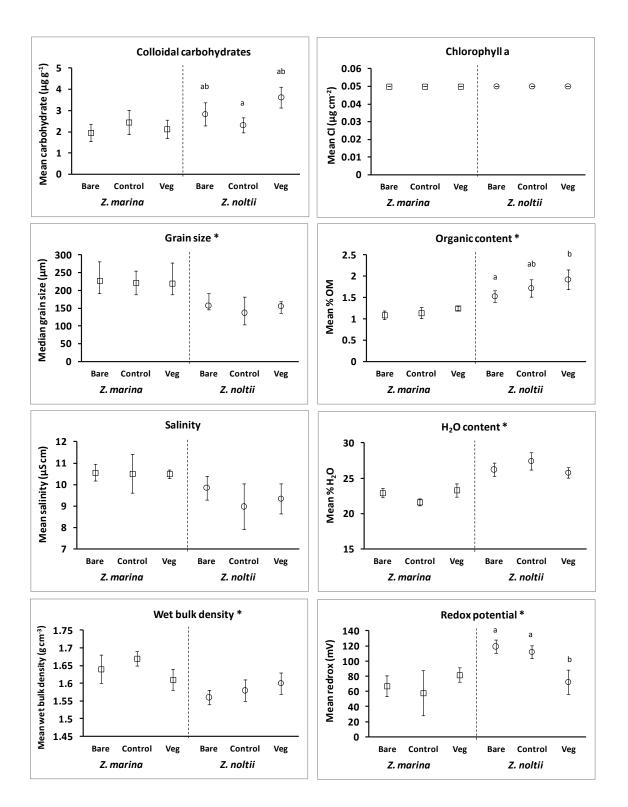
1.68, p = 0.05, n = 21). Z. noltii vegetated sediment had significantly higher colloidal carbohydrate content (two-sample t-test, t = -2.39, p = 0.01, n = 24) than the control plots. Organic content was significantly higher in vegetated plots than in bare ones.

**Table 2.1**: Two-way ANOVA comparing the physical sediment characteristics of sites where *Z. marina* and *Z. noltii* seagrass beds occur in the Tay estuary; and within these sites, comparing plots of vegetated sediment, bare sediment patches within seagrass beds, and bare sediment 2 m outside each bed (control).

					Residual
	Variable	n	F	p	d.f.
0/ organia contant	Diet type	54	1.74	0.185	50
% organic content	Plot type Species	54 54	13.61	<0.183	50 50
	1				
% water content	Plot type	54	0.01	0.994	36
	Species	54	21.73	<0.001	36
Wet bulk density (g cm <sup>-3</sup> )	Plot type	53	0.27	0.767	49
	Species	53	5.00	0.030	49
Chlorophyll (mg l)	Plot type	54	1.11	0.337	50
	Species	54	0.99	0.325	50
Colloidal carbohydrate (µg g)	Plot type	54	1.60	0.211	50
	Species	54	2.44	0.125	50
Redox (mV)	Plot type	42	0.59	0.562	38
	Species	42	12.79	< 0.001	38
Salinity (µS cm)	Plot type	44	0.30	0.745	40
	Species	44	1.27	0.267	40

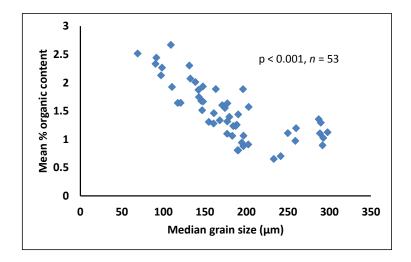
**Table 2.2**: Kruskal-Wallis tests comparing the median sediment grain size ( $\mu$ m) of sites where *Z. marina* and *Z. noltii* seagrass beds occur in the Tay estuary; and within these sites, comparing plots of vegetated sediment, bare sediment patches within seagrass beds, and bare sediment 2 m outside each bed (control).

	Variable		Н		Residual d.f.
	variable	n	П	p	u.1.
Grain size (µm)	Plot type	54	0.96	0.619	2
	Species	54	22.76	< 0.001	1
	Site	54	40.28	<0.001	5



**Figure 2.6:** Comparisons of the physical sediment characteristics in *Z. marina* and *Z. noltii* seagrass beds, bare sediment patches within these beds, and bare sediment 2 m outside each bed (control). Significant differences between species are indicated by \*. Where indicated, data that do not share a letter denote a significant difference between plot types. Standard error bars are shown for all variables except grain size, where the interquartile range is used.

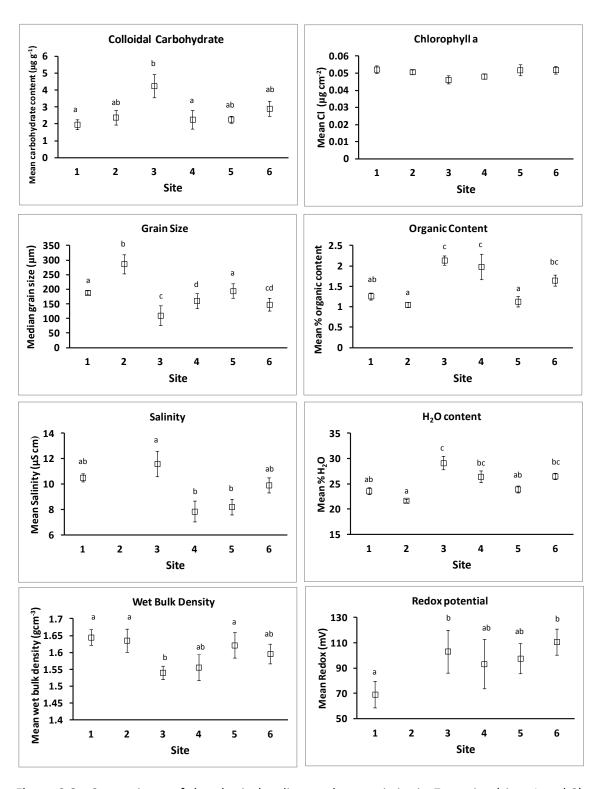
In the *Z. marina* sites, both the physical and chemical conditions appeared to be relatively uniform in vegetated and bare plots. Only at *Z. noltii* sites did the presence of vegetation significantly affect any grain size fractions (Mann Whitney, W = 115, p = 0.05, n = 24), with median 53% (IQR: 49%, 59%) of grains 125-250  $\mu$ m in vegetated plots compared to 40% (IQR: 33%, 55%) in control plots.



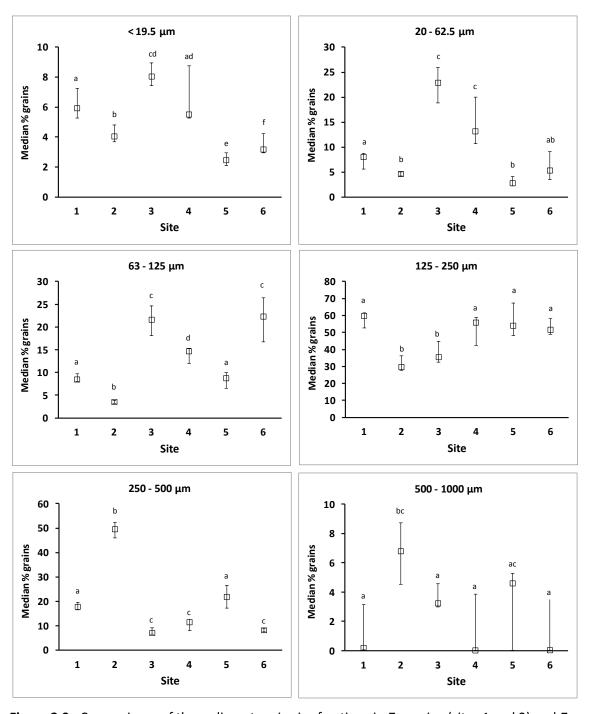
**Figure 2.7**: The correlation between sediment organic content and grain size.  $R^2 = 0.5255$ ,  $y = 3.0313e^{-0.004x}$ 

**Table 2.3**: One-way ANOVA comparing the physical sediment characteristics of sites where *Z. marina* and *Z. noltii* seagrass beds occur in the Tay estuary.

Variable (vs sampling site)	n	F	p	d.f.	Residual d.f.
Zostera noltii					
% organic content	36	9.10	< 0.001	3	32
% water content	36	3.92	0.017	3	32
Wet bulk density (g cm <sup>-3</sup> )	35	1.26	0.305	3	31
Chlorophyll (mg l)	36	1.39	0.263	3	32
Colloidal carbohydrate (µg g)	36	3.37	0.030	3	32
Redox (mV)	34	1.02	0.396	3	30
Salinity (µS cm)	36	4.82	0.007	3	32
Zostera marina					
% organic content	18	3.42	0.083	1	16
% water content	18	6.83	0.019	1	16
Wet bulk density (g cm <sup>-3</sup> )	18	0.07	0.797	1	16
Chlorophyll (mg l)	18	0.30	0.591	1	16
Colloidal carbohydrate (µg g)	18	0.57	0.460	1	16



**Figure 2.8**: Comparisons of the physical sediment characteristics in *Z. marina* (sites 1 and 2) and *Z. noltii* seagrass beds (sites 4 to 6). Where indicated, data that do not share a letter denote a significant difference between sites. Standard error bars are shown for all variables except grain size, where the interquartile range is used.



**Figure 2.9**: Comparisons of the sediment grain size fractions in *Z. marina* (sites 1 and 2) and *Z. noltii* seagrass beds (sites 4 to 6), with interquartile range bars. Data that do not share a letter indicate a significant difference between sites.

When data were further analysed according to sampling site, there were significant differences between sites for both species in mean organic content and water content (Table 2.3; Figure 2.8) with highest percentages of both in sites 3 and 4 (*Z. noltii*), and lowest in both the *Z. marina* sites (sites 1 and 2). Mean colloidal carbohydrate content and salinity also differed between *Z. noltii* sites. Salinity was lower in sites higher up the shoreline. For both species, there were significant differences in median grain size between sites. The greatest difference (Mann Whitney, W = 45.0, p < 0.001, n = 18) was found for *Z. marina*, where the median grain size in plot 2 was 287  $\mu$ m (IQR: 259  $\mu$ m, 291  $\mu$ m) compared to 188  $\mu$ m (IQR: 183  $\mu$ m, 190  $\mu$ m) in plot 1 (Figure 2.9). At *Z. noltii* sites the median grain size ranged from 110  $\mu$ m (IQR: 98  $\mu$ m. 131  $\mu$ m) in site 3 to 195  $\mu$ m (IQR: 177  $\mu$ m, 202  $\mu$ m) in site 5. Chlorophyll *a* concentrations were similar in all plots.

#### 2.4 Discussion

### 2.4.1 Distribution of intertidal Zostera spp.

The two species of intertidal seagrass, *Z. marina* and *Z. noltii*, found in the Tay Estuary grow in largely monospecific beds, with distinctly different habitat preferences. The *Z. marina* sites were subject to low riverine currents when submerged, associated with low tidal flow, and low wave energy (Green 1975). The sediments consisted of medium and fine sand, with a median size of 222  $\mu$ m and mean wet bulk density of 1.64 g cm<sup>-3</sup>  $\pm$  0.02 SE. The *Z. noltii* sites were not subject to riverine currents, only tidal ebb and flow with low wave energy (Green 1975). The sediments also comprised fine sand, median sediment grain size 151  $\mu$ m, but with a high silt fraction and mean wet bulk density of 1.58 g cm<sup>-3</sup>  $\pm$  0.02 SE. Contrary to the findings of previous studies (e.g. Kenworthy *et al.* 1982; Bos *et al.* 2007; van Katwijk *et al.* 2010) the presence or absence of seagrass did not increase the fraction of small particles. At Tayport, the deposition and sorting of sediment grains appears to be governed primarily by the hydrodynamic conditions within the estuary rather than the influence of seagrass canopies (see Chapter 6, section

6.4.1), as both current velocities and median grain sizes exhibit a landward decrease (Yang *et al.* 2008). These results do not support the hypothesis  $H_1$ , that intertidal Z. *marina* and Z. *noltii* beds modify their physical sediment environment.

Z. marina occurred mainly in two large meadows (~ 0.4 ha) although higher up the shore sparse growth also occurred. Where the grass grew in large meadows, shoot densities were highest towards the centre of the meadow, decreasing towards the edges. The edge is the most dynamic zone of the meadow with regards to current flow (Fonseca et al. 1982) and this disturbance leads to lower shoot densities and biomass at the meadow edge (Brun et al. 2003). Z. noltii did not form large beds, and occurred in a mosaic of small discrete patches. This patchiness is common in seagrasses (e.g. Brun et al. 2003; Frederiksen et al. 2004; Hirst & Attrill 2008). The fragmented Z. noltii community at Tayport may be the result of biotic or abiotic stressors higher up the shoreline such as sediment disruption by grazing of leaves and rhizomes by wild fowl in autumn (Vermaat & Verhagen 1996; Zipperle et al. 2010), frost damage (Davison & Hughes 1998) or ice scouring over winter (van Katwijk & Hermus 2000). Recurring periods of disturbance may cause only partial mortality, and may allow only the partial recovery of the species from these disturbances (Marbà & Duarte 1995; Cunha et al, 2004). Highest Z. noltii shoot densities were found at the top of the shoreline. It can be assumed that high shoot densities are a sign of a healthy seagrass bed but, counter intuitively, the clumping of intertidal vegetation may actually be an indication of stress (Glynn 1965; Hruby & Norton 1979; Phillips & Lewis 1983). Intertidal seagrasses are exposed to the air at twice-daily intervals of up to six hours, thus high shoot densities reduce the risk of desiccation as water is retained between and under leaves lying flat on the sediment surface (Pérez-Lloréns & Niell 1993; Hemminga & Duarte 2000). Sediment water content was lower in Z. marina sites than in Z. noltii, though differences were not significant. In both species there was no significant difference between water content in bare or vegetated plots, suggesting that the plants do not reduce water losses from sediment through evaporation, although it could be speculated that the effect may be evident in hotter, drier climates than that of eastern Scotland. Water content was greater in the Z. noltii sites, corresponding with lower wet bulk densities and higher fractions of finer sediment grains than in Z. marina sites. The clear shoreline zonation between the two species suggests that *Z. marina* is less tolerant of desiccation than *Z. noltii* (Borum & Greve 2004; Davison & Hughes 1998).

Porewater was brackish at all sites. Porewater salinity was generally lower in *Z. noltii* sites with a mean of 9.39  $\mu$ S cm  $\pm$  0.05 SE (6.3 psu) compared to 10.52  $\mu$ S cm  $\pm$  0.31 SE (7.1 psu) in *Z. marina*. Both species are tolerant of fluctuations in salinity (Davison & Hughes 1998) and although both species are covered at high tide, where salinity commonly ranges from 11 to 32 psu in the seaward reaches of the estuary (Maitland *et al.* 1994; Bates *et al.* 2003; McManus 2005), the *Z. noltii* sites higher up the shore (sites 4 to 6) are subject to freshwater inputs from streams and storm drains when uncovered at low tide.

### 2.4.2 Comparison between the sediments of *Z. marina* and *Z. noltii* beds within the Tay estuary

Sediments within seagrass meadows are generally richer in organic matter than bare sediment outwith the beds (eg. Kenworthy et al. 1982). As well as sestonic particles deposited from the water column, organic content comprises leaf fragments, epibionts, root and rhizome parts, and root exudates (Miyajima et al. 1998; Papadimitriou et al. 2005; Marbà et al. 2006). Seagrass sediments generally comprise < 6 % dry weight organic concentration (Hemminga & Duarte 2000), and in previous studies organic content in Z. noltii surface sediment has been measured at 2.2% to 2.6 % (Laugier et al. 1999; Peralta et al. 2000), while measurements range from 3.4% to 11.4 % for intertidal Z. marina (Laugier et al. 1999). At Tayport, organic content was lower than this for sites colonised by both species. Organic content was greater in Z. noltii sites (mean  $1.73\% \pm 0.11$  SE) than in Z. marina sites (1.16%  $\pm 0.06$  SE). There was no difference between vegetated and bare Z. marina plots, but organic content was significantly higher in vegetated Z. noltii plots, supporting hypothesis H<sub>1</sub>. This may be because at Tayport Z. marina has shoots present year-round, while Z noltii undergoes leaf loss in winter. Density dependent regulation requires the mortality of seagrass leaves to avoid overcrowding of the population, as predicted by the self-thinning rule (Westoby 1986). During the summer, high Z. noltii shoot densities lead to increasing shoot mortality,

which increases further during autumn and winter (Hemminga & Duarte 2000). Thus, higher rates of leaf mortality may have contributed to the higher levels of organic matter. *Z. noltii* sites 3 and 4, with the highest organic content, were also subject to inputs from a stream running through agricultural land, and a pipeline carrying wastewater from a nearby factory.

Benthic microalgae and their exudates also contribute to sedimentary organic content (Spivak *et al.* 2009). The deposition and accumulation of organic matter and seagrass detritus within the meadow increases the pool of organic substrate available for microbial decomposition compared to unvegetated areas (Gacia & Duarte 2001; Marbà *et al.* 2006). Carbohydrate content was higher in vegetated *Z. noltii* plots, and in bare plots within *Z. noltii* meadows compared to control plots, supporting hypothesis H<sub>1</sub>, and was highest at site 3 where wastewater inputs occur. However, chlorophyll *a* concentrations, an indication of algal (diatom) biomass, were similar in all plots, suggesting than the presence of seagrass does not influence the presence of autotrophic algae.

Sediments with high organic content support high bacterial activity, leading to reducing conditions within the sediment, shown as negative redox potential (Mateo et al. 2006). Seagrasses grow in sediments with redox potentials ranging from moderately reduced to highly oxidised (-175 to + 300 mV) (Terrados et al 1999; Enríquez et al. 2001; Màrba & Duarte 2001). At Tayport, redox potential was lower in vegetated Z. noltii sediment (72.4 mV  $\pm$  16.0 SE) compared to bare (119.3 mV  $\pm$  8.6 SE) and control plots (112.0  $mV \pm 8.3$  SE), indicating that reducing conditions were more evident in vegetated This contradicts some previous studies, where the release of O<sub>2</sub> from seagrass roots has led to higher redox potential in vegetated sites (e.g. Isaksen & Finster 1996; Màrba & Duarte 2001). However, where organic matter is increased by the presence of seagrass, respiration in the sediments can lead to the development of a heterotrophic bacterial community and a lower sediment redox potential in comparison to non-vegetated sediments (Enriquez et al. 2001; Barrón et al. 2004; Neubauer et al. 2004; Vichkovitten & Holmer 2005). Deposited organic matter in the seagrass beds feeds bacterial decomposition processes such as sulphate reduction (Holmer & Nielsen 1997) which dominates the mineralisation of organic matter in seagrass beds (Vichkovitten & Holmer 2005). Decomposition *in situ* increases oxygen demand (Stevenson 1988), and alternative electron acceptors: nitrate; manganese; iron; sulphate; and carbon dioxide are utilised after the oxygen supply is exhausted. In seagrass beds, the sediment can become anaerobic up to the surface when organic input is high (Thayer *et al.* 1984). It has been estimated that 50% of organic matter oxidation takes place under anaerobic conditions in Danish coastal sediments (Vichkovitten & Holmer 2005). Additionally, Terrados and Duarte (2000) suggest that resuspension of surface sediments in bare sediments could increase the exchange of pore waters with overlying oxygenated waters. Therefore, sediment stability in vegetated areas may lead to a decrease in redox potential. There were no differences between redox potentials in vegetated and bare *Z. marina* plots at site 1, perhaps due to lower sediment organic content.

### 2.5 Conclusions

Seagrasses can be ecologically plastic and populations of *Z. marina* and *Z. noltii* can be found living across a range of habitat variations (Tubbs 1995; Valle *et al.* 2011) as demonstrated by physical differences between sites containing the same species ((sites 1 and 2 (*Z. marina*) and 3 to 6 (*Z. noltii*)). Although some habitat requirements are satisfied by hydrodynamic and topographical conditions of the site, seagrasses also modify their physical environment to some degree.

A survey of intertidal seagrass in the Tay estuary recorded  $\sim 3$  ha of Z. noltii growing high up the shoreline at Tayport. It occurred at sites with smaller median grain size with a high silt fraction, and lower wet bulk density than Z. marina. Around 1 ha of Z. marina was found growing on poorly drained fine sand, and its distribution was limited by its intolerance to desiccation. Substratum type and tidal inundation/exposure time appear to be the primary factors influencing the distribution of these two species on the shoreline. At Tayport, the sorting of sediment grains appears to be governed by the hydrodynamic conditions within the estuary, rather than the presence of seagrass shoots. Seagrass also had no influence on sediment water content.

Sediment organic content increased within vegetated plots of *Z. noltii*, leading to a decrease in redox potential. This effect was not evident in *Z. marina*, however the low sample size may have led to results unrepresentative of the community. Carbohydrate concentration was higher in vegetated *Z. noltii* plots, and bare sediment within *Z. noltii* patches, suggesting the increased pool of organic matter leads to higher microbial decomposition. Chlorophyll *a*, an indication of the abundance of photosynthesising microalgae, was not affected by the presence or absence of seagrass.

This study has provided a basic overview of the environmental variables the grasses prefer – to what extent these are already present, or whether are they due to ecosystem engineering by the seagrass.

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### Additional data

Botanical Society of the British Isles

### **Chapter 3**

# The influence of *Zostera* spp. on sediment deposition and stability in the Tay estuary

### Abstract

Sedimentation in estuaries is a continuous process regulated by sediment inputs and controlled by prevailing atmospheric and hydrodynamic forces. Short-term studies of annual cycles of sedimentation have demonstrated that on tidal flats there are phases of accretion, mainly during spring and summer, and surface erosion during winter. These general conditions can be altered by episodic storm events. The biota of estuarine systems have adapted to these conditions and are resilient to sediment changes. For example, seagrasses stabilise their sediment environment by altering the hydrodynamic conditions near the sediment surface, dissipating flow energy and increasing sediment deposition and retention. The greatest sediment accretion occurs in the densest patches under low flow conditions. The dense three-dimensional network of roots and rhizomes binds sediment, further enhancing the retention of sediment particles.

In the Tay Estuary, the influence of intertidal *Z. marina* and *Z. noltii* on sediment deposition and stability was explored. Sediment accretion in areas of *Z. marina*, *Z. noltii* and bare sediment was measured between July 2009 and August 2010. During summer and autumn, accretion was greater in vegetated areas compared to bare areas. In vegetated areas accretion peaked in November 2009, with 18 mm (IQR: 8 mm, 25 mm) in *Z. noltii* compared to 5 mm (IQR: -9 mm, 15 mm) in bare sediment. In *Z. marina* there was accretion of 11 mm (IQR: 3 mm, 16 mm) (high density) and 10 mm (IQR: 2 mm, 14 mm) (low density) compared to a loss of 4 mm (IQR: -8 mm, 2 mm) in bare sediment. During winter, erosion of the vegetated areas of both species was comparable to, and sometimes greater than in bare areas, although sediment levels above the baseline measurement remained higher. This is evidence that over winter the root-rhizome system of *Z. noltii* did not reduce erosion in denuded *Z. noltii* beds

compared to areas of permanently bare sediment. Alternatively, rhizomes may not be intact due to both abiotic and biotic disturbances such as ice scouring or grazing by wildfowl.

Sediment accretion was greater in areas of high density *Z. marina* compared to areas with low shoot densities or bare sediment. This may be due to sediment retention rather than sediment deposition, as sediment which becomes suspended under the canopy is caught up in the leaf blades and re-deposited. Low density *Z. marina* occurs mainly round the edges of the meadow, where edge effects may lead to lower deposition.

After one year, sediment levels in low density *Z. marina* and its corresponding bare sediment were similar to those at the beginning of the year. Sediment levels in high density *Z. marina* and in *Z. noltii* were significantly higher than the baseline, and in bare sediment (*Z. noltii*) were significantly lower. This variability may be attributable to short-term influences on sediment supply and transport; therefore, long-term patterns of sedimentation on tidal flats in the Tay Estuary should not be extrapolated from these results.

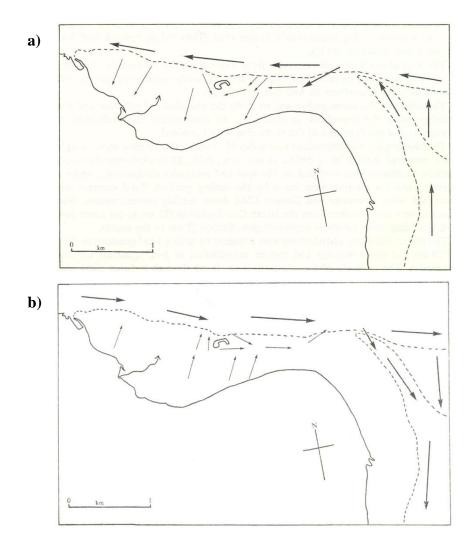
### 3.1 Introduction

### 3.1.1 Processes influencing sedimentation at Tayport

Sedimentation in estuaries is a continuous process regulated by sediment inputs from fluvial and marine sources, combined with resuspended materials transported by tidal circulation (McManus 1998; Little 2000). Sediment deposition and resuspension is controlled by prevailing atmospheric and hydrodynamic forces (Dyer 1997; Quaresma et al. 2007). Low frequency, high magnitude events, such as storms, can cause sudden and large changes to sediment type or distribution (Cahoon, 2006; Bramato et al. 2010); however, long-term observations confirm that most changes happen gradually, influenced by high frequency, low magnitude events such as tides, seasonal fluctuations in sea level or river discharge (Day et al. 1995; McManus 1998). Short-term studies (one to four years) of annual cycles of sedimentation, involving repeated measurements at fixed points, have demonstrated that on tidal flats there are phases of accretion, the raising of the sediment surface by the external addition of new material (Lawrence 1998), mainly during spring and summer, but that surface levels may also erode during periods of higher winds and in winter (e.g. McManus & Alizai 1987; Andersen & Pejrup 2001; Marion et al. 2009). The sediment surface may change in level by several centimetres during a complete annual cycle, the degree of change being dependent upon the duration of tidal inundation (Ralston & Stacey 2007), and the strength and direction of winds and waves moving across the tidal flat (McManus & Alizai 1987; Yang et al. 2003).

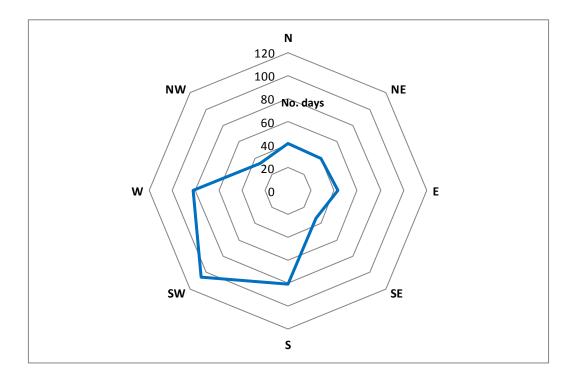
Tayport beach, at the southern entrance to the Tay Estuary, forms an area of extensive inter-tidal sedimentation. The tidal range is ~ 5 m during spring tides, falling to 2.5 m on neap tides (Charlton *et al.* 1975; Charlton 1980) and the flats at Tayport are flooded for periods not exceeding six hours over any tide. Tidal currents decrease in energy and duration with greater distance from the main channel of the river, allowing the deposition of fine-grained sediments over the entire beach. Waves have a limited influence on sediment processes on Tayport beach, due to shelter from the prevailing winds provided by high land to the south-west and afforested land to the south-east

(Green 1975). The estuary is protected from North Sea waves by its west-to-east orientation, and by narrowing of the estuary mouth between Broughty Ferry to the North and Tayport to the south (Charlton *et al.* 1975). This constriction concentrates the flood flow of the tide on the south side of the estuary (Charlton *et al.*1975; Figure 3.1).



**Figure 3.1**: The pattern of water movemement during the a) flood and b) ebb tides at Tayport (from Green 1975). The large arrows indicate tidal flows in excess of 1 ms<sup>-1</sup> at a) low water plus 3 hours, and b) high water plus 3 hours. The shoreline is illustrated (——) and the low water mark (LWS) is illustrated (- - - -).

Fetch is greatest when the wind direction has a northerly component, but this is rare as the prevailing winds are south-westerly (Figure 3.2). The dominant wave direction is the same as the wind direction (Green 1975). The tidal flats comprise sand, quartz, feldspar, and lithic fragments derived mainly from the sea (Mishra 1969; Al-Dabbas & McManus 1980). Wave refraction and sediment transport in the nearshore zones of the North Sea are almost always toward the mouth of the Tay Estuary (Sarrikostis & McManus 1987). With the exception of periods of North Sea storms, marine tidal waters penetrating the lower reaches of the estuary contain little suspended sediment, with concentrations of less than 10 mg l<sup>-1</sup> (McManus & Alizai 1987).



**Figure 3.2**: Wind rose for the period 1<sup>st</sup> April 2009 to 13<sup>th</sup> August 2010, indicating the frequency distribution of wind origins, based on data for Carnoustie 56° 5′ N, 2° 69′W, 4 km north of the Tay estuary mouth, and 11 km north-east of Tayport (© www. windguru.cz).

Although the River Tay drains an upland catchment area of  $\sim 6500 \text{ km}^2$ , little sediment is carried into the estuary as it is generally trapped in freshwater lochs within the drainage basin (McManus 1998). In consequence, the suspended sediment concentrations of fluvial inputs are often less than 10 mg  $I^{-1}$  (McManus & Alizai 1987). The River Earn, which has fewer lochs within its catchment, carries some sand and gravel into the estuary above Newburgh (Al-Ansari & McManus 1979); however, the quantities are too small to contribute significantly to the overall sediment budget (Al-

Jabbari et al. 1980). In the estuary, suspended material is mainly derived from the resuspension of small grains from the surface of mudflats, and bank erosion along the estuary margins (Buller 1975). The quantities of these sediments entering the system are controlled by tidal state (ebb/flow), amplitude, and wind velocity and direction. During windy weather, increased wind-induced wave activity over the tidal flats is associated with raised suspended sediment concentrations (Dobereiner & McManus 1983; Weir & McManus 1987) as sediments are resuspended by oscillatory currents before being transported and deposited elsewhere (Alizai & McManus 1980). Sediment resuspension, or erosion, and sediment accretion on tidal flats generally follows an annual cycle, with deposition in spring and summer followed by lowering of the surface level in winter (McManus & Alizai 1987; Marion et al. 2009). Lowering of the sediment surface level can also occur at other times of the year during the episodic occurrence of stormy weather and strong winds (Andersen & Pejrup 2001; Cahoon 2006).

# 3.1.2 The influence of intertidal seagrasses on sediment composition, deposition and retention

Although sediment deposition on the seashore and in shallow-waters is largely controlled by physical factors such as the speed and direction of waves and currents (Le Hir *et al.* 2000; Marion *et al.* 2009), sediment processes can also be modified by benthic communities such as seagrass meadows (Bouma *et al.* 2005; Bos *et al.* 2007). Seagrasses have been described as ecosystem engineers, shaping and stabilising their sediment environment by increasing sediment deposition and increasing grain retention (Hemminga *et al.* 1991; Terrados & Duarte 2000; Gacia & Duarte 2001; Bouma *et al.* 2008). Seagrass canopies alter the hydrodynamic conditions near the sediment surface, dissipating flow energy (Gambi *et al.* 1990; Widdows *et al.* 2008). As flow is reduced, the capacity of the water to carry suspended particles decreases (Hjulstrom 1939), enhancing particle deposition (Scoffin 1970; Gacia *et al.* 1999) and reducing resuspension (Almasi *et al.* 1987; Herkül & Kotta 2009), with greatest accretion in the densest patches in low flow conditions (Gacia & Duarte 2001; Bos *et al.* 2007; Peralta

et al. 2008). The mechanisms governing particle trapping and retention by seagrasses are discussed in detail in Chapter 4.

The deposition and accumulation of organic matter and seagrass detritus within the meadow increases the pool of organic substrate available for microbial decomposition compared to unvegetated areas (Gacia & Duarte 2001; Marbà *et al.* 2006). In dense meadows, a large proportion of detrital matter is retained within the canopy, providing a source of organic particles (Hemminga & Duarte 2000; Mateo *et al.* 2006). Burial can lead to the long-term storage of deposited organic matter, either imported or produced within the seagrass bed (Romero *et al.* 1992; Hemminga & Duarte 2000). The degree to which this occurs depends on a number of factors, including the flux of organic matter to the sediment surface, and losses through resuspension (Henrichs 1993; Gacia *et al.* 1999). The dense three-dimensional network of roots and rhizomes binds sediment, further enhancing the retention of particles (Scoffin 1970; Hemminga & Duarte 2000; Marbà *et al.* 2006) and accumulating organic matter (Dauby *et al.* 1995).

At Tayport, while the *Z. marina* population is evergreen, with leaves present all year round, the *Z. noltii* population is deciduous, with only the root-rhizome system left over winter. In November, *Z. noltii* leaves are lost due to senescence and as a result of other stressors high up the shoreline such as sediment disruption by storms (Herkül & Kotta 2009) or ice scouring (van Katwijk & Hermus 2000), and grazing by wild fowl (Vermaat & Verhagen 1996; Zipperle *et al.* 2010).

Seagrass meadows would ultimately develop into emergent islands if deposition was continuous (Koch 1999); however, even under normal, non-extreme conditions this does not happen. In seagrass beds the sediment surface may become elevated compared to adjacent unvegetated areas, due to higher sedimentation rates within the meadow, but over time a relatively constant level is maintained. For this to occur, equilibrium between sediment deposition and erosion must be reached (Fonseca *et al.* 1983; Koch 1999). Nevertheless, large short-term accretion/depositional events in seagrass beds can result in relatively sizeable depth fluctuations, with too high levels of sediment deposition having negative effects on seagrasses. When 25 % (cf 40 mm) of the plant height is buried, *Z. marina* experiences greater than 50 % mortality (Mills & Fonseca

2003), while the loss of all shoots occurs in *Z. noltii* at this depth (Cabaço & Santos 2007). Thus, sediment resuspension is requisite to maintain healthy seagrass beds.

#### 3.1.3 Aims

This study explores the role of the intertidal seagrasses *Z. noltii* and *Z. marina* in sediment accretion in the Tay Estuary, comparing the variations in sediment levels in vegetated and bare areas over one year. It also investigates whether the rhizome system of *Z. noltii* reduces sediment erosion in winter, when leaves are not present.

The following hypotheses were tested in order to investigate the annual cycle of sedimentation:

- H<sub>1</sub> Sediment accretion will be greater in *Zostera* spp. beds compared to bare areas.
- H<sub>2</sub> Sediment accretion will be greater in areas of high density *Z. marina* compared to areas with low shoot densities or bare sediment.
- H<sub>3</sub> Over winter there will be less erosion in denuded *Z. noltii* beds compared to areas of permanently bare sediment.
- H<sub>4</sub> After one year, sediment levels in both *Zostera* spp. beds and areas of bare sediment will be statistically similar to those at the beginning of the year.

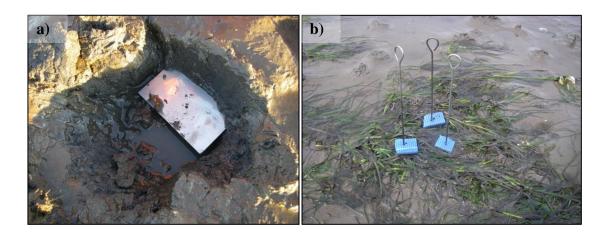
### 3.2 Material and methods

### 3.2.1 Sediment deposition/erosion measurements

The method for measuring sediment deposition and erosion was adapted from Brown (1998). Steel plates  $15 \times 15 \times 0.15$  cm were buried within patches of *Z. nolti* and *Z. marina*, and in bare sediment of similar tidal heights relative to the shore line. Before plate burial, the absence of any other metal objects at the site was confirmed using a metal detector. A hole was dug adjacent to the area of sediment to be measured, and the steel plate was inserted horizontally at a depth of  $\sim 10$  cm, using a spirit level to ensure

the plate was flat (Figure 3.3). The plate locations were recorded using GPS and a bamboo cane was placed 2 m west of each plate to aid future plate relocation, thus minimising disturbance to the site.

Plates were left to settle for one month before baseline measurements were taken, and then measurements taken every six-to-eight weeks thereafter. A metal detector was used to locate the metal plates. The sediment height over each plate was measured at five points using a thin metal rod pushed through a foam disc (Figure 3.3). Any leaves were carefully moved aside using the metal rod. The rod was inserted into the sediment over the plate at a right angle. The disc was pushed down to the sediment surface, and the rod and disc were carefully removed. The distance from the base of the disc to the tip of the rod was measured with a ruler, giving the sediment height over the plate.



**Figure 3.3:** a) A 150 x 150 x 1.5 mm steel plate being inserted under the patch of sediment to be measured, and b) the height of sediment above a plate being measured using metal rods.

### Z. marina site

The *Z. marina* plates were situated 550 m offshore from Tentsmuir forest (central grid reference NO 47550, 28290) on the inter-tidal sand flat (Figure 3.4). Patches of low (< 100 shoots m<sup>-2</sup>) and high (> 100 shoots m<sup>-2</sup>) density *Z. marina*, and bare sediment were compared, with 20 measurement points for each sediment type (Figure 3.5).

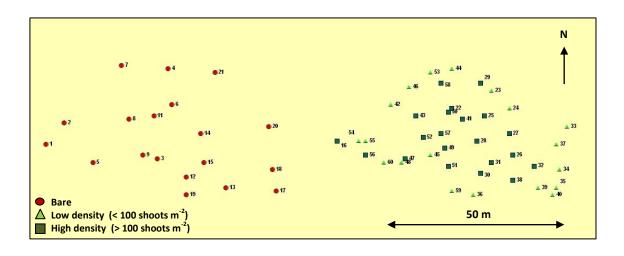
Measurement locations within the meadow were chosen at random from 2008 survey data (Chapter 2, section 2.3.1). Because the *Z. marina* grew in a large meadow with few bare areas, measurements for unvegetated sediment were taken directly west of the *Z. marina* meadow, at a similar height relative to the shore line.

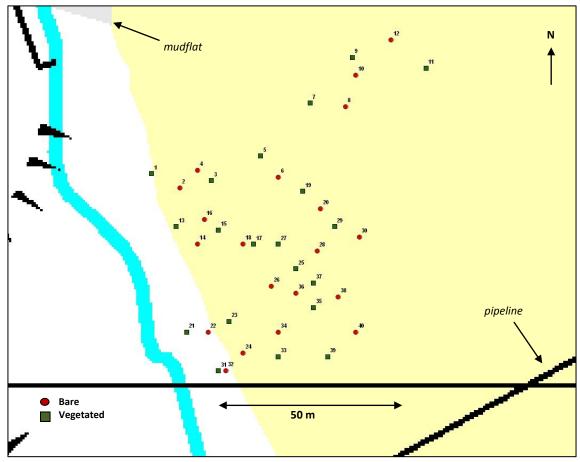


**Figure 3.4**: The Tay Estuary, Scotland showing the location of the *Zostera noltii* and *Z. marina* beds where measurements of changes in sediment height were made between April 2009 and August 2010.

### Z. noltii site

The *Z. noltii* plates were situated between Tayport Beach and Tentsmuir (central grid reference NO 46785, 28040) on the inter-tidal muddy sandflat (Figure 3.4). The distance from the shore varied from 1 m to 90 m. Patches of *Z. noltii*, and bare sediment were compared. Measurement locations within the meadow were initially chosen at random from 2008 survey data (Chapter 2, section 2.3.1), and were located as closely as possible to these grid references following regrowth in spring 2009. The *Z. noltii* grew in a mosaic, with patches (0.05 m² to 25 m²) surrounded by bare sediment, rather than in a large meadow. It was not possible to take discrete bare sediment measurements from the seagrass site, due to the presence of a pipeline immediately to





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**Figure 3.5**: The position of measurements taken within a) the *Z. marina* meadow and in adjacent bare sediment, central grid reference NO47550, 28290; and b) the *Z. noltii* meadow, central grid reference NO46785, 28040. Low density *Z. marina* occurred mainly at the meadow-edge, with high shoot densities inside the meadow.

the south of the study area, and muddy sediment and a stream to the north. Measurements for bare sediment were taken within the *Z. noltii* study area, at a similar height relative to the shore line as the nearest *Z. noltii*-covered plate, and ensuring no grasses were present within 2 m of the plate. There were twenty measurement points for each sediment type (Figure 3.5).

### Intensity of water motion - comparison between plates

A comparison of the intensity of water motion, or exposure to flow, was carried out at the *Z. noltii* site only, supported by a BSc Honours project, (Cross 2010). The method was adapted from Muus (1968). Plaster of Paris blocks were made in ice trays to ensure uniformity of block shape. Each block weighed 10 g. Silicon was applied to the base of each block to prevent erosion through movement against the sediment surface, and to attach string for fastening the blocks down. Blocks were pinned to the sediment surface 20 cm from each plate and 1 per site left *in situ* for 24 hours (2 tides) on 3<sup>rd</sup> February, and on 3<sup>rd</sup> March 2010. The blocks were dried at 40 °C for 48 h and reweighed, and the difference in mass was calculated as a proxy for exposure.

### 3.2.2 Statistical analysis

The statistical software used was Minitab 16.

The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. The data for changes in plaster block mass were log transformed, and compared using a t-test. Sediment deposition and erosion data could not be transformed to fulfil the requirements of homogeneity of the variance and statistical analysis of sediment height data was carried out using a Scheirer-Ray-Hare test – a non-parametric equivalent of a two-way ANOVA with replication (Dythan 2003). Further differences between groups were explored using *post hoc* Mann-Whitney tests. Results were considered significant at p = 0.05.

Daily deposition and erosion measurements began in April 2009 for *Z. marina*, and in July 2009 for *Z. noltii* as the interval for *Z. noltii* re-growth delayed plate burial. To allow comparison over one year, the baseline measurements for total deposition and erosion were taken from July 2009 for both species.

### 3.3 Results

There was no significant difference between weight lost from plaster blocks throughout the *Z. noltii* site, therefore there was no difference between flow intensity over measurement points in either February or March.

At both the *Z. noltii* (Scheirer-Ray-Hare test, p < 0.001, d.f. 7, n = 320; Table 3.1) and the *Z. marina* (Scheirer-Ray-Hare test, p < 0.001, d.f. 10, n = 639) comparison sites the estimated daily rate of deposition or erosion was strongly influenced by the time of year that the measurement was taken. For both species, most sediment accretion occurred in summer and autumn, and there was no significant difference in sedimentation between the two species (Figure 3.6). There was little change in the height of bare sediment during the same months. In the *Z. marina* meadow, the presence or absence of vegetation was also highly significant factor for sediment behaviour (Scheirer-Ray-Hare test, p < 0.001, d.f. 2, n = 639) with accretion continuing over vegetated plates, but not bare sediment, throughout autumn 2009; however, there was no difference between vegetated *Z. noltii* and bare sediment during this period.

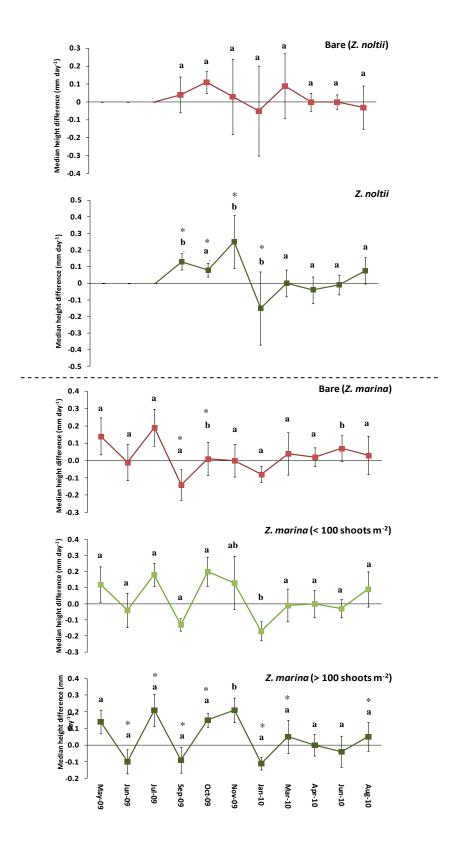
In winter there were notable sediment losses. Estimated daily erosion in *Z. noltii* was greater than for bare sediment over most of winter, with daily losses of 0.15 mm  $\pm$  0.22 SE from vegetated plots compared to losses of 0.05 mm  $\pm$  0.25 SE from bare from late November to the beginning of February, though the difference was not significant. Over the same period, winter sediment losses in both low (0.17 mm  $\pm$  0.06 SE) and high density *Z. marina* (0.11 mm  $\pm$  0.06 SE) areas were greater than for bare sediment (0.08 mm  $\pm$  0.05 SE), although the overall sediment level above the baseline remained higher for seagrass regions than the unvegetated areas (Figure 3.7). By January 2010 *Z. noltii* had lost all its leaves, but *Z. marina* had not. There was greater erosion in *Z. noltii* 

patches compared to the corresponding bare sediments. The low density *Z. marina* areas experienced significantly higher losses than bare sediment and high density *Z. marina* areas. Erosion from high density *Z. marina* areas was also significantly lower than that in the *Z. noltii* beds.

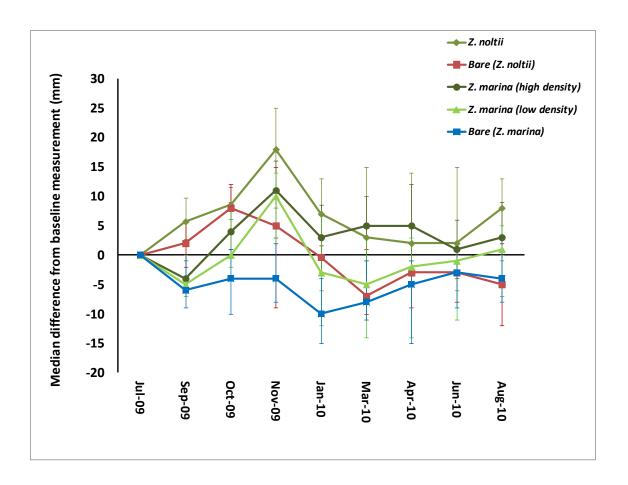
Sediment levels remained stable throughout spring despite events such as a storm on March 31<sup>st</sup> 2010, combined with a high spring tide and onshore winds (Figure 3.8), and there were no significant differences in sedimentation between the two species. *Z. noltii* leaves grew back in June, and deposition took place in beds of both species between June and August, although sediment levels in bare sediment at both sites did not change significantly.

**Table 3.1**: Scheirer-Ray-Hare tests comparing the estimated median daily sediment deposition or erosion between measurements, and comparing the median sediment levels relative to the baseline measurement.

Variable	d.f.	Cumulative chi-square value	n	р	
Daily depostion/erosion					
Z. noltii					
Measurement type	1	0.679	320	0.321	
Measurement date	7	1.000	320	< 0.001	
Z. marina					
Measurement type	2	0.999	639	< 0.001	
Measurement date	10	1.000	639	<0.001	
Total difference from baseline					
Z. noltii					
Measurement type	1	1.000	320	< 0.001	
Measurement date	7	0.997	320	0.003	
Z. marina					
Measurement type	2	1.000	640	< 0.001	
Measurement date	10	1.000	640	<0.001	

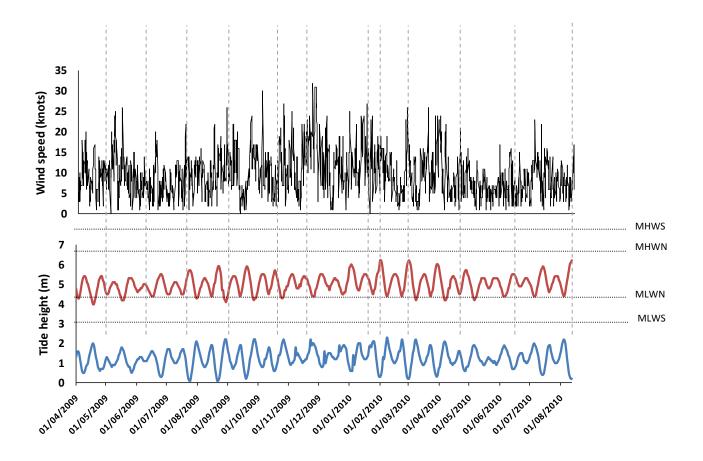


**Figure 3.6**: A comparison of median daily sediment deposition or erosion rates (mm) in *Zostera noltii* and *Z. marina* beds and bare sediment, with interquartile range bars. Data points marked (\*) indicate a significant difference in the daily sedimentation rate compared with the previous measurement date. Within each species, data that do not share a letter in the same month indicate a significant difference in sedimentation rates between plot types for that measurement period only.



**Figure 3.7**: A comparison of sediment heights (mm) relative to the baseline measurement (taken from July 2009), in *Zostera noltii* and *Z. marina* beds and bare sediment, with interquartile range bars.

For both species, the total change in sediment height above the baseline was strongly influenced (p < 0.01; Tables 3.1 & 3.2) by both the measurement date and the presence or absence of vegetation. The sediment depth was consistently greater in both *Z. marina* and *Z. noltii* areas compared to bare sediment throughout the year. In vegetated areas, sediment deposition peaked in November 2009. In vegetated *Z. noltii* deposition was 18 mm (IQR: 8 mm, 25 mm) compared to 5 mm (IQR: -9 mm, 15 mm) in bare sediment, and in *Z. marina* it was 11 mm (IQR: 3 mm, 16 mm) (high density) and 10 mm (IQR: 2 mm, 14 mm) (low density) compared to erosion of 4 mm (IQR: -8 mm, 2 mm) in bare sediment. Sediment levels in vegetated areas followed a similar pattern of deposition until the end of November 2009, following which there were losses of 12 mm (IQR: -16 mm, -8 mm) from low density regions and 8 mm (IQR: -10 mm, -4 mm)



**Figure 3.8**: Wind velocities (knots) based on data for Carnoustie 56° 5′ N, 2° 69′W (© www. windguru.cz) and tide heights (m) based on tidal statistics for Dundee, for the period 1<sup>st</sup> April 2009 to 13<sup>th</sup> August 2010. Measurement dates (----) are indicated.

from high density *Z. marina* areas over two months to February, compared to -5 mm (IQR: -9 mm, -3 mm) from bare sediment. During the same period there was erosion of 8 mm (IQR: -16 mm, 6 mm) from *Z. noltii* site, compared to losses of -3 mm (IQR: -5 mm, 7 mm) from bare sediment. Although there were no major differences between daily deposition in *Z. noltii* and *Z. marina* sites, the total accretion in *Z. noltii* was higher over much of the trial period (Figure 3.7).

After one year sediment levels were significantly higher in both *Z. noltii* (Mann Whitney, p = 0.022, n = 20; Table 3.2) and in high density *Z. marina* (p = 0.005, n = 21). The reduction in the sediment level in bare sediment at the *Z. noltii* site was also significant (p = 0.022, n = 20).

**Table 3.2**: Mann-Whitney tests comparing median (with interquartile range) total change in sediment height above the baseline measurement between measurement dates.

Measurement type	Measurement date 1	n	Median	$IQR(Q_1, Q_3)$	Measurement date 2	n	Median	$IQR(Q_1, Q_3)$	p
Z. noltii (Bare)	02 September 2009	20	2	(-2, 6)	19 October 2009	20	8	(1, 12)	0.032
	19 October 2009	20	8	(1, 12)	20 November 2009	20	5	(-9, 15)	0.860
	20 November 2009	20	5	(-9, 15)	20 January 2010	20	0	(-3, 2)	0.208
	20 January 2010	20	0	(-3, 2)	02 March 2010	20	-7	(-10, 1)	0.044
	02 March 2010	20	-7	(-10, 1)	22 April 2010	20	-3	(-9, 2)	0.882
	22 April 2010	20	-3	(-9, 2)	14 June 2010	20	-3	(-8, 0)	0.978
	14 June 2010	20	-3	(-8, 0)	11 August 2010	20	-5	(-12, 2)	0.409
	21 July 2009	20	0		11 August 2010	20	-5	(-12, 2)	0.022
Z. noltii (Vegetated)	02 September 2009	20	6	(3, 7)	19 October 2009	20	9	(6, 12)	0.023
	19 October 2009	20	9	(6, 12)	20 November 2009	20	18	(8, 25)	0.041
	20 November 2009	20	18	(8, 25)	20 January 2010	20	7	(-6, 13)	0.068
	20 January 2010	20	7	(-6, 13)	02 March 2010	20	3	(-1, 15)	0.705
	02 March 2010	20	3	(-1, 15)	22 April 2010	20	2	(-3, 14)	0.665
	22 April 2010	20	2	(-3, 14)	14 June 2010	20	2	(-6, 15)	0.860
	14 June 2010	20	2	(-6, 15)	11 August 2010	20	8	(-1, 13)	0.543
	21 July 2009	20	0	( -, -,	11 August 2010	20	8	(-1, 13)	0.022
Z. marina (Bare)	03 September 2009	19	-6	(-9, -1)	23 October 2009	19	-4	(-10, 1)	0.609
	23 October 2009	19	-4	(-10, 1)	23 November 2009	19	-4	(-8, 2)	0.827
	23 November 2009	19	-4	(-8, 2)	01 February 2010	19	-10	(-15, 4)	0.096
	01 February 2010	19	-10	(-15, 4)	01 March 2010	19	-8	(-11, -1)	0.640
	01 March 2010	19	-8	(-11, -1)	23 April 2010	19	-5	(-15, 1)	0.850
	23 April 2010	19	-5	(-15, 1)	28 June 2010	18	-3	(-9, 2)	0.261
	28 June 2010	18	-3	(-9, 2)	13 August 2010	18	-4	(-8, 3)	0.693
	22 July 2009	19	0	( - , ,	13 August 2010	18	-4	(-8, 3)	0.135
Z. marina (High density)	03 September 2009	21	-4	(-6, 0)	23 October 2009	21	4	(0, 9)	0.000
	23 October 2009	21	4	(0, 9)	23 November 2009	21	11	(3 16)	0.005
	23 November 2009	21	11	(3 16)	01 February 2010	21	3	(-3, 9)	0.002
	01 February 2010	21	3	(-3, 9)	01 March 2010	21	5	(-5, 10)	0.642
	01 March 2010	21	5	(-5, 10)	23 April 2010	21	5	(-1, 12)	0.473
	23 April 2010	21	5	(-1, 12)	28 June 2010	21	1	(-4, 6)	0.232
	28 June 2010	21	1	(-4, 6)	13 August 2010	21	3	(0, 9)	0.450
	22 July 2009	21	0		13 August 2010	21	3	(0, 9)	0.005
Z. marina (Low density)	03 September 2009	19	-5	(-7, -4)	23 October 2009	19	0	(-2, 8)	0.015
	23 October 2009	19	0	(-2, 8)	23 November 2009	18	10	(2, 14)	0.176
	23 November 2009	18	10	(2, 14)	01 February 2010	18	-3	(-12, 7)	0.020
	01 February 2010	18	-3	(-12, 7)	01 March 2010	18	-5	(-14, 3)	0.704
	01 March 2010	18	-5	(-14, 3)	23 April 2010	18	-2	(-14, 3)	0.975
	23 April 2010	18	-2	(-14, 3)	28 June 2010	17	-1	(-11, 1)	0.792
	28 June 2010	17	-1	(-11, 1)	13 August 2010	17	1	(-7, 5)	0.380
	22 July 2009	19	0		13 August 2010	17	1	(-7, 5)	0.511

**Table 3.3**: Mann-Whitney tests comparing median (with interquartile range) total change in sediment height above the baseline measurement between plot types.

Measurement date	Measurement type 1	n	Median	$IQR(Q_1,Q_3)$	Measurement type 2	n	Median	$IQR(Q_1, Q_3)$	p
02 September 2009	Z. noltii	20	6	(3, 7)	Bare	20	2	(-2, 6)	0.037
19 October 2009		20	9	(6, 12)		20	8	(1, 12)	0.285
20 November 2009		20	18	(8, 25)		20	5	(-9, 15)	0.117
20 January 2010		20	7	(-6, 13)		20	0	(-3, 2)	0.133
02 March 2010		20	3	(-1, 15)		20	-7	(-10, 1)	0.011
22 April 2010		20	2	(-3, 14)		20	-3	(-9, 2)	0.033
14 June 2010		20	2	(-6, 15)		20	-3	(-8, 0)	0.072
11 August 2010		20	8	(-1, 13)		20	-5	(-12, 2)	0.002
03 September 2009	Z. marina (High density)	21	-4	(-6, 0)	Bare	19	-6	(-9, -1)	0.336
23 October 2009		21	4	(0, 9)		19	-4	(-10, 1)	0.004
23 November 2009		21	11	(3 16)		19	-4	(-8, 2)	0.000
01 February 2010		21	3	(-3, 9)		19	-10	(-15, 4)	0.001
01 March 2010		21	5	(-5, 10)		19	-8	(-11, -1)	0.007
23 April 2010		21	5	(-1, 12)		19	-5	(-15, 1)	0.001
28 June 2010		21	1	(-4, 6)		18	-3	(-9, 2)	0.091
13 August 2010		21	3	(0, 9)		18	-4	(-8, 3)	0.007
03 September 2009	Z. marina (Low density)	19	-5	(-7, -4)	Bare	19	-6	(-9, -1)	0.942
23 October 2009		19	0	(-2, 8)		19	-4	(-10, 1)	0.018
23 November 2009		18	10	(2, 14)		19	-4	(-8, 2)	0.000
01 February 2010		18	-3	(-12, 7)		19	-10	(-15, 4)	0.162
01 March 2010		18	-5	(-14, 3)		19	-8	(-11, -1)	0.485
23 April 2010		18	-2	(-14, 3)		19	-5	(-15, 1)	0.386
28 June 2010		17	-1	(-11, 1)		18	-3	(-9, 2)	0.895
13 August 2010		17	1	(-7, 5)		19	-4	(-8, 3)	0.261
03 September 2009	Z. marina (Low density)	19	-5	(-7, -4)	Z. marina (High density)	21	-4	(-6, 0)	0.193
23 October 2009	•	19	0	(-2, 8)		21	4	(0, 9)	0.735
23 November 2009		18	10	(2, 14)		21	11	(3 16)	0.564
01 February 2010		18	-3	(-12, 7)		21	3	(-3, 9)	0.118
01 March 2010		18	-5	(-14, 3)		21	5	(-5, 10)	0.031
23 April 2010		18	-2	(-14, 3)		21	5	(-1, 12)	0.012
28 June 2010		17	-1	(-11, 1)		21	1	(-4, 6)	0.094
13 August 2010		17	1	(-7, 5)		21	3	(0, 9)	0.177

### 3.4 Discussion

In autumn (September to November 2009) and summer (June to August 2010) daily sediment deposition and sediment heights above the baseline measurement were greater in both *Z. noltii* and intertidal *Z. marina* compared to bare areas, partly supporting hypothesis H<sub>1</sub>. The highest levels of sediment deposition and retention would be expected to occur when foliage is present, and in both species leaf growth is greatest in spring and summer, declining in autumn (Mukai *et al.* 1979; Nienhuis & De bree 1980; Vermaat *et al.* 1987, Vermaat & Verhagen 1996; Philippart 1995). However, over winter, erosion of the vegetated areas of both species was comparable, and sometimes

greater than in bare areas. This may be expected in *Z. noltii*, where plants are denuded over winter. In the Dutch Wadden Sea, significant winter erosion in areas formerly vegetated with *Z. marina* in comparison to bare areas has also been observed (Bos *et al.* 2007). The reasons for this are as yet unknown, but Bos *et al.* (2007) speculated that decaying leaves and rhizomes may have increased erosion through scouring, or attracted bioturbating fauna, destablising the sediments. Alternatively, areas of raised sediment may be more prone to flow energy during periods of high flow (Chanson 2004). Conversely, *Z. marina* populations in the Tay estuary are present year-round, and the *Z. marina* leaves were still in place over winter at Tayport.

In winter, tidal flat levels generally undergo surface level lowering (e.g. Andersen & Pejrup 2001; Marion *et al.* 2009) and this has previously been observed in the Tay (MacManus & Alizai 1987), possibly in conjunction with winter storms. McManus & Alizai (1987) observed a reduction in sediment surface level at Invergowrie and Kingoodie, 6.5 km and 8 km west of Tayport, was always associated with the occurrence of strong winds immediately before the day of measurement. This resuspended material may be deposited elsewhere within the estuary during calmer conditions. For example, in the days following the storm on March 31<sup>st</sup> 2010, which combined onshore winds gusting up to 23.2 ms<sup>-1</sup> (Met Office) with a high spring tide (6 m), sediment levels remained similar to, or were slightly higher than the previous month for all sediment types. During storms both turbidity and sedimentation are increased by the combination of increased sediment supply through storm-induced resuspension and river run-off and (Dauby *et al.* 1995; Ralph *et al.* 2006; Marion *et al.* 2009). In spring the sediment load within the estuary is also raised by sediment release from within the catchment from snow-melt (Dyer 1994; McManus 1998).

The effects of storms are not limited to winter months. From 29<sup>th</sup> August to 2<sup>nd</sup> September there were high offshore winds, gusting up to 17.5 ms<sup>-1</sup>. On 2<sup>nd</sup> September 2009 decreased sediment levels were recorded in *Z. marina*, but on 3<sup>rd</sup> September an increase in sediment height was measured in *Z. noltii*, coinciding with a change in the wind direction from south-westerly to northerly (onshore). Resuspended sediment from the *Z. marina* site on the lower shore may have been transferred further up the shore to the *Z. noltii* site. It can be speculated that if measurements had been made at the *Z.* 

*noltii* site a day earlier, erosion would also have been observed there. However, the occurrence of relatively calm weather – the sustained wind speed was lower, although there were still gusts of 15.4 ms<sup>-1</sup> – and an onshore wind between measurements may have led to accretion.

The increased sediment levels in bare sediment in summer, and losses over winter from both bare and vegetated sediments suggest that biogenic activity, i.e., biofilms may also be a contributing factor to sediment stability (Ginsberg & Lowenstam 1958: McManus & Aliza 1987; Paterson 1997). This concept is further explored in Chapter 5 of this thesis. However, total sediment heights above the baseline measurement were still higher in vegetated areas compared to bare sediment sites over winter in both species. Overall, lower sediment losses from seagrass beds compared to unvegetated areas demonstrates that vegetation reduces sediment erosion (Almasi *et al.* 1987; Koch 1999).

The hypothesis H<sub>2</sub>, sediment accretion is greater in areas of high density Z. marina compared to areas with low shoot densities or bare sediment, was supported throughout the year. Although it has been predicted, using models and flume studies, that sediment trapping and deposition is positively related to plant density (e.g. Gambi et al. 1990; Bouma et al. 2005; Hendriks et al. 2008; Peralta et al. 2008), high shoot densities may not have increased sediment deposition (Chapter 4, Section 4.3.1) but may rather have enhanced sediment retention (Chapter 4, Section 4.3.2) as the reconfiguration of submerged leaves forms a protective layer above the sediment surface. Any suspended sediment under the canopy is caught up in the leaf blades and settles out where it cannot easily be resuspended by tidal flow (Ginsberg & Lowenstam 1958; Scoffin 1970; Gacia et al. 1999). Low density Z. marina is found mainly at the outer margins of the meadow, where it is subject to the meadow-edge effects of scour and erosion (Fonseca et al. 1982), and where the trapping and deposition of sediment are impeded (Fonseca & Koehl 2006). The edge is the most dynamic zone of the meadow with regards to current flow, as flexing of the leaves re-directs the current over and under the canopy, reducing the current velocity further into the bed (Fonseca et al. 1982).

Over winter there was greater erosion from Z. noltii compared to the corresponding bare sediments, thus the hypothesis H<sub>3</sub> is rejected. The results suggest that Z. noltii rhizomes

do not stabilise sediments over winter when leaves are absent. Rhizomes may be too deep to prevent erosion: the mean rhizome depths in the Tay species are  $23 \text{ mm} \pm 0.1$  SE (n = 30) for *Z. noltii* and  $23 \text{ mm} \pm 0.2$  SE (n = 30) for *Z. marina* (pers. comms.) Leaf presence is important in sustaining the rhizome system. Vermaat & Verhagen (1996) observed 40% mortality of *Z. noltii* after subjecting plants to partial defoliation, similar to grazing damage, illustrating the importance of photosynthetic capability in sustaining winter seagrass populations. Rhizomes may be damaged due to both abiotic and biotic disturbances such as ice scouring (van Katwijk & Hermus 2000), grazing of both leaves and rhizomes by wild fowl (Vermaat & Verhagen 1996; Zipperle *et al.* 2010), and bioturbation by polychaete worms disturbing the sediment around the plants and exposing their roots (Philippart 1994; Hughes & Paramor 2004). However, grazing wildfowl also promote seedling recruitment by accumulating and burying seeds in the feeding pits they create (Zipperle *et al.* 2010). This may be a reason why *Z. noltii* patches in the Tay are so variable in their location and size from year to year.

After one year, sediment levels in low density Z. marina (median 1 mm, IQR: -7 mm, 5 mm; Table 3.3) and its corresponding bare sediment (-4 mm, IQR: -8 mm, 3 mm) were similar to those at the beginning of the year, partially supporting hypothesis H<sub>4</sub>. Conversely, sediment levels in high density Z. marina (3 mm, IQR: 0 mm, 9 mm) and in Z. noltii (8 mm, IQR: -1 mm, 13 mm) were significantly higher than the baseline, and in bare sediment (Z. noltii) (-5 mm, IQR: -12 mm, 2 mm) were significantly lower than the baseline, thus the hypothesis H<sub>4</sub> is rejected. This variability may be attributable to short-term influences on sediment supply and transport. Accretion of 5-7 mm has previously been measured in annual intertidal patches of Z. marina during the growing season (Bos et al. 2007). Between 10<sup>th</sup> June and 22<sup>nd</sup> July 2009 the bed level rose by a median of 7 mm (IQR: 5 mm, 11 mm) in low density Z. marina and by 9 mm (IQR: 4 mm, 12 mm) in high density grass, giving a daily deposition of ~ 0.2 mm for both densities. However, from 28th June to 13th August 2010 daily accretion was less than half that of the previous year, < 0.1 mm for both densities, highlighting differences between years. Therefore, long-term patterns of sedimentation on tidal flats should not be extrapolated from these results.

Due to their differences in both leaf size and in meadow location it is not appropriate to make direct comparisons of sediment accretion between the two Zostera species. Z. marina leaves are three to four times larger than those of Z. noltii; however, the proportion of the water column they occupy relative to depth when submerged may be comparable, meaning both species would have similar influences on near-bed flow dynamics and flow reduction at their location (Gacia et al. 1999; Koch 1999). Although ambient flow conditions have been recorded as 0.04 ms<sup>-1</sup> at 0.1 m above both Zostera beds (pers. comms.), the Z. marina meadow, being ~ 500 m further offshore, is exposed to additionallow riverine currents associated with tidal flow and low wave energy, while the Z. noltii site is subject only to tidal ebb and flow with low wave energy (Green 1975). Generally hydrodynamic dynamism increases with distance seawards (Yang et al. 2008), although distance from the shore did not significantly affect flow velocity in the Z. noltii site. Differences in grain size and OM between the two species areas (Chapter 2, section 2.3.2) suggest sediment stability may also be explained by differences in sediment cohesivity, as well as hydrodynamic forces between the sites. Overall, however, sediment accretion was highest in areas of high density Z. marina and in Z. noltii patches.

There was no significant difference between weight loss from plaster blocks at the Z. *noltii* site, therefore there was no difference between flow intensity over measurement points in either February or March (one-sample t-test, t = 0.00, p = 1.00, n = 40). Therefore proximity to the shoreline did not significantly influence sediment deposition over the 90 m over which the measurements were taken although blocks closer to the shore were immersed for shorter periods than those further off-shore, with a difference of around 20 minutes between the highest and lowest plates for each tidal cycle.

#### 3.5 Conclusions

In areas of intertidal *Z. marina* and *Z. noltii*, sediment accretion was greater in vegetated areas compared to bare areas during summer and autumn months. During winter, erosion of the vegetated areas of both species was comparable to, and sometimes greater

than in bare areas, although sediment levels above the baseline measurement remained higher. The reasons for this are not known, but storms, reduced biogenic (biofilm) activity or bioturbating polychaetes may contribute to winter erosion in *Zostera* meadows. Over winter, the root-rhizome system of *Z. noltii* did not reduce erosion in denuded *Z. noltii* beds compared to areas of permanently bare sediment. Rhizomes may be damaged due to both abiotic and biotic disturbances.

Sediment accretion was greater in areas of high density *Z. marina* compared to areas with low shoot densities or bare sediment. This may be due to a higher degree of sediment retention rather than to greater sediment deposition, as suspended sediment under the canopy is caught up in the leaf blades and re-deposited. Low density *Z. marina* occurs mainly round the edges of the meadow, where edge effects may lead to lower sediment deposition rates.

After one year, sediment levels in low density *Z. marina areas* and its corresponding bare sediment were similar to those at the beginning of the year. Sediment levels in high density *Z. marina* and in *Z. noltii* regions were significantly higher than those at the beginning of the year, and in bare sediment (*Z. noltii*) were significantly lower than the baseline. A comparison of deposition rates between June-August 2009 and 2010 demonstrate that variability may be attributable to short-term influences on sediment supply and transport, therefore, long-term patterns of sedimentation on tidal flats in the Tay Estuary should not be extrapolated from these results. Net changes are relatively small but variation in vegetated areas is greater, i.e. the sediment can build up but is eventually eroded again. To fully assess whether the area is undergoing net accretion or erosion, measurements over several years are required.

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# **Chapter 4**

# Particle trapping and retention by Zostera noltii: a flume and field study

#### Abstract

The relative particle trapping ability of *Zostera noltii* was examined in an 8 m seawater flume. Two size classes of fluorescent tracer particles (150-250  $\mu$ m and < 63  $\mu$ m) were used to determine the number of particles captured by bare sediment (control) and three densities of *Z. noltii* (200, 600 and 1000 shoots m<sup>-2</sup>) at three flow velocities (0.05, 0.16, 0.44 ms<sup>-1</sup>). Particle sizes, plant densities and flow velocities were all within the range of conditions observed in the field.

Fewer large particles were trapped at higher flow rates (ANOVA,  $F_{2,30} = 23.29$ , p < 0.001, n = 36), with the mean number of particles decreasing with increasing flow. The numbers of large particles trapped in both low and medium flows were similar in vegetated and bare sediments, suggesting that particles were deposited due to low energy conditions rather than due to the presence of vegetation. At low flow, low density seagrass beds trapped more particles of both sizes on the sediment surface than higher density beds. This result was explained in hydrodynamic terms because skimming flow develops at higher shoot densities, carrying suspended particles over the plants following leaf reconfiguration in flow.

This work was extended to the field and sediment retention in high density *Z. noltii* (Tay Estuary, Scotland, UK) was compared to bare sediment over one week (14 tides). Tracer particles were added directly among natural seagrass beds exposed at low tide. After large losses due to initial particle resuspension, *Z. noltii* retained large particles (150-250  $\mu$ m) more effectively than bare plots over time. Particle retention for small particles (< 63  $\mu$ m) was similar for both plot types.

Mechanisms underlying the results are suggested and the findings discussed.

# 4.1 Introduction

# 4.1.1 Seagrass as an ecosystem engineer

Sediment deposition on the seashore and in shallow-waters is greatly controlled by physical factors such as topography, tidal range, and the speed and direction of waves and currents (Ginsburg & Lowenstam 1958; Marion *et al.* 2009). However, water circulation and sediment processes can also be modified by benthic communities such as biofilms and seagrass meadows (Ginsburg & Lowenstam 1958; Paterson & Black 1999; Andersen & Pejrup 2001; Bouma *et al.* 2005; Bos *et al.* 2007). Seagrasses have been described as ecosystem engineers because they shape and stabilise their sediment environment by increasing sediment deposition and retention (Hemminga *et al.* 1991; Terrados & Duarte 2000; Gacia & Duarte 2001; Bouma *et al.* 2008). These physical state changes in the biotic and abiotic environment are caused indirectly, by the movement, structure and spatial distribution of shoots and leaves; therefore seagrasses are autogenic ecosystem engineers (Jones *et al.* 1994, 1997).

As water flows through a seagrass meadow suspended particles may be trapped by adhesion to leaves or uptake by epibionts (Scoffin 1970; Agawin & Duarte 2002), and collisions with leaves reduces momentum causing particles to drop out of suspension (Hendriks *et al.* 2008). The plant structures attenuate hydrodynamic energy and modify current flow (Gambi *et al.* 1990; Fonseca & Cahalan 1992; Widdows *et al.* 2008). Decreased flow velocity enhances particle settling and deposition within the meadow (Scoffin 1970; Bos *et al.* 2007) and energy dissipation by the leaves buffers erosion and resuspension processes below the canopy (Fonseca & Fisher 1986; Gacia *et al.* 1999; Gacia & Duarte 2001; Herkül & Kotta 2009). The reduction of flow allows organic matter (OM) to accumulate, creating a nutrient-rich substratum (Fonseca & Fisher 1986; Koch & Gust 1999). The decrease in porewater flux in lower flow promotes anaerobic conditions, where heterotrophic sulphate-reducing bacteria convert molecular nitrogen into ammonia gas (NH<sub>3</sub>) and then to ammonium (NH<sub>4</sub><sup>+</sup>). Ammonium, produced in the rhizosphere by sulphate-reducing bacteria (Welsh *et al.* 1996, 1997; McGlathery *et al.* 1998), is the form of N most readily assimilated by seagrasses (Short 1987). The

influence of seagrasses on nutrient fluxes is vital to the maintenance of ecosystem function (Duffy 2006; Holmer & Marbà 2010).

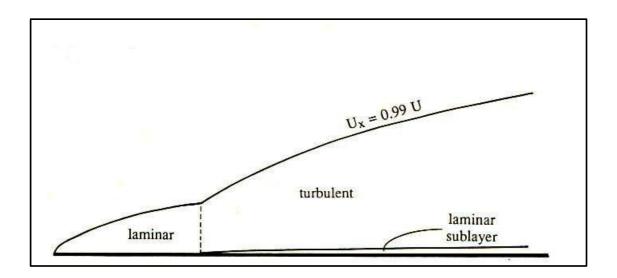
#### 4.1.2 The effect of seagrass on water flow

It has been predicted, using models and flume studies, that sediment trapping and deposition is positively related to plant density (e.g. Gambi et al. 1990; Bouma et al. 2005; Hendriks et al. 2008; Peralta et al. 2008). However, it has also been demonstrated in flume studies that above certain plant densities (Koch & Gust 1999; Peralta et al. 2008) and at the meadow-edge (Fonseca & Koehl 2006) these effects may be impeded. The force exerted by water flow causes the shoot canopy to bend, even at low current speeds. In unidirectional flows the leaves intermesh forming a dense layer that redirects flow from the edge over the bed in a "skimming flow" (sensu Morris 1955; Eckman 1982; Nowell & Jumars 1984). Skimming flow enhances sediment stability and retention by reducing mixing between the water above and within the meadow (Koch & Gust 1999), decreasing in-meadow current flow and turbulence, enhancing the settling of sediment (Fonseca et al. 1982). The barrier of leaves and the reduced flow conditions protect roots from scour. Scoffin (1970) observed that Thalassia testudinum could withstand flow up to 1.5 ms<sup>-1</sup> before in-meadow scour, although scouring could occur at the meadow-edge (Fonseca et al. 1982).

When water moves over sediment some of the energy from the movements of waves and tidal currents is transferred to the movement of sediment grains. To move sediment the fluid force must overcome the frictional drag between grains and the underlying sediment (Koch 1999). Water flow is slowed down by friction as it moves over a surface, and the lower part of the water column subjected to frictional retardation is known as the boundary layer. The boundary layer takes on a logarithmic velocity profile as friction decreases and velocity increases with distance from the bed, so that the free stream velocity is greater than that occurring near the bed (Brown *et al.* 1989; Vogel 1994). Seagrass increases the height of the boundary layer (Bryan *et al.* 2007), facilitating the settling of suspended matter in lower velocity flow near the bed. The

frictional plane is moved from the bed to the surface of the leaves, reducing frictional forces on the sediment surface and decreasing erosion (Koch 1999).

Flows in the sea and all natural water flows are almost always turbulent (Brown *et al.* 1989). However, there is a thin layer close to the bed where frictional retardation is greatest and this creates laminar flow (Figure 4.1). Within this laminar sublayer there is negligible mixing of fluid particles so it acts as a barrier to the exchange of material, such as sediment grains, with the water column (Vogel 1994; Potter 2009). When the velocity profile has a logarithmic variation with height, roughness length ( $z_0$ ) is the height above the bed where the mean flow speed theoretically becomes zero (Denny 1988; Vogel 1994). A thick  $z_0$  increases the percentage of the water column available for the entrapment of deposited sediment, therefore the potential for trapping more suspended particles is greater and sediment deposition is enhanced (Fonseca *et al.* 1982; Gacia & Duarte 2001). Within the boundary layer turbulence increases with increasing velocity and with distance from the bed (Brown *et al.* 1989). However, within a seagrass meadow turbulence intensity is reduced below the canopy (Gambi *et al.* 1990; Ackerman & Okubo 1993), buffering the resuspension of sediment and particulate OM, and reducing porewater flux (Koch & Gust 1999; Gacia & Duarte 2001).



**Figure 4.1**: Boundary layer in flow over a flat surface. Flow is laminar upstream, but downstream becomes increasingly turbulent with a laminar sublayer. (Taken from Vogel, 1994)

# 4.1.3 The use of flumes and tracer particles

Flumes allow the controlled simulation, manipulation and measurement of near bottom flow conditions found in the natural environment. The stress imposed by fluid forces on the sediment surface and organisms growing on it can be assessed. Controlled flow experiments using flumes have been used to determine the influence of organisms, such as biofilms or seagrasses, on sediment deposition and stability. Flumes have previously been used to describe the mechanics of current flow around artificial seagrass meadows (Fonseca *et al.* 1982) and natural beds (e.g. Scoffin 1970; Fonseca & Fisher 1986; Gambi *et al.* 1990; Fonseca & Cahalan 1992; Peterson *et al.* 2004; Widdows *et al.* 2008). A number of flume types are available (Denny 1988) producing unidirectional flow (e.g. Nowell & Jumars 1984; Muschenheim *et al.* 1986), oscillating flow (Svoboda 1970) or waves (Fonseca & Cahalan 1992; Bouma *et al.* 2005). Unidirectional, single-flow channels are useful for determining flow conditions in the principal flow direction (*x*), cross-stream direction (*y*) and vertical direction (*z*).

Dye, phytoplankton and leaf fragments have been used as tracers to track water flow (Anderson & Charters 1982; Muschenheim *et al.* 1986) or to measure particle retention (Terrados & Duarte 2000). Yasso (1965) studied foreshore sediment transport using fluorescent particles of similar size classes and mass to those found in the natural environment. In this study we used magnetic fluorescent particles as described in section 4.2.1.

#### 4.1.4 Relevance of the study

Although studies of the effects of other benthic species (seagrasses, macroalgae, invertebrate tube fields, mussel beds) on the erosion and deposition of sediment are common (e.g. Gacia & Duarte 2001; Romano *et al.* 2003; Eckman 1982; Widdows & Brinsley 2002), relatively few studies have been conducted on *Z. noltii*. Despite *Z. noltii* being the only seagrass species found growing in the high intertidal zone in the U.K. relatively little is known about its ecology and its role as an ecosystem engineer.

Most research has taken place in the last two decades, and on continental Europe (e.g. Welsh *et al.* 1996, 1997; Bouma *et al.* 2005, 2009; Peralta *et al.* 2008; Widdows *et al.* 2008; Lebreton *et al.* 2009).

When considering habitat restoration, either of *Z noltii* beds or in conjunction with other sediment habitats, e.g. as part of managed realignment, it is important to know what density of grass will give the greatest trapping and retention for the flow conditions of the site. Replication of flow and suspended sediment conditions for the site can allow informed habitat management decisions to be made.

#### **4.1.5** Aims

In this chapter the physical conditions which lead to the deposition and retention of sediments are considered for conditions present in the Tay Estuary. The influence of the seagrass *Z. noltii* on water flow in shallow water environments was also explored.

The following hypotheses will be tested in order to determine the functional role of *Z. noltii* on sand and mud flats:

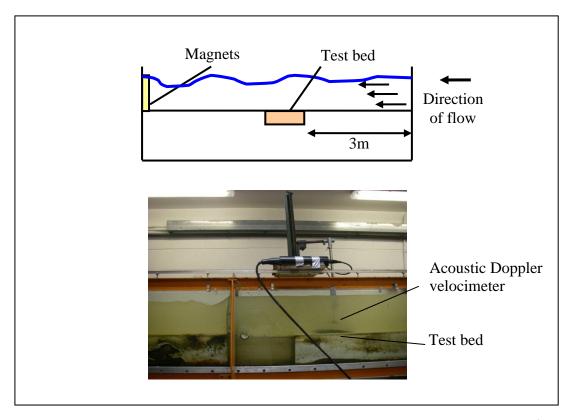
- H<sub>1</sub> Patches of *Z. noltii* will trap more particles than patches of bare sediment.
- H<sub>2</sub> Particle trapping by Z. noltii will increase with increasing grass density.
- H<sub>3</sub> Sediment trapping by *Z. noltii* will decrease with increasing flow.
- H<sub>4</sub> Z. noltii patches will decrease the loss of sediment through resuspension.

# 4.2 Materials and methods

# 4.2.1 Flume study

Flume studies were carried out using an 8 m linear saltwater flume (Armfield Ltd, Ringwood, England; Figure 4.2) modified with a false bed engineered to accept

rectangular sample box cores (0.25 m x 0.2 m x 0.11 m). Flow velocity was monitored using an acoustic Doppler velocimeter (ADV) at 1 Hz (Gratoit *et al.* 2000; Chanson 2008) and velocity profiles were measured to characterise the flow over the test bed.



**Figure 4.2**: Experimental set-up. Experiments were conducted in an 8 m linear tilting flume (Armfield Ltd). Experimental set up consisted of a test bed 3 m downstream of the flow input. An array of magnets was placed at the outflow of the flume to capture any particles not retained by the test area. Flow velocity was recorded using an acoustic Doppler velocimeter.

# Sediment trapping capacity of Zostera noltii

The experiment consisted of four sediment conditions (bare, low, medium or high density *Z. noltii*) against three flow levels (Table 4.1) resulting in 36 experimental flume runs. Shoot densities and flow velocities were representative of those found in the field (see Chapter 2, section 2.3.1 and Chapter 3, section 3.4). Low density treatments comprised 10 shoots per box core (200 m<sup>-2</sup>), medium 30 shoots (600 m<sup>-2</sup>) and high 50 shoots (1000 m<sup>-2</sup>). Flow conditions (average free-stream velocities) calculated from

ADV measurements were low 0.05 ms<sup>-1</sup>  $\pm$  0.001, medium 0.16 ms<sup>-1</sup>  $\pm$  0.002 and high 0.44 ms<sup>-1</sup>  $\pm$  0.004.

**Table 4.1:** Experimental design: the 12 treatments used in the flume to study particle trapping by bare sediment and patches of *Zostera noltii* at different shoot densities and under different flow regimes typical of those found in the Tay Estuary, Scotland.

	Flow conditions $(u)$					
Shoot density (ρ)	Low	Medium	High			
Bare (control)	Bare, low <i>u</i>	Bare, med <i>u</i>	Bare, high u			
Low	Low $\rho$ , low $u$	Low $\rho$ , med $u$	Low $\rho$ , high $u$			
Medium	Low $\rho$ , low $u$ Med $\rho$ , low $u$	Med $\rho$ , med $u$	Med $\rho$ , high $u$			
High	High $\rho$ , low $u$	High $\rho$ , med $u$	High $\rho$ , high $u$			

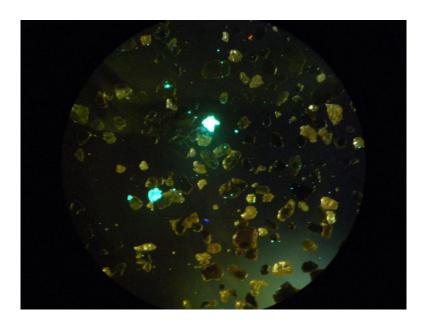
n = 3

A box core (0.25 m x 0.20 m x 0.11 m) of bare or vegetated sediment was taken from Tayport. *Z. noltii* shoots were removed where necessary to obtain the required shoot density. The core was inserted into a core-well in the floor of the adapted fume, 2m upstream of the flume exit. The core height was adjusted until the sediment surface was flush with the floor. The test bed was covered with a protective steel mesh (1.5 mm steel, aperture size 6 x 6 mm) to prevent erosion and the flume was filled with saltwater. The velocity of the flow was increased in increments until the required water speed and height (0.23 m) were reached. Free stream velocity was measured using the ADV at 0.15 m height, 1.5 m upstream of the bed. The mesh and the ADV probe were removed before the addition of tracer particles.

A known mass (6.5 g) of specially made fluorescent ferrous particles (Partrac Ltd, http://www.partrac.co.uk) was injected into the flow 1 m upstream of the bed. The particles used were a mixture of 1.5 g small ( $<63 \mu m$ ) (9 x  $10^5$  particles  $\pm$  1.6 x  $10^5$  SE) and 5.0 g large (150-250  $\mu m$ ) (8.4 x  $10^5$  particles  $\pm$  1.3 x  $10^5$  SE), and are a composite including iron oxide fragments and pigment particles in a binding agent. During manufacture the particles are ground to the desired size and their density adjusted.

After 1 h, flow in the flume was decelerated to zero and the test bed was removed. The water was drained from the flume, magnets were used to remove any remaining particles from the flume, and it was then rinsed with clean water. Five small sediment cores  $(3.1~{\rm cm}^2)$  and five leaves were taken from each bed. Water was added to each sediment core in a small dish. Magnetically responsive particles, comprising the added fluorescent tracer particles and other ferrous particles present in the estuarine sediment, were separated out using a molybdenum magnet, dried and weighed. The samples contained too many grains to place in the counting chamber at one time, so the number of fluorescent particles in a weighed sub-sample  $(0.026~{\rm g}~\pm~0.001~{\rm SE})$  was counted using fluorescence microscopy (Figure 4.3). The number of particles per core was calculated using Equation 4.1. and from this the mean number of particles per core per test bed could be calculated.

Particles per core = 
$$\left(\frac{\text{Weight of sample}}{\text{Weight of sub-sample}}\right) * particle count$$



**Figure 4.3**: Fluorescent ferrous tracer particles (Partrac Ltd, http://www.partrac.co.uk) were counted using fluorescence microscopy. Large particles (150-250  $\mu$ m) are green while small particles (< 63  $\mu$ m) are pink.

# Near-bed hydrodynamics

Near-bed hydrodynamic conditions were determined using flow velocities obtained with the ADV. The boundary layer velocity profile was determined at 1 cm increments above the centre of the test bed from the base into the area of free stream velocity. These velocity values within the boundary layer were plotted against the natural log of water depth. Using the law of the wall (von Kármán 1930) the shear velocity ( $U_*$ ) acting upon the base was determined from the slope of the line  $m = 2.5U_*$  (Equation 4.2).

$$U_* = \frac{m}{2.5}$$
(Equation 4.2)
$$U_* = \text{Shear velocity (ms}^{-1})$$
 $m = \text{Slope of the best fit}$ 

Once  $U_*$  had been obtained this value was used to estimate the boundary layer shear stress ( $\tau_0$ ) using Equation 4.3.

$$\tau_0 = \rho U_*^2$$
 (Equation 4.3)  
 $\tau_0 = \text{Boundary layer shear stress (Nm}^{-2})$   
 $\rho = \text{Density of the fluid (1027 kgm}^{-3} \text{ at 35 psu, 20 °C)}$   
 $U_* = \text{Shear velocity (ms}^{-1})$ 

From this stage the roughness length  $z_0$  was also derived from intercept of the slope of the semi-log plot (Equation 4.4).

$$\frac{b}{\ln z_0 = -2.5U*}$$
(Equation 4.4)
$$z_0 = \text{Roughness length (m)}$$

$$b = y \text{ intercept of the best fit}$$

$$U* = \text{Shear velocity (ms}^{-1})$$

The boundary roughness Reynolds number (Equation 4.5) and the Froude number (Equation 4.6) were calculated to characterise the flow conditions during the experiment.

$$Re_{r} = \frac{\bar{u}l}{v}$$

$$Re_{r} = Boundary roughness Reynolds number$$

$$\bar{u} = Mean velocity of the flow (ms-1) (boundary layer)$$

$$l = Length (of test bed) (m)$$

$$v = Viscosity (m2s-1)$$
(Equation 4.5)

```
Fr = \frac{\bar{u}}{\sqrt{gD}} (Equation 4.6)

Fr = Froude number

\bar{u} = Mean velocity of the flow (ms<sup>-1</sup>) (boundary layer)

g = Acceleration due to gravity (9.81 ms<sup>-1</sup>)

D = Depth (m)
```

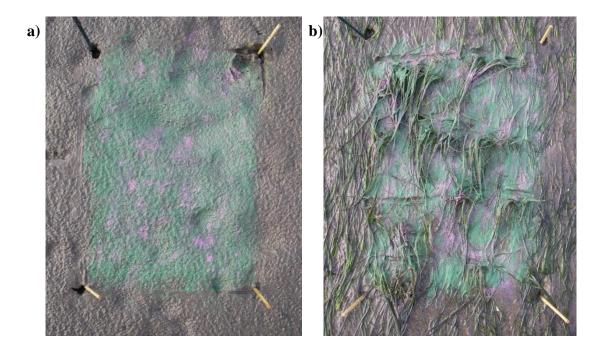
# 4.2.2 Field study

# Sediment retaining capacity of Zostera noltii

The experiment was carried out at Tayport in October 2009 and consisted of five replicates of two experimental conditions:

- Bare sediment (control)
- Vegetated sediment (1000 shoots m<sup>-2</sup>)

The treated beds were 0.25 m x 0.20 m (0.05 m<sup>2</sup>). A known mass of the fluorescent ferrous particles (Partrac Ltd, http://www.partrac.co.uk) were mixed together in a plastic bag and then sprinkled as evenly as possible over each bed (Figure 4.4). Particles used were small (< 63 µm) 1.5 g (9 x  $10^5$  particles  $\pm$  1.6 x  $10^5$  SE) and large (150-250 µm) 5.0 g (8.4 x  $10^5$  particles  $\pm$  1.3 x  $10^5$  SE). Five small sediment cores (3.1 cm<sup>2</sup>) were taken after zero, 2 and 14 tides. Water was added to each sediment core in a small dish. Magnetically responsive particles, comprising the added fluorescent tracer particles and other ferrous particles present in the estuarine sediment, were separated out using a molybdenum magnet, dried and weighed. The samples contained too many grains to place in the counting chamber at one time, so the number of fluorescent particles in a weighed sub-sample (0.026 g  $\pm$  0.001 SE) was counted using fluorescence microscopy (Figure 4.3). The number of particles per core was calculated using Equation 4.1 and used to calculate the mean and percentage particles remaining per test bed.



**Figure 4.4**: Experimental set-up. A known mass of small (< 63  $\mu$ m) and large (150-250  $\mu$ m) fluorescent ferrous tracer particles (Partrac Ltd, http://www.partrac.co.uk) were mixed together in a plastic bag and then sprinkled as evenly as possible over five 0.25 m x 0.20 m (0.05 m²) beds of a) bare sediment and b) *Zostera noltii*. Samples were taken, and particle retention calculated, after zero, 2 and 14 tides.

#### 4.2.3 Statistical analysis

The statistical software used was Minitab 16. *Post hoc* power analysis was carried out using G\*Power 3 (Faul *et al.* 2007). Power analysis calculates the minimum sample size needed to detect an effect of a given size.

# Sediment trapping capacity of Zostera noltii – Flume

The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. Statistical analysis was carried out using two-way ANOVA (Analysis of Variance) and Tukey's *post hoc* test was used to determine where there were significant differences between means. Results were considered significant at p < 0.05.

# Near-bed hydrodynamics; percentage flow reduction - Flume

The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. Statistical analysis was carried out using two-way ANOVA (Analysis of Variance) and Tukey's *post hoc* test was used to determine where there were significant differences between means. Results were considered significant at p < 0.05.

#### Sediment retaining capacity of Zostera noltii

Data for particles counted after 2 and 14 tides were examined. Data expressed as percentage was converted to proportional data. The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. Data were transformed using  $\log_e(x)$  to fulfil the requirements of homogeneity of the variance. Statistical analysis was carried out using two-way ANOVA (Analysis of Variance). Tukey's *post hoc* test was used to determine where there were significant differences between means. Results were considered significant at p < 0.05.

# 4.3 Results

# 4.3.1 Flume study

# Sediment trapping capacity of Zostera noltii

The trapping of particles was mainly influenced by flow velocity (Figure 4.5). Flow had a highly significant effect on the number of large particles (two-way ANOVA,  $F_{2,30} = 23.29$ , p < 0.001, n = 36) (Table 4.2) trapped in test beds. The mean number of particles trapped within the test beds decreased significantly as flow velocity increased (Table 4.3).

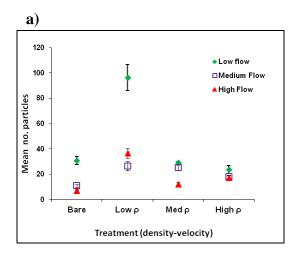
**Table 4.2**: Two-way ANOVA of the influence of flow velocity and *Zostera noltii* shoot density on the trapping of small ( $< 63 \mu m$ ) and large ( $150-250 \mu m$ ) particles.

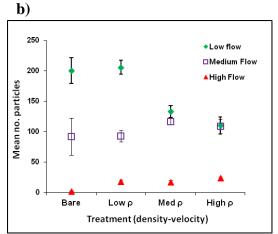
Particle size	Variable	n	F	р	d.f.
< 63 µm	Flow velocity	36	3.12	0.062	2
	Shoot density	36	4.19	0.016	3
	Interaction	36	0.67	0.678	6
150-250 μm	Flow velocity	36	23.29	< 0.001	2
	Shoot density	36	0.2	0.894	3
	Interaction	36	1.02	0.437	6

Deposition of large particles at low and medium flow in vegetated and bare treatments was similar suggesting that deposition was due to particle dropping out of suspension in the low flow conditions, rather than the presence of vegetation. Statistical analysis suggests that shoot density significantly influenced the trapping of small particles (two-way ANOVA,  $F_{3,30} = 4.19$ , p = 0.016, n = 36) (Table 4.2) however the effect was not consistent with increasing density and there was no influence on large particles.

**Table 4.3:** Tukey's *post hoc* tests of the influence of flow velocity and *Zostera noltii* shoot density on the trapping of small (< 63  $\mu$ m) and large (150-250  $\mu$ m) particles. Plots comprised of a combination of low (200 ms<sup>-2</sup>), medium (600 ms<sup>-2</sup>), and high (1000 ms<sup>-2</sup>) *Zostera noltii* shoot densities at low (~0.05 ms<sup>-1</sup>), medium (~0.15 ms<sup>-1</sup>), and high (~0.4 ms<sup>-1</sup>) flow velocities. Particles counts were carried out on five small sediment cores (3.1 cm<sup>2</sup>) taken from each test bed. Means that do not share a letter are significantly different (p < 0.05).

				Mean no. particles			
Particle size		Variable $n$ per core $\pm$ SE Grounds		Variable $n$ per core $\pm$ SE $G$		oupi	ng
< 63 μm	Density	Low	9	$38.84 \pm 8.27$	A		
		Medium	9	$22.56 \pm 2.81$	A	В	
		High	9	$19.55 \pm 2.67$	A	В	
		Bare	9	$16.27 \pm 4.39$		В	
150-250 μm	Flow	Low	12	$156.29 \pm 18.27$	A		
		Medium	12	$102.37 \pm 16.65$		В	
		High	12	$13.88 \pm 3.15$			C

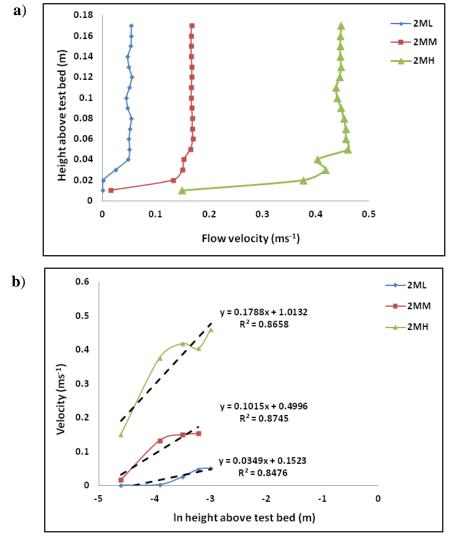




**Figure 4.5**: The mean number of (a) small (<63  $\mu$ m) and (b) large (150-250  $\mu$ m) fluorescent particles per 3.14 cm<sup>2</sup> core retained in test plots in a flume experiment, with SE bars. The study compared the particle trapping properties of bare sediment, low, medium and high density *Zostera noltii* at low, medium and high velocities.

# Near-bed hydrodynamics

Vegetated beds showed more complex vertical velocity profiles than bare sediment. Velocity profiles at higher shoot densities and higher flow regimes also showed accelerated flow over the canopy, consistent with skimming flow (example given as Figure 4.6).



**Figure 4.6**: Examples of a) boundary layer profiles and b) semi-log plots for medium (M) (600 m<sup>-2</sup>) density *Zostera noltii* beds at low (L) (~0.05 ms<sup>-1</sup>), medium (M) (~0.15 ms<sup>-1</sup>) and high (H) (~0.45 ms<sup>-1</sup>) free stream velocities. Plots are labelled for shoot density/flow velocity, e.g. MM is a medium shoot density plot at medium flow. In the boundary layer profiles an increase in flow velocity can be seen as height above the bed increases until the flow reaches free stream velocity. The points of measurement within the boundary layer can be plotted against a log scale of the height above the test bed, allowing shear stress, Reynolds number and roughness length to be calculated. Accelerated flow over the canopy is consistent with skimming flow.

The percentage flow reduction at 1 cm above the bed and within the boundary layer were strongly influenced by both shoot density and flow (two-way ANOVA, p < 0.001, n = 36) (Tables 4.4, & 4.5). At all velocities the reduction in flow was greater in vegetated beds than over bare sediment, with greater reductions at higher densities (Figure 4.7).

**Table 4.4:** Two-way ANOVA of the influence of flow velocity and *Zostera noltii* shoot density on the percentage flow reduction 1 cm above the bed, and within the boundary layer.

Height above	bed Variable	n	F	p	d.f.
1 cm	Flow velocity	36	25.83	< 0.001	2
1 CIII	Shoot density	36	24.54	< 0.001	3
	Interaction	36	2.41	0.058	6
Boundary	Flow velocity	36	40.76	< 0.001	2
•	Shoot density	36	11.27	< 0.001	3
	Interaction	36	1.27	0.308	6

There was a strong positive linear relationship between  $z_0$  and percentage flow reduction (Figure 4.8).  $z_0$  increased with shoot density, but was lower with increasing velocity (Table 4.6). It was highest in low flow, ranging from 6.53 mm  $\pm$  1.49 SE in bare sediment to 13.87 mm  $\pm$  1.15 SE in high shoot densities. In medium and high flow velocities there was a marked increase in boundary layer  $\tau_0$  in vegetation compared to over bare sediment (Figure 4.9), and  $\tau_0$  increased with velocity.

Flow was least turbulent at low velocity with  $Re_r$  values from 2480  $\pm$  212 SE at high shoot density to 4111  $\pm$  202 SE over bare sediment.  $Re_r$  was highest over bare sediment and generally decreased with increasing shoot density. For all treatments Froude number was < 1. Both  $Re_r$  and Fr increased four-fold between low and medium velocities, and by over ten-fold at high flow. There was a weak negative correlation

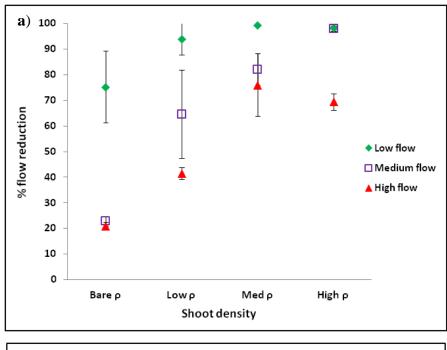
between  $z_0$  and Re<sub>r</sub> (Figure 4.10), with the layer of near-bed laminar flow becoming thinner in more turbulent flow.

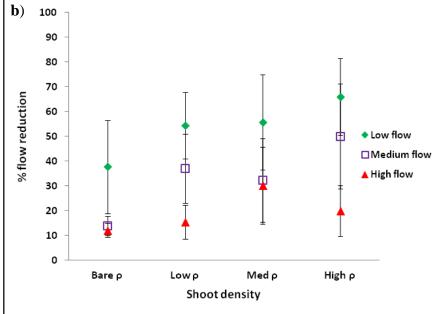
# Post hoc power analysis

Two-way ANOVA had the power  $(1 - \beta)$  of 0.71 for flow velocity and 0.63 for shoot density where effect size f = 0.5 (high), but only 0.22 for flow velocity and 0.19 for shoot density where effect size f = 0.25 (medium).

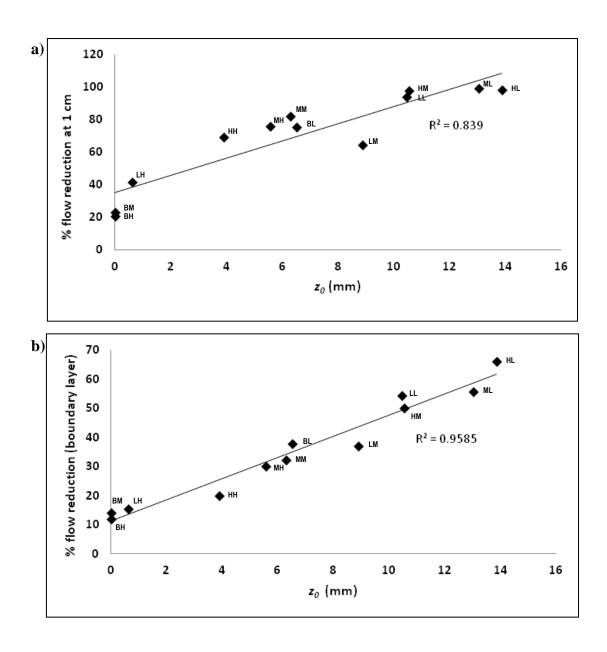
**Table 4.5:** Tukey's *post hoc* tests of the influence of *Zostera noltii* on flow reduction 1 cm above the sediment surface and within the boundary layer. Plots comprised of a combination of low (200 ms<sup>-2</sup>), medium (600 ms<sup>-2</sup>), and high (1000 ms<sup>-2</sup>) *Zostera noltii* shoot densities at low ( $^{\circ}0.05$  ms<sup>-1</sup>), medium ( $^{\circ}0.15$  ms<sup>-1</sup>), and high ( $^{\circ}0.4$  ms<sup>-1</sup>) flow velocities. Means that do not share a letter are significantly different (p < 0.05).

	Shoot			Mean flow					
Height above bed	density	Flow velocity	n	reduction ± SE		Gt	oupi	ng	
1 cm	Med	Low	3	$99.5 \pm 0.24$	A				
	High	Low	3	$98.3 \pm 1.4$	A				
	High	Med	3	$98.0 \pm 1.1$	A				
	Low	Low	3	$93.9 \pm 6.2$	A				
	Med	Med	3	$82.2 \pm 6.2$	A				
	Med	High	3	$76.1 \pm 12.3$	A	В			
	Bare	Low	3	$75.3 \pm 14.1$	A	В			
	High	High	3	$69.5 \pm 3.2$	A	В			
	Low	med	3	$64.7 \pm 17.2$	A	В			
	Low	High	3	$41.5 \pm 2.3$		В	C		
	Bare	Med	3	$22.9 \pm 2.0$			C		
	Bare	High	3	$21.0\pm1.5$			C		
Boundary layer	High	Low	3	$65.8 \pm 0.9$	Α				F F F F F
	Med	Low	3	$55.2 \pm 0.6$	A	В			
	Low	Low	3	$50.8 \pm 8.5$	A	В	C		
	High	Med	3	$49.5 \pm 1.2$	A	В	C		
	Med	Med	3	$39.9 \pm 10.1$	A	В	C	D	
	Bare	Low	3	$38.1 \pm 3.4$		В	C	D	F
	Low	Med	3	$35.9 \pm 10.2$		В	C	D	F
	Med	High	3	$28.3 \pm 4.7$			C	D	I
	High	High	3	$20.9 \pm 1.4$				D	I
	Bare	Med	3	$16.5 \pm 0.3$				D	I
	Low	High	3	$15.4 \pm 2.5$				D	I
	Bare	High	3	$12.5 \pm 1.8$					





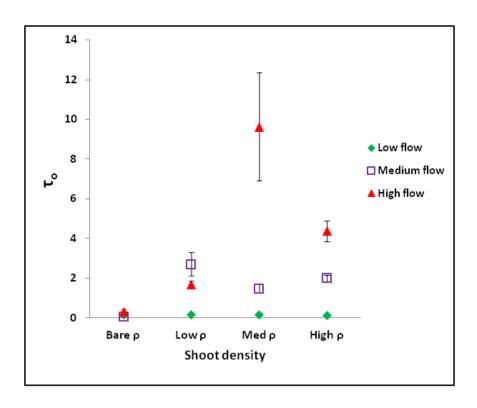
**Figure 4.7**: The mean percentage flow reduction with SE bars at a) 1 cm above the sediment surface and b) within the boundary layer above test plots of bare sediment, low, medium and high density *Zostera noltii* at low, medium and high velocities.



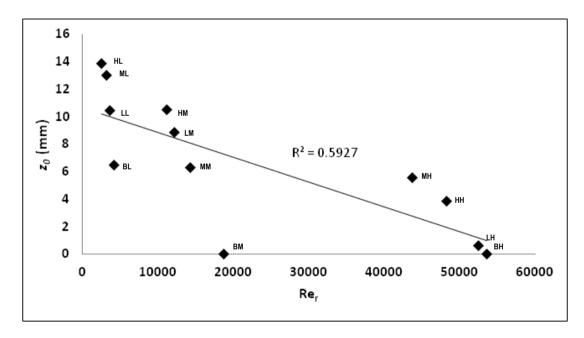
**Figure 4.8**: The linear relationship between roughness length ( $z_0$ ) and percentage flow reduction at a) 1 cm and b) within the boundary layer of test beds consisting of bare sediment or *Zostera noltii* at varying shoot densities and free stream flow velocities. Data points are labelled for shoot density/flow velocity: shoot densities are bare (B), low (L) (200 m<sup>-2</sup>), medium (M) (600 m<sup>-2</sup>) and high (H) (1000 m<sup>-2</sup>); flow velocities are low (L) (~0.05 ms<sup>-1</sup>), medium (M) (~0.15 ms<sup>-1</sup>) and high (H) (~0.45 ms<sup>-1</sup>), e.g. HM is a high shoot density plot at medium flow.

**Table 4.6:** Summary of shear velocity  $U_*$  (ms<sup>-1</sup>), shear stress  $\tau_0$  (Nm<sup>-2</sup>), boundary roughness Reynolds number (Re<sub>r</sub>), percentage flow reduction 1 cm above the flume base and within the boundary layer, roughness length  $z_0$  (mm) and Froude number (Fr) for different *Zostera noltii* shoot densities ( $\rho$ ) and flow velocities (u). Mean  $\pm$  SE.

Plot type	U* (ms <sup>-1</sup> )	$\tau_{\rm o}~({\rm Nm}^{-2})$	Re <sub>r</sub>	% flow reduction (1cm)	% flow reduction (boundary layer)	$z_0$ (mm)	Fr
Bare, low $u$	$0.013 \pm 0.002$	$0.17 \pm 0.05$	$4111 \pm 202$	$75.3 \pm 14.1$	$37.8 \pm 18.8$	$6.53 \pm 1.49$	$0.006 \pm 0.001$
Low $\rho$ , low $u$	$0.013 \pm 0.002$	$0.19 \pm 0.05$	$3507 \pm 614$	$93.9 \pm 6.2$	$54.4 \pm 13.4$	$10.47 \pm 2.13$	$0.004 \pm 0.001$
Med $\rho$ , low $u$	$0.013 \pm 0.001$	$0.19 \pm 0.02$	$3159 \pm 143$	$99.5 \pm 0.2$	$55.7 \pm 19.2$	$13.04 \pm 0.70$	$0.003 \pm 0.00$
High $\rho$ , low $u$	$0.012 \pm 0.001$	$0.15 \pm 0.02$	$2480 \pm 212$	$98.3 \pm 1.4$	$66.0 \pm 15.5$	$13.87\pm1.15$	$0.003 \pm 0.00$
Bare, med u	$0.008 \pm 0.000$	$0.06 \pm 0.01$	$18708 \pm 734$	$22.9 \pm 1.97$	$14.0 \pm 3.8$	$0.02 \pm 0.01$	$0.022 \pm 0.001$
Low $\rho$ , med $u$	$0.051 \pm 0.006$	$2.71 \pm 0.59$	$12094 \pm 1034$	$64.7 \pm 17.2$	$37.1 \pm 14.0$	$8.89 \pm 0.87$	$0.017 \pm 0.003$
Med $\rho$ , med $u$	$0.037 \pm 0.003$	$1.46\pm0.22$	$14222 \pm 2204$	$82.2 \pm 6.2$	$32.3 \pm 16.8$	$6.29 \pm 0.93$	$0.017 \pm 0.002$
High $\rho$ , med $u$	$0.044 \pm 0.002$	$2.00\pm0.15$	$11133 \pm 154$	$98.0 \pm 1.0$	$50.0 \pm 21.2$	$10.54 \pm 0.36$	$0.013 \pm 0.00$
Bare, high u	$0.017 \pm 0.003$	$0.32 \pm 0.10$	$53585 \pm 2023$	$21.0\pm1.5$	$12.1 \pm 2.8$	$0.01 \pm 0.01$	$0.058 \pm 0.001$
Low $\rho$ , high $u$	$0.041 \pm 0.002$	$1.70\pm0.17$	$52425 \pm 1449$	$41.5 \pm 2.3$	$15.4 \pm 6.8$	$0.63 \pm 0.16$	$0.055 \pm 0.002$
Med $\rho$ , high $u$	$0.095 \pm 0.014$	$9.63 \pm 2.72$	$43694 \pm 3205$	$76.1 \pm 12.3$	$30.2 \pm 15.6$	$5.56 \pm 1.22$	$0.052 \pm 0.006$
High $\rho$ , high $u$	$0.065 \pm 0.004$	$4.38 \pm 0.51$	$48207 \pm 1332$	$69.5 \pm 3.2$	$19.9 \pm 10.3$	$3.89 \pm 0.64$	$0.048 \pm 0.003$



**Figure 4.9**: Shear stress ( $\tau_0$ ) with SE bars within the boundary layer above test plots of bare sediment, low, medium and high density *Zostera noltii* at low, medium and high velocities.

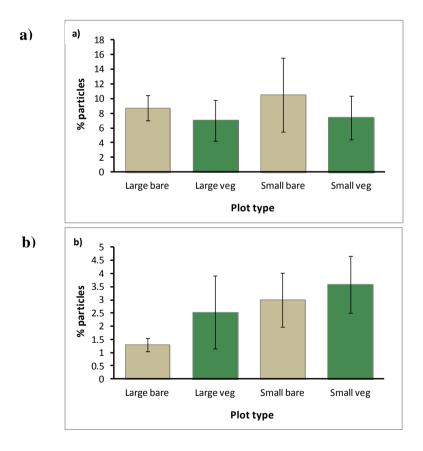


**Figure 4.10**: The linear relationship between Reynolds number (Re<sub>r</sub>) and roughness length ( $z_0$ ) within the boundary layer of test beds consisting of bare sediment or *Zostera noltii* at varying shoot densities and free stream flow velocities. Data points are labelled for shoot density/flow velocity: shoot densities are bare (B), low (L) (200 m<sup>-2</sup>), medium (M) (600 m<sup>-2</sup>) and high (H) (1000 m<sup>-2</sup>); flow velocities are low (L) (~0.05 ms<sup>-1</sup>), medium (M) (~0.15 ms<sup>-1</sup>) and high (H) (~0.45 ms<sup>-1</sup>), e.g. HM is a high shoot density plot at medium flow.

# 4.3.2 Field study

# Sediment retaining capacity of Zostera noltii

After two tides the percentage of particles of both size categories in bare and vegetated treatments had decreased by > 90%. After 14 tides 1.9 %  $\pm$  2.2 SE of large and 3.3 %  $\pm$  2.2 SE of small particles remained. A significant number of small particles (two-way ANOVA,  $F_{1,16} = 5.84$ , p = 0.028, n = 20) and a highly significantly number of large particles ( $F_{1,16} = 17.67$ , p = 0.001, n = 20) (Table 4.7) were lost over this 12 tide period. Over the 12 tides there was no significant difference between the percentage of small or large particles retained in bare and vegetated plots. However, there were greater losses of large particles from bare plots than from vegetated plots.



**Figure 4.11**: The percentage, with error bars, of small (< 63  $\mu$ m) and large (150-250  $\mu$ m) fluorescent particles retained in plots where *Zostera noltii* (veg) is growing and bare plots at Tayport over a) two and b) 14 tides.

**Table 4.7**: Two-way ANOVA of the influence *Zostera noltii* presence or absence, and the number of tidal inundations on particle loss. The loss of two size classes of particle were investigated: small (< 63  $\mu$ m) and large (150-250  $\mu$ m).

Particle size	Variable	n	F	p	d.f.
< 63 μm	No. tides	10	5.84	0.028	1
	Plot type	10	0.01	0.91	1
	Interaction	10	0.77	0.39	1
150-250 μm	No. tides	10	17.67	0.001	1
	Plot type	10	0.05	0.83	1
	Interaction	10	0.86	0.36	1

**Table 4.8:** Tukey's *post hoc* tests of the influence *Zostera noltii* presence or absence, and the number of tidal inundations on particle loss. The loss of two size classes of particle were investigated: small (< 63  $\mu$ m) and large (150-250  $\mu$ m). Particles counts were carried out on five small sediment cores (3.1 cm²) taken from each test bed. Means that do not share a letter are significantly different (p < 0.05).

Particle size	No. tides	Veg/bare	n	Mean % particles remaining per core	Grou	ping
150-250 μm	2	Bare	5	$8.7 \pm 1.7$	A	
< 63 µm	2	Bare	5	$10.5 \pm 5.0$	A	
< 63 µm	2	Veg	5	$7.4 \pm 3.0$	A	В
150-250 μm	2	Veg	5	$7.0 \pm 2.8$	A	В
< 63 µm	14	Veg	5	$3.6 \pm 1.1$	A	В
< 63 µm	14	Bare	5	$3.0 \pm 1.0$		В
150-250 μm	14	Veg	5	$2.5 \pm 1.4$		В
150-250 μm	14	Bare	5	$1.3 \pm 0.3$		В

# 4.4 Discussion

# 4.4.1 Particle trapping and flow reduction

The Tayport sand and mudflats are a tide-dominated system, and although wind generated waves will resuspend sediment and increase the exchange between the sediment surface and the water column, only unidirectional flow was considered in the laboratory due to the constraints of the facilities: flume studies were carried out using a linear flume with a unidirectional, single-flow channel.

Flow velocity was the main influence on particle deposition during this experiment, with most particle deposition occurring at lower flow velocities, supporting H<sub>3</sub>. Most particle deposition occurred in low water flow (~0.05 ms<sup>-1</sup>), typical of ambient conditions in Z. noltii beds in the Tay estuary (pers. comms. - ad hoc flow measurements were made at Tayport throughout the project using a SENSA RC2 ADS water velocity meter). In low flow treatments the large reduction in flow within the boundary layer and near the bed enhanced deposition and inhibited resuspension of trapped particles.  $z_0$  was greatest in low flow (< 6.5 mm) therefore the turbulent water layer was not low enough in the bed to remove deposited particles from the sediment surface. Boundary layer  $Re_r$  (turbulence) and  $\tau_0$  were both low. The similarity between boundary  $\tau_0$  calculated for bare and vegetated beds suggests that the presence of Z. noltii does not greatly increase friction in the water column in low flow conditions. Although Z. noltii altered the shape of the velocity profile by extending the boundary layer upwards, the mean boundary layer height only increased from 3.8 cm over bare sediment to 4.4 cm in high density treatments. The number of large particles (150-250 μm) was similar in both bare and low shoot density beds. This may be due to larger, heavier particles settling out of suspension in low energy conditions rather than being trapped by seagrass leaves. There were fewer particles in medium and high density beds, thus disproving H<sub>1</sub> and H<sub>2</sub>; however particles may have been adsorbed to leaf blades instead of reaching the sediment surface (see Chapter 5, section 5.3.2).

As flow velocity increased to medium flow ( $\sim 0.15 \text{ ms}^{-1}$ ) particle deposition was lower for both particle size classes. More particles were trapped on the bed when vegetation was present, although increasing shoot density had no significant influence. Flow reduction and  $z_0$  showed a marked increase in vegetated beds compared with bare sediment, although they were lower than those found in slower flow.

At very high flows (> 0.4 ms<sup>-1</sup>) the surface of bare sediment was badly scoured, removing biofilms that could have potentially adsorbed particles (see Chapter 5). The critical velocity was exceeded for the erosion and transport of estuarine sediment particles and any deposited fluorescent particles of both sizes (Hjulstrom 1939; Gordon *et al.* 1992). The presence of vegetation reduced scouring but had little effect on the trapping of suspended particles. Although  $\tau_0$  was not influenced by shoot density in low flow,  $\tau_0$  increased with flow and with the presence of leaves. In larger beds this may facilitate the settling of suspended matter (Koch 1999) but the effect was not evident at the small-scale of this experiment. Although the sediments and their corresponding bed roughness were similar in all treatments, the presence of the *Z. noltii* canopy in the benthic boundary layer altered the roughness of the bottom. Vegetated beds were exposed to high boundary  $\tau_0$  and highly turbulent flow, although in medium and high density beds this may have been buffered by large reductions in near-bed flow and relatively high  $z_0$  compared to bare and low density treatments.

At all flow speeds vegetated beds showed more complex vertical flow profiles, with the development of skimming flow at higher shoot densities and higher flow velocities, which was indicated by accelerated flow over the canopy. Many particles of both size classes were carried over, rather than through the canopy by this skimming flow. Skimming flow has been noted in previous studies of flow in seagrass meadows (e.g. Fonseca *et al.* 1982; Worcester 1995; Gacia *et al.* 1999; Koch & Gust 1999; Peralta *et al.* 2008) and Scoffin (1970) observed that dense *T. testudinum* beds could withstand free stream velocities of up to 1.5 ms<sup>-1</sup> before in-meadow scour occurred due to the combination of skimming flow, and decreased current velocity under the canopy. Overall, fewer small particles were trapped in the beds - on average < 50 per core compared with up to 200 per core of large ones. The particles were injected into flowing water where smaller particles, having a lower critical velocity, would have remained in suspension and been carried over the bed in all flow velocities, whereas some of the largest particles would be deposited in low flow (Hjulstrom 1939; Gordon *et al.* 1992).

Fluorescent particles were adsorbed onto the leaf surfaces and a count of the particles trapped by the leaves would have greatly improved this study. Although samples were taken, on this occasion it was not possible to count them due to equipment failure at the time of the experiment and subsequent degradation of the samples. However, particle capture by leaves is discussed in Chapter 5.

Both  $Re_r$  and Fr were typical of conditions found in estuaries; flows over tidal flats are mostly subcritical (Fr < 1) (Nowell & Jumars 1987) and  $Re_r$  in (tidal) estuaries has been measured as ranging from  $10^4$  to  $10^8$  (Chanson & Trevethan 2006; Trevethan & Chanson 2009).  $Re_r$  increased with increasing velocity, but decreased in the presence of leaves. Leaves suppress turbulence in the main flow while generating their own microturbulence. This may be an adaptive trait to increase local turbulence to a level where gas exchange and nutrient uptake can take place (Anderson & Charters 1982).

Scaling issues were considered during the experimental design, but as the flume depth and patch size were similar to conditions in areas of Z. noltii in the Tay Estuary no scaling adjustments were required. However, when making measurements in a flume it is important to consider that small bed size and edge effects will influence results, and that these results might not be extrapolated to a full-size meadow elsewhere. The edge is the most dynamic zone of the meadow with regards to current flow, as flexing of the leaves re-directs the current over and under the canopy, reducing the current velocity further into the bed (Fonseca et al. 1982). For example, Peterson et al. (2004) found that in dense Zostera marina beds flow velocity was reduced by 60% at 0.02 m height, 0.25 m into the bed, and that this reduction increased with distance into the meadow. Fonseca et al. (1982) found that the reduction of current began further into the meadow with increasing velocity, and so were unable to exactly replicate the effect of natural Z. marina on current flow in a flume. In this experiment the high number of particles trapped in low density beds compared to higher densities is likely to be due to edgeeffects and skimming flow rather than the sediment trapping properties of leaves. We suggest that more particles would have been present in medium and high density beds if the test beds had been longer.

As well as influencing the type of flow the flexing of *Z. noltii* leaves also affected the extent to which flow energy was attenuated, as this is dependent on the water depth and the portion of the water column taken up by the leaves (Fonseca & Cahalan 1992). For example Fonseca & Cahalan (1992) measured 40% wave reduction caused by four species when leaf length was similar to water depth. While larger seagrass species occupy more of the water column, *Z. noltii* only influences flow in the bottom few cm, although this is important high up the intertidal zone in shallow waters. Widdows *et al.* (2008) observed that near-bed (0.05 ms<sup>-1</sup>) current velocities were reduced by 40% in high density *Z. noltii* beds at velocities > 0.1 ms<sup>-1</sup> while we observed a 70% reduction. Larger seagrasses may affect greater sediment deposition (Fonseca & Fisher 1986), but smaller species, for example *Halophila decipiens* (Fonseca 1985, 1989), and *Z. muelleri* (Connolly 1995) also modify their sediment environment.

# 4.4.2 Sediment retaining capacity of Z. noltii in the field

There was a large loss (> 90%) of particles between application to the test beds and first sampling after two tides, and for both particle size classes there were significant further losses over 12 tides. For small particles there was no difference between vegetated and bare plots, but *Z. noltii* retained large particles more effectively than bare plots over time, partly supporting H<sub>4</sub>. In the low energy estuarine environment it would be expected that fewer large particles would be lost as it takes more energy to reach their critical threshold, moving them over the bed or into suspension, than for smaller ones. However, large particles are not adsorbed as strongly to biofilms as small particles (see Chapter 5, section 5.3) and less force is needed to remove them from the sediment surface. In low energy conditions, beds of the seagrasses *Thalassia testudinum* and *Posidonia oceanica* also demonstrate significantly greater particle retention than bare sediment (Koch 1999; Gacia *et al.* 1999; Terrados & Duarte 2000; Gacia & Duarte 2001). Most previous studies did not consider the retention of grains of different sizes; however Koch (1999) noted that beds trap finer particles which are suspended at lower flow intensities.

During the experiment there were low velocity flow conditions in the estuary, with an average offshore windspeed of 7.4 ms<sup>-1</sup> (ranging from 2.1 to 13.9 ms<sup>-1</sup>) (www.windguru.cz). Test beds contained *Z. noltii* at high shoot densities and although flow was not fast enough for skimming flow to develop, the leaves will have reconfigurated, forming a protective layer above the sediment surface. Any suspended sediment under the canopy is caught up in the leaf blades and settles out where it cannot easily be resuspended by tidal flow (Ginsberg & Lowenstam 1958; Scoffin 1970; Gacia *et al.* 1999). In low flow in the flume there was a 98% reduction in flow velocity at 1cm height, with a  $z_o$  of < 10 mm. If similar conditions were replicated *in situ* particle erosion from the sediment surface would have been inhibited.

It is important to remember that seagrass beds are sources of suspended matter as well as sinks. It is vital that the burial of the plants is avoided as mortality can occur at low burial levels (Cabaço *et al.* 2008), therefore resuspension is an integral part of the sediment dynamics of seagrass systems.

#### 4.5 Conclusions

For small patches of *Z. noltii* similar to those found in the Tay Estuary, the following conclusions can be made from the results of these studies:

- Patches of Z. noltii do not trap more particles than patches of bare sediment.
- Particle trapping by Z. noltii does not increase with increasing grass density.
- Sediment trapping by Z. noltii decreases with increasing flow.

The number of large particles trapped in both low and medium flows was similar in vegetated and bare treatments, suggesting that particles were deposited out of suspension in low energy conditions rather than due to the presence of vegetation. In low flow, low density seagrass beds trapped more particles on the sediment surface than higher density beds. This result was explained in hydrodynamic terms because skimming flow develops at higher shoot densities, carrying suspended particles over the

plants following leaf reconfiguration in flow. Particles may have adsorbed to leaf surfaces. In low energy flow conditions *Z. noltii* did not greatly increase the height of the boundary layer or shear stress within the bed, and turbulence within the main flow is supressed. Importantly, it should be considered that these results may represent meadow-edge effects only, and might not be extrapolated to a full-size meadow.

• Z. noltii decreases the loss of large particles (>150  $\mu$ m) through resuspension, but does not influence the loss of small particles (<63  $\mu$ m).

Although smaller particles should be more readily resuspended than larger particles in low flows, they are trapped on the sediment surface by biofilms; this is explored in Chapter 5.

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# Chapter 5

# Measurement of particle adhesion to sediment and leaf biofilms using magnetic particle induction (MagPI)

#### Abstract

Both sediment and leaf biofilms play an important role in the retention of sediment particles within intertidal seagrass beds. Sediment biofilms increase sediment cohesion and stability, and particle retention on sediment and leaf surfaces is also increased by the adhesive properties of extracellular polymeric substances (EPS) associated with biofilm development. Larson et al. (2009) reported the method of magnetic particle induction (MagPI) whereby the surface adhesive capacity of sediment biofilms could be analysed without reaching the critical erosion threshold for surface erosion. This method was used to compare the adhesive strength of sediment biofilms in bare sediment and Z. noltii beds. MagPI was also used to determine the adhesive capacity of EPS secreted by epiphytes on leaves of Z. noltii. The adhesion of particles to leaf biofilms was further explored using a flume system, comparing different parts of the leaves at three different flow velocities (0.05, 0.15, 0.45 ms<sup>-1</sup>). During these experiments, particles of two different sizes (< 63 µm and 150-250 µm), typical of those found in Z. noltii beds in the Tay estuary, were compared. Small particles adhered more strongly to leaf and sediment surface biofilms than large ones. Sediment biofilms in patches of Z. noltii and bare sediment were similarly adhesive. Neither flow velocity nor position on the leaf (base, middle or tip) significantly influenced the trapping of small particles. However more particles were trapped where leaf surfaces had a large covering of epiphytic diatoms.

#### 5.1 Introduction

## 5.1.1 Adhesion of sediment particles to aquatic biofilms

It has been shown that macrophytes, such as seagrasses, influence sediment deposition and habitat stability by reducing flow within the bed (Gambi et al. 1990; Widdows et al. 2008), and by reflecting, trapping and resettling particles (Gacia & Duarte 2001; Hendriks et al. 2008). However, sediment stabilisation is also influenced at a microscopic scale by microphytobenthos, in the form of biofilms and microbial mats (Holland et al. 1974; Grant & Gust 1987; Dade et al. 1990; Paterson 1997). The microorganisms, such as diatoms and cyanobacteria, that comprise sediment biofilms, secrete "Extracellular Polymeric Substances" (EPS) (Stal 1995; Underwood et al. 1995; de Winder et al. 1999). These mucilaginous secretions form a cohesive matrix surrounding the particles of intertidal sediments or a flexible coating over plant surfaces (Decho 2000). EPS performs a number of functions, for example it protects microbial cells from environmental changes such as desiccation or changes in salinity (Decho 1990); it acts as a mechanism for the locomotion of diatoms (Edgar & Pickett-Heaps 1984); and it is responsible for the cohesion of cells to particulate materials and their adhesion to the substratum (Characklis & Wilderer 1989; Wingender 1999). EPS binds sediment particles together because of chemical bonds and electrostatic attractions (Paterson & Hagerthey 2001; Salant, 2011).

The adhesion of particles to biofilms is common in aquatic systems, and occurs on a wide range of substrata including surfaces in freshwater streams (Battin *et al.* 2003), marine mudflats (Westall & Rinse 1994), and on the leaves of aquatic macrophytes such as seagrasses (Scoffin 1970; den Hartog & Phillips 2001; Agawin & Duarte 2002). Seagrass leaves act as a substratum for diatom attachment (den Hartog & Phillips 2001; Lebreton *et al.* 2009), protozoans (Agawin & Duarte 2002), calcareous and soft algae, and bryozoans (Scoffin 1970).

Both sediment and leaf biofilms may play an important role in the retention of sediment within intertidal seagrass beds. Sediment biofilms increase sediment cohesion and

stability (Grant & Gust 1987; Dade *et al.* 1990; Madsen *et al.* 1993; Sutherland *et al.* 1998; Underwood & Paterson 2003), and the retention of particles deposited on sediment and leaf surfaces is increased by the adhesive properties of EPS (Staats *et al.* 2001; Agawin & Duarte 2002; Gacia *et al.* 2003; Salant 2011).

In turbulent flow conditions biofilms can be eroded by flow-induced disturbance (Paterson & Hagerthey 2001; Battin *et al.* 2007). The erosional behaviour of sediments has previously been measured in terms of critical erosion threshold ( $\tau_{crit}$ ) for sediment transport and erosion rates ( $\epsilon$ ) using several different methods including a propeller (EROMES, Schuenemann & Kuehl 1991), water jets (CSM, Paterson 1989), water flow (e.g., Sedflume, McNeil *et al.* 1996; SETEG, Kern *et al.* 1999), or combined suction and flow (Gust-Microcosm, Gust & Mueller 1997). These approaches all require bed failure, therefore intermediate changes to the surface before the point of erosion cannot be measured (Tolhurst *et al.* 2000). However, sediment grains can be removed from biofilms without reaching  $\tau_{crit}$  (Larson *et al.* 2009).

Larson *et al.* (2009) reported the method of magnetic particle induction (MagPI) whereby the surface adhesive capacity of sediment biofilms could be analysed without reaching the critical erosion threshold – the point of bed failure. This method was used to compare the adhesive strength of sediment biofilms on bare sediment with those within *Z. noltii* beds. *In situ* comparisons of sediment colloidal carbohydrate and chlorophyll *a* content in bare sediment and *Z. noltii* beds in the Tay Estuary (Chapter 2, section 2.3.2), indicate that biofilm adhesiveness should be comparable between these two treatments. Furthermore, similarities in the retention of small particles (< 63  $\mu$ m) in both bare and vegetated sediments, in low to high flow velocities (Chapter 4, section 4.3.1), suggest that smaller particles are more cohesive than larger ones (150-250  $\mu$ m), while the retention of larger particles is related to energy conditions.

MagPI was also used to determine the adhesive capacity of EPS secreted by epiphytes on leaves of *Z. noltii*, to explore how strongly sestonic particles were attached to leaf surfaces. The adhesion of particles to leaf biofilms was further explored using a flume, comparing different parts of the leaves at three different flow velocities. Both these

experimental methods introduce novel techniques to investigate the attachment of sestonic particles to seagrass leaf surfaces.

#### **5.1.2** Aims

In this chapter the adhesive properties of sediment and leaf biofilms present in the Tay Estuary were explored. The forces required to remove ferrous particles from sediment biofilms in bare and vegetated sediment, and also from the surface of leaves of *Z. noltii* were compared. Two different size classes of grain were used; small ( $< 63 \mu m$ ) and large (150-250  $\mu m$ ). Particle sizes were indicative of those found in the *Zostera* beds in the Tay Estuary (Chapter 2, section 2.3.2).

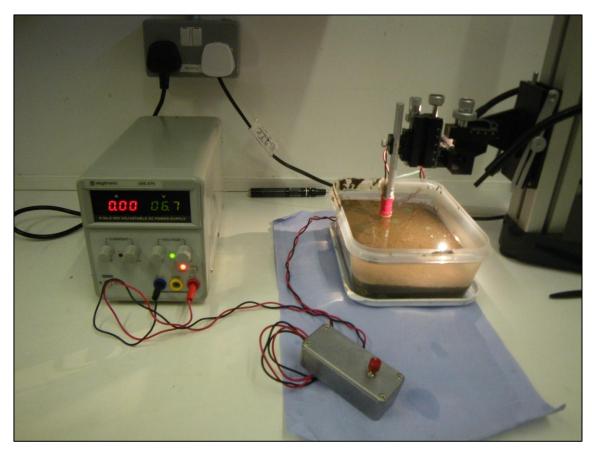
The following hypotheses, formulated using experience gained in the previous chapters, were tested in order to determine the function of aquatic biofilms, found in *Z. noltii* beds in the Tay Estuary, in sediment retention and stabilisation:

- H<sub>1</sub> Sediment biofilms in patches of *Z. noltii* are more adhesive that biofilms in bare sediment.
- H<sub>2</sub> Small particles will adhere more strongly to leaf and sediment surface biofilms than large ones.

# **5.2** Materials and Methods

## 5.2.1 MagPI – sediment and leaf biofilms

The MagPI equipment comprises an electromagnet, with a tip 9.621 mm<sup>2</sup>, linked to a precision power supply (Skytronic 650.676 0-3 A, 0-30 V adjustable DC power supply) (Figure 5.1). The electromagnet was made of copper wire (26 standard wire gauge), wrapped 400 times around a 9 mm diameter electrical steel core.



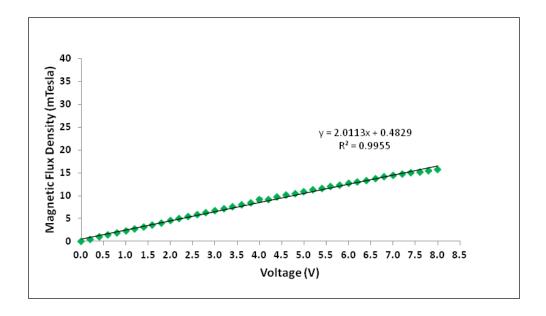
**Figure 5.1:** The MagPI equipment comprises an electromagnet, with a tip 9.621 mm<sup>2</sup>, linked to a precision power supply (Skytronic 650.676 0-3 A, 0-30 V adjustable DC power supply).

#### Calibration

To calibrate the device, the magnet was placed 10 mm above a gauss meter (Hengtong magnetoelectricity Co. Ltd. HT201 Gauss meter). The calibrations were performed in saltwater using a waterproof sensor. The voltage and current were increased in small increments (0.2 V/  $\sim$  0.05 A), and the Magnetic Flux Density (MFD) for each increase was recorded. The calibration curve was determined from the slope of the line (Figure 5.2). There was a strong correlation between (MFD) (mTesla) and the current supplied to the electromagnet ( $r^2 = > 0.95$ ) (Larson *et al.* 2009).

A very low power (MFD) permanent magnet (0.5 mTesla) was used during experimental set-up to remove any particles that were loose, or had very poor contact

with the sediment surface. The MFD of this magnet was also measured using the gauss meter.



**Figure 5.2:** The calibration curve in saltwater at 10 mm above sediment is determined from the slope of the line. There is a strong correlation between Magnetic Flux Density (MFD) (mTesla) and the current supplied to the electromagnet ( $r^2 = > 0.95$ ).

## Sediment biofilms

Five box cores (0.20 m x 0.20 m x 0.10 m) of bare sediment and sediment vegetated with *Z. noltii* were taken from Tayport on 31<sup>st</sup> August 2010. The cores were stored in un-lidded plastic containers in the laboratory so that the sediment was exposed to light. Seawater was carefully added to the containers, avoiding further disturbance to the sediment, and the cores were left to settle for 24 hours.

The test particles used (Partrac Ltd, http://www.partrac.co.uk) are a composite including iron fragments and pigment particles in a binding agent. During manufacture, the particles are ground to the desired size and their density adjusted. Two different size classes of ferrous particles were used; small ( $< 63 \mu m$ ) and large (150-250  $\mu m$ ). Particle sizes were indicative of those found the *Zostera* beds in the Tay Estuary. There

were three test patches of each grain size per box core. Test particles were suspended in water, and then drawn into a plastic pipette. The particles were allowed to settle towards the tip of the pipette before being released as a single drop into the water above the sediment surface. A cut-off 2 ml syringe, submerged into the water and held ~ 1-2 mm above the test surface, confined the particles to a small test area of ~ 10 mm diameter. A low MFD magnet (0.5 mTesla) was held 10 mm above the test area to remove any particles that were loose, or had very poor contact with the sediment surface. This achieved a relatively even single layer of particles on the test surface.

The electromagnet was positioned underwater, with the tip 10 mm above the particles. The magnetic field was increased in increments until (i) a small number of particles (around 5) were attracted to the magnet; and (ii) total removal of particles under the magnet occurred. The MFD was determined using the calibration curve, and the mean MFD per box core for each sediment size was calculated. Results were compared to a control, where the force required to remove particles from a clean ceramic tile immersed in seawater was measured.

## Leaf biofilms

 $Z.\ noltii$  leaves were removed from the aforementioned cores at the leaf base using forceps. They were sprinkled with small (< 63 µm) or large (150-250 µm) ferrous particles until the entire leaf surface was covered. Leaves were immersed in seawater and gently moved back and forth to dislodge any loose particles. Each leaf was secured to a ceramic tile using elastic bands and placed at the bottom of a container of seawater. The electromagnet was positioned underwater, with the tip 10 mm above the leaf and its particles. The magnetic field was increased in increments until total removal of particles under the magnet was achieved. Results were compared to a control, where the force required to remove particles from a clean ceramic tile immersed in seawater was measured.

# 5.2.2 Flume study - leaf biofilms

Flume studies were carried out using an 8 m linear saltwater flume (Armfield Ltd, Ringwood, England; Chapter 4, Figure 4.3) modified with a false bed engineered to accept rectangular sample box cores (0.25 m x 0.2 m x 0.11 m). Free stream velocity was monitored using an acoustic Doppler velocimeter (ADV) at 1 Hz (Gratoit *et al.* 2000; Chanson 2008) placed 0.5 m in front of the test bed.

The experiment consisted of three treatments, composed of high density *Z. noltii*, 50 shoots per core (1000 m<sup>-2</sup>), under low, medium and high flow conditions. Shoot densities and flow velocities were representative of those found in the field. Flow conditions were low  $\sim 0.05 \text{ ms}^{-1}$ , medium  $\sim 0.15 \text{ ms}^{-1}$  and high  $\sim 0.45 \text{ ms}^{-1}$ .

A box core (0.25 m x 0.20 m x 0.11 m) of sediment vegetated with *Z. noltii* was taken from Tayport on  $31^{\text{st}}$  August 2010. Excess *Z. noltii* shoots were excised to obtain the required shoot density. The core was inserted into a core-well in the floor of the flume, 2 m upstream of the flume exit. The core height was adjusted until the sediment surface was flush with the floor. The test bed was covered with a protective steel mesh (1.5 mm steel), aperture size  $6 \times 6 \text{ mm}$  to prevent erosion and the flume was filled with saltwater. The velocity of the flow was increased in increments until the required water speed  $(\sim 0.05 \text{ ms}^{-1})$  and height (0.23 m) were reached. The mesh and the ADV probe were removed before the addition of tracer particles.

A known mass of fluorescent ferrous particles was injected into the flow directly upstream of the bed. The particles used were small (< 63  $\mu$ m) 1.5 g (9 x 10<sup>5</sup> particles  $\pm$  1.6 x 10<sup>5</sup> SE) and large (150-250  $\mu$ m) 5.0 g (8.4 x 10<sup>5</sup> particles  $\pm$  1.3 x 10<sup>5</sup> SE).

After thirty minutes under flow, five leaves were removed at their base with tweezers and stored in a vial of salt water. This was repeated for medium and high flows. Three 2 cm sections were taken from each leaf blade – from the apical-end (tip), middle and base. The number of fluorescent particles adhered to each leaf section was counted using fluorescence microscopy. Only one side of each leaf section was examined.

# **5.2.3** Statistical analysis

The statistical software used was Minitab 16.

## Sediment biofilms - MagPI

The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. Since this was the case, statistical analysis was then carried out for each particle size class using two-way ANOVA (Analysis of Variance) and Tukey's *post hoc* test was used to find which means were significantly different from one another. Results were considered significant at p < 0.05.

#### Comparison of sediment vs. leaf biofilms - MagPI

The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. Since this was the case, statistical analysis was carried out using one-way ANOVA (Analysis of Variance) and Tukey's *post hoc* test was used to find which means were significantly different from one another. Results were considered significant at p < 0.05.

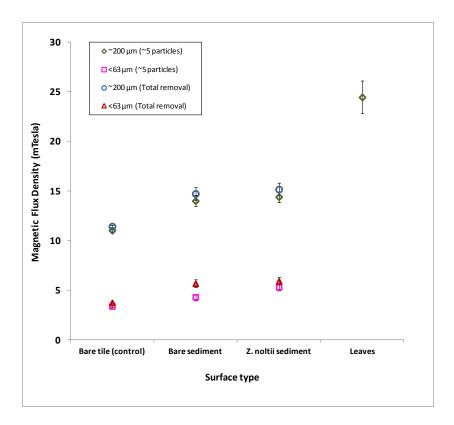
# *Leaf biofilms – flume study*

The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. Data were square root transformed to fulfil the requirements of homogeneity of the variance. Statistical analysis was carried out using two-way ANOVA (Analysis of Variance). Results were considered significant at p < 0.05.

#### 5.3 Results

# 5.3.1 MagPI – sediment and leaf biofilms

Significantly more force was needed to lift both large and small particles from sediment than from the ceramic tile (two-way ANOVA,  $F_{2,56} = 37.72$ , p < 0.001, n = 60) (Tables 5.1 & 5.2), however there was no difference between the adhesive properties of biofilms on vegetated and bare sediments. Substantially greater force was required to completely remove large particles (14.72 mTesla  $\pm$  0.65 SE) from bare sediment than for small ones (5.67 mTesla  $\pm$  0.40 SE) (Figure 5.3), with similar results in vegetated treatments. However, the small particles were not detached from the bed; bed failure occurred and the area of biofilm subjected to magnetic force detached from the sediment below with the particles still adhered to it.



**Figure 5.3**: The magnetic force, or magnetic flux density, measured in mTesla, required to remove ferrous particles from a clean ceramic tile (control), bare sediment, sediment from a *Zostera noltii* patch and a *Z. noltii* leaf. The particles used were indicative of grain sizes found in *Z. noltii* beds in the Tay Estuary and were small (< 63  $\mu$ m) and large (150-250  $\mu$ m).

**Table 5.1**: Two-way ANOVA of the magnetic force, or MFD, required to either partially ( $^{\sim}$ 5 particles) or totally remove small (< 63  $\mu$ m) and large (150-250  $\mu$ m) ferrous particles from different substrata: a clean ceramic tile (control), and sediment in patches of *Zostera noltii* and bare sediment.

					Residual
Particle size	Variable	n	F	p	d.f.
Both sizes	Particle size	30	1025.72	< 0.001	54
	Substrate	30	37.72	< 0.001	54
	Interaction	30	4.73	0.013	54
< 63 μm	Removal (total or partial)	30	14.32	0.001	24
	Substrate	30	21.65	< 0.001	24
	Interaction	30	3.65	0.041	24
150-250 μm	Removal (total or partial)	30	1.98	0.172	24
	Substrate	30	25.14	< 0.001	24
	Interaction	30	0.07	0.937	24

**Table 5.2:** Tukey's *post hoc* tests of the magnetic force, or MFD, required to either partially (~5 particles) or totally remove small (< 63  $\mu$ m) and large (150-250  $\mu$ m) ferrous particles from different substrata: a clean ceramic tile (control), and sediment in patches of *Zostera noltii* and bare sediment. Means that do not share a letter are significantly different (p < 0.05).

				Mean MFD			
Particle size	Variable		n	$(MTesla) \pm SE$	Gro	Grouping	
Both sizes	Particle size	150-250 μm	30	$13.46\pm0.36$	A		
		< 63 μm	30	$4.63 \pm 0.22$		В	
Both sizes	Substrate	vegetated	20	$10.20 \pm 1.09$	A		
		bare	20	$9.53 \pm 1.15$	A		
		control	20	$7.39 \pm 0.89$	A		
< 63 μm	Removal	total	15	$5.11 \pm 0.33$	A		
		partial	15	$4.14 \pm 0.26$		В	
< 63 µm	Substrate	vegetated	10	$5.61 \pm 0.30$	A		
		bare	10	$4.71 \pm 0.39$	A		
		control	10	$3.56 \pm 0.09$		В	
150-250 µm	Removal	total	15	$13.14 \pm 0.49$	A		
		partial	15	$13.77\pm0.55$	A		
150-250 μm	Substrate	vegetated	10	$14.78 \pm 0.45$	A		
		bare	10	$14.36 \pm 0.47$	A		
		control	10	$11.22\pm0.10$		В	

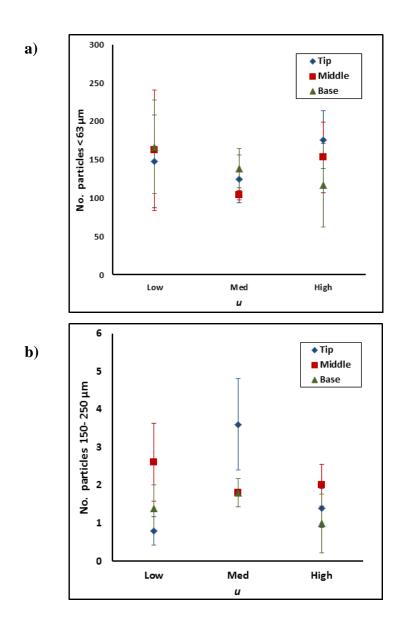
There was some difficulty in getting large particles to stick to leaf surfaces but, when it was achieved, the removal of large particles from leaves required greater force than for removal from the sediment surface (24.45 mTesla  $\pm$  1.63 SE) (ANOVA F<sub>3,17</sub> 29.67, p < 0.001, n = 21) (Table 5.3). It was not possible to generate enough magnetic force with the current MagPI system to remove small particles from leaves.

**Table 5.3**: One-way ANOVA of the magnetic force, or MFD, required to remove large (150-250  $\mu$ m) ferrous particles from different substrata: a clean ceramic tile (control), and sediment in patches of *Zostera noltii* and bare sediment. Results of the Tukey's *post hoc* tests are also shown. Means that do not share a letter are significantly different (p < 0.05).

Variable	n	F	p	Residual d.f.	Mean MFD ± SE	Grouping
Substrate	21	29.67	< 0.001	17		
Z. noltii leaf	6				$24.45 \pm 1.79$	A
Z. noltii sediment	5				$14.40 \pm 0.60$	В
bare sediment	5				$14.00 \pm 0.63$	В
control	5				$11.02 \pm 0.12$	В

#### 5.3.2 Flume study - leaf biofilms

A greater number of small particles ( $< 63 \mu m$ ) were trapped by leaf biofilms than large particles (150-250  $\mu m$ ) (Figure 5.4). While up to 439 small particles were counted on one leaf section, the highest number of large particles trapped was seven. Statistical analysis suggests that neither flow velocity nor position on the leaf (base, middle or tip) significantly influenced the trapping of small particles (two-way ANOVA, Table 5.4). However more particles were trapped where leaf surfaces had a large covering of epiphytic diatoms (Figures 5.5 and 5.6).



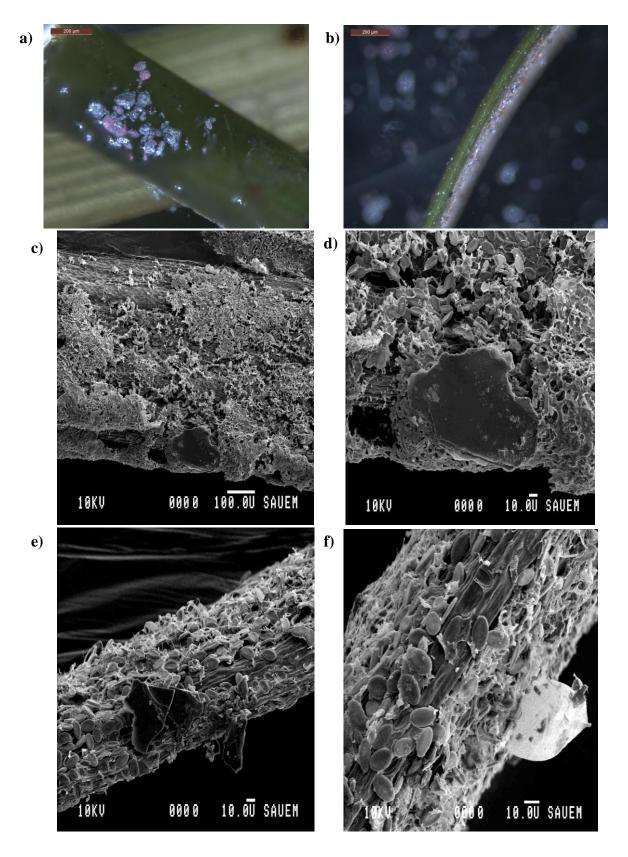
**Figure 5.4**: A greater number of (a) small particles (< 63  $\mu$ m) were trapped on *Z. noltii* leaf surfaces than (b) large particles (150-250  $\mu$ m). Neither flow velocity nor position on the leaf (base, middle or tip) significantly influenced the trapping of particles.

**Table 5.4**: Two-way ANOVA of the influence of flow velocity and position on the *Zostera noltii* leaf (base, middle or tip) on the trapping of small (< 63  $\mu$ m) particles.

					Residual
Particle Size	Variable	n	F	p	d.f.
< 63 μm	Flow velocity	15	0.11	0.897	36
	Leaf section	15	0.09	0.914	36
	Interaction	15	0.3	0.877	36



**Figure 5.5**: Using fluorescence microscopy, a *Zostera noltii* leaf tip (a) with a covering of epiphytic diatoms and bryozoans, and leaf basal sections demonstrating that more particles were trapped where leaf surfaces had a large covering of epiphytic diatoms (b) in comparison to those with low diatom cover (c).



**Figure 5.6**: The adhesion of particles to *Zostera noltii* leaf biofilms under fluorescence microscopy (figures 5.6a and 5.6b) and a low temperature scanning electron microscope (figures 5.6c to 5.6f). The layer of epiphytic diatoms is visible in figures 5.6c to 5.6f.

#### 5.4 Discussion

There were observable differences in the capacity of biofilms to retain sestonic particles of different sizes. Although large particles did adhere to sediment and leaf biofilms, they could be easily removed, suggesting that their critical mass is more relevant to resuspension than the adhesive strength of the sediment surface. Small particles adhered much more strongly to leaf and sediment surface biofilms than large ones, supporting the hypothesis ( $H_2$ ). Their removal from sediment required the failure of the biofilm, and they could not be removed from leaf surfaces by either using MagPI or by increasing flow velocity. Fine particles can be captured and retained in the spaces between large particles in the substratum (Salant 2011) or attached by fibrils from the biofilm (Westall & Rincé 1994). Furthermore, particles less than 63  $\mu$ m in diameter have a greater surface-to-volume ratio than larger particles. In consequence, the charge distribution over the grain's surface causes cohesive interactions (inter-particle attraction) through Van der Waals forces, binding the particles strongly to each other (Paterson & Hagerthey 2001).

When the equipment was being tested two weeks earlier (mid-August 2010), on sediment taken from the same *Zostera* meadow, it was not possible to separate the sediment biofilms covered with small particles, although large particles could be removed. The equipment test took place in the afternoon, while our experiment was carried out during the morning. In sediments, diatoms typically exhibit a diel pattern of vertical migration and photosynthetic production (Madsen *et al.* 1993) with corresponding fluctuations in sediment microalgal biomass. Biomass is greatest during daytime exposures and lower during dark periods, due to migration (Blanchard *et al.* 2001). Sediment microbial density and biomass also exhibit seasonal variations (Danovaro & Fabiano 1995), with highest values in warmer months (Underwood & Paterson 1993; Widdows *et al.* 2000). Therefore, cohesiveness of the microbial mat and bed stability may vary depending on the time of day (Madsen *et al.* 1993; de Winder *et al.* 1999) and the stage of mat formation (Decho 2000). For these reasons these experimental results should not be taken as definitive values for the adhesive strength of

aquatic biofilms, rather they should be regarded as indicative of the biofilms present at that time.

Leaf biofilms were more adhesive than sediment biofilms. This may be due to the constant presence of epiphytic diatoms, compared to the motile nature of sediment diatoms. Lebreton et al. (2009) observed a monolayer of epiphytic diatoms on Z. noltii leaves, comprising only Cocconeis scutellum and C. placentula, present throughout the year. When examining leaf surfaces during this study, only epiphytic diatoms and bryozoans were observed, with no animals or macroalgae. However, epiphytic species vary according to seagrass species and locality (e.g. Scoffin 1970; Novak 1984; Agawin & Duarte 2002; Lebreton et al, 2009). Although there was no difference in the number of particles adhering to different parts of the leaves, there appeared to be more particles when diatom density was higher (Figure 5.4), on older leaves (Novak 1984; Gacia et al. 2003; Lebreton et al. 2009). Particle retention is enhanced by complexity of the biofilm surface (Battin et al, 2003; Salant 2011) and by the surface area available (Palmer et al. 2004). Small particles (<< 63 µm) may be trapped between protruding diatoms (Figure 5.6), while larger particles (> 150 µm) do not fit into these spaces. Sediment or detrital matter adhering to diatom exudates may form a crust covering the leaf entirely (Lebreton et al. 2009), impeding both the photosynthetic capacity and nutrient uptake of the seagrass (Hemminga & Duarte 2000; den Hartog & Phillips 2001). The relatively short life span of seagrass leaves in comparison to terrestrial plants (with means of 88 and 170 days respectively) (Hemminga et al. 1999) may be explained by the combination of the coverage of leaf surfaces and leaf senescence (Hemminga & Duarte 2000). However, within Z. noltii beds microphytobenthic diatoms comprise a large proportion of the diet of grazing invertebrates (Lebreton et al. 2011), which may help to keep leaf surfaces clear (Valentine & Duffy 2006), and small particles on leaves may also be ingested by phagotrophic protozoans (Agawin & Duarte 2002). Any remaining particles will be returned to the water column or deposited on the substratum as the leaves decay (Gacia et al. 2003).

The adhesive properties of biofilms in bare and vegetated sediment were similar, thus the hypothesis  $(H_1)$  can be rejected. In Chapter 2 of this thesis there were no significant

differences in the chlorophyll and carbohydrate content of either treatment. Both of these variables are good indicators of diatom biomass and therefore sediment stability (Underwood & Paterson 1993; Underwood *et al.* 1995; de Winder *et al.* 1999).

The results of the experiments in this chapter show that both sediment and leaf biofilms play an important role in the retention of sediment within intertidal seagrass beds, and in bare sediment systems. Greater force was required to remove particles from surfaces where biofilms were present, demonstrating that biofilms increase sediment stability and cohesion (Dade *et al.* 1990; Underwood & Paterson 2003). This ecosystem service of particle trapping and retention by biofilms is crucial for the replacement of material lost by tidal or wave erosion (Verney *et al.* 2006; Andersen et al. 2007).

In high density *Z. noltii* a greater number of small particles (< 63 μm) was trapped on each 2 cm leaf section than in a 3.1 cm<sup>2</sup> core taken from the sediment surface (Chapter 4, section 4.3.1). Seagrass leaves enhance particle settling and deposition within the meadow by decreasing flow velocity (Scoffin 1970; Bos *et al.* 2007); however, the direct trapping of sestonic particles by leaves may also contribute to a higher particulate organic fraction (Marbà *et al.* 2006) and higher sediment accretion in seagrass beds (Gacia *et al.* 2003). Despite the large number of studies into the role of seagrasses in flow reduction and resulting sediment deposition (e.g. Gambi *et al.* 1990; Fonseca & Cahalan 1992; Bos *et al.* 2007; Widdows *et al.* 2008), there is currently little quantitative data for the role of seagrass canopies in direct passive particle trapping (e.g. Lemmens *et al.* 1996; Gacia *et al.* 2003).

## 5.5 Conclusions

For patches of *Z. noltii* and bare sediment found in the Tay Estuary, the following conclusions can be made from the results of these studies:

• Sediment biofilms in patches of *Z. noltii* and bare sediment are similarly adhesive.

- Small particles ( $<63~\mu m$ ) adhere more strongly to leaf and sediment surface biofilms than large ones ( $150\text{-}250~\mu m$ ).
- Leaf biofilms are more adhesive than sediment biofilms.

Due to diel and seasonal fluctuations in microbial biomass, these experimental results should not be taken as definitive values for the adhesive strength of aquatic biofilms in the Tay Estuary; rather they should be regarded as indicative of the biofilms present at the time the experiment took place.

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# Chapter 6

# Can artificial seagrass be used to restore sediment habitats? A comparison of restoration techniques

#### Abstract

The efficacy of artificial seagrass beds and transplantation of *Zostera noltii* as habitat restoration techniques were compared over one year in the Tay Estuary. Sediment accretion and physical sediment properties were compared in plots of artificial and transplanted seagrass, naturally growing *Z. noltii* and bare sediment. Flow reduction in *Z. noltii* and artificial seagrass was compared in an 8 m seawater flume.

In artificial seagrass beds there was sediment deposition all year round, demonstrating that, in principle, the technique could be used to increase sediment deposition in front of saltmarsh. During a seven-week period artificial beds accumulated  $21 \text{ mm} \pm 8 \text{ SE}$  of sediment, and this could have been greater with design improvements. Flow reduction and sediment deposition in artificial plots was greater than in *Z. noltii*. This may be due to increased shoot stiffness. The stiffer shoots incurred trade-offs such as increased drag on the artificial structures during storms, and trapping of *Enteromopha*, preventing sediment exchange between the water column and the bed.

Sediment deposition and erosion over one year was similar in patches of naturally occurring *Z. noltii* and transplanted *Z. noltii*. However, only two out of the five transplanted plots were successful, with regrowth two-years after planting, and expansion out of the plot boundaries. Pre-trial sediment characteristics at the experimental site were similar to those of the donor site, but plot elevation was not considered during the experimental design stage, and transplanted seagrass may have succumbed to desiccation. *Z. noltii* transplantation cannot be considered a fast-acting technique for increasing sediment deposition in front of saltmarshes. Transplantation

may be considered as a tool for *Z. noltii* habitat restoration, but the elevation and hydrodynamic conditions of the planting site must be similar to those of the donor site for the seagrass to survive.

Over the relatively short time-span of one year it was not possible to say whether either of these restoration techniques offer protection to the saltmarsh cliff behind.

#### 6.1 Introduction

#### 6.1.1 The decline of saltmarsh habitat in the U.K.

In Great Britain 25% of our natural intertidal areas, including saltmarsh, have been lost in the last century (Doody 2008) and over 67% of the eastern coastline has shown a landward retreat of the low-water mark (Taylor *et al.* 2004). In 2008 there remained approximately 45500 ha of saltmarsh around the coast of the United Kingdom, with only around 6700 ha of this in Scotland (UK Biodiversity Group 2008). For several centuries marshes have been enclosed behind seawalls and drained, primarily to provide land for grazing and agriculture, but also for sediment extraction and turf cutting (Doody 2008). Today saltmarsh is valued for a variety of ecosystem functions. As well as supporting a wide range of flora and fauna, saltmarsh habitat plays a vital role as a natural sea defence; saltmarsh protects the coastline from scour and wave erosion by dissipating the energy of tidal currents and waves (Möller *et al.* 1999).

Human activities such as dredging and the construction of sea walls and groynes, which reduce sediment erosion and transport from cliffs and beaches, have contributed to recent saltmarsh losses by decreasing the amount of recharging sediment available for natural recharging (Hughes 2001). Additionally, many areas of saltmarsh are rapidly being eroded as a result of strong winds, high tides and increased wave height (Hughes & Paramor 2004; Wolters *et al.* 2005). Over the next few decades increased wave energy will erode saltmarshes, exposing man-made sea defences to the full force of the sea and lessening their effectiveness. For example, in some places loss of saltmarsh has led to the undermining of the toe of the sea wall (Doody 1996).

Although sea-level is rising as a result of climate change, saltmarshes have the inherent ability to cope with sea-level rise, and have kept pace with increases in relative sea-level over the past few thousand years (Adam 1990; Doody 2008). Spencer *et al.* (2008) measured a sediment accumulation rate of ~0.75 cm y<sup>-1</sup> after the sea defences at Orplands Farm managed realignment site in the Blackwater Estuary, Essex, were

breached, compared to a regional sea-level rise of 0.1–0.3 cm y<sup>-1</sup>. The rate of vertical accretion at both Tollesbury and Dengie Marshes, Essex, are also keeping pace with, or exceeding, sea-level rise (Reed 1988; Cahoon *et al.* 2000) and a recent study in the Eden Estuary found that tidal range had a significant and positive effect on sediment deposition (Maynard *et al.* 2011). The predicted rise of sea-level is also within the vertical growth rate of most seagrass species (Hemminga & Duarte 2000). However, rising sea-level combined with the loss of recharging sediment could have a significant negative impact on saltmarsh development.

Most of the losses of saltmarsh vegetation occur on the seaward margins, in the pioneer zone, increasing lateral erosion at the seaward edge of the marsh (Hughes 2001; Hughes & Paramor 2004). The loss of intertidal seagrass from in front of the marshes may increase sediment erosion and exposure to wave action. For example, in the River Stour the decline of *Zostera marina* led to losses of 15 million m<sup>3</sup> of sediment, and increased its tidal volume by 30% (Beardall *et al.* 1988). Due to the extent of the losses resulting from erosion and human activities it may be insufficient to maintain our remaining saltmarshes. Habitat restoration is required to sustain the ecosystem services of saltmarsh, such as coastal defence (Doody 2008).

## 6.1.2 Saltmarsh development: Zostera noltii as an ecosystem engineer

The first step in the development of saltmarsh is the colonisation of mudflats by pioneer species (Boorman 2003). On the upper shore, seagrasses such as *Zostera noltii* trap suspended particles and elevate the sediment surface, which may lead to saltmarsh succession (Hughes *et al.* 2000). Where sufficient sediment has built up, and germinating plants are undisturbed by tidal action for several days, halophytic species such as *Salicornia europea* and *Spartina anglica* can begin to establish (Gray 1992; 1990; Doody 2008). While individual plants have little effect on water flow, and may cause scour (Sumer *et al.* 2001), dense vegetation increases the depth of the boundary layer of low velocity water above the sediment surface, and the deposition of suspended particles is enhanced (Hughes 2001).

The velocity of water flow over the marsh is critical to its vertical development (Boorman 2003) and seagrass can significantly reduce the velocity, increasing sediment deposition (Ward *et al.* 1984; Hemminga *et al.* 1991; Fonseca and Cahalan 1992; Agawin & Duarte 2002). Möller *et al.* (1999) measured wave dissipation at an average of 29% over sandflat and 82% over saltmarsh, and experiments have shown that seagrasses reduce water flow within the canopy by 20 to 40%, depending on patch width (Gambi *et al.* 1990; Fonseca & Koehl 2006). Thus, the loss of intertidal seagrass from directly in front of a saltmarsh may increase its vulnerability to erosion caused by wave action (Hughes *et al.* 2000; Hughes & Paramor 2004).

Once vegetative cover within the marsh has become established, the rate of sediment accretion increases (Stumpf 1983; Stevenson *et al.* 1988; Hughes 1999). Nutrients diffusing from the sediment to the overlying water column, or released from decomposing organic matter produced by seagrass leaves and by other primary producers, such as epiphytes and benthic algae, are recaptured (Hemminga *et al.* 1991; Pedersen & Borum 1997). Thus, nutrients and organic matter are added to the sediment surface, further enhancing marsh development (Allen & Pye 1992). Once established, saltmarshes are naturally self-repairing, as erosion during storms is restored by accretion and plant recolonisation (Boorman 2003).

The timescale for the natural development of saltmarsh from pioneer to lower marsh has been estimated at between 46 to 140 years, depending on sediment accretion rates (Boorman 2003). However, the immediacy of coastal erosion and climate change means that faster ways to establish saltmarsh habitat are needed.

# 6.1.3 Habitat restoration: seagrass and saltmarsh

As the requirement for sustainable coastal defence increases, coastal managers are seeking cost-effective and relatively fast-acting ways to restore marshes where losses have taken place, or reduce erosion in front of marshes. The Joint Nature Conservation

Committee (JNCC) has highlighted the need for research and trials of innovative ways to accelerate saltmarsh succession (Boorman 2003).

## Seagrass restoration

Numerous seagrass restoration techniques have been developed for different species and environments, with most large-scale programs taking place in the U.S.A. and Australia, although some have been implemented in Europe (Paling et al. 2009). The most common restoration methods involve transplantation of mature plants, either with or without associated sediments (Fonseca et al. 1998; Wear 2006). Although in many cases Z. noltii transplantation trials have had limited success in terms of survival and coverage, trials on sheltered estuarine mudflats in Norfolk and Suffolk (Ranwell et al. 1974; Nicholls 2003), and on the Wadden Sea coast (van Katwijk et al. 2009) have produced encouraging results. The transplantation of intertidal Zostera species in the UK would be beneficial, not only to Zostera as a Biodiversity Action Plan species, but also for the restoration and maintenance of saltmarsh habitat (Hughes & Paramor 2004). However, seagrass transplantation requires considerable effort and expense, and is destructive to otherwise healthy meadows, which may be slow to recover (Ranwell et al. 1974; Fonseca et al. 1998). These factors, in combination with the poor survival of transplants, limit the area that can be restored (Wear 2006). With increasing natural and human-induced pressures placed upon seagrass meadows, it may be unrealistic to consider large-scale transplantation as a 'quick-fix' for habitat restoration.

## Saltmarsh restoration and managed realignment

Where sea-level rise and saltmarsh loss have led to the erosion of sea defences, 'soft' engineering options such as managed realignment are often advocated to maintain flood protection, restore saltmarsh habitat, increase biodiversity and help the recovery of threatened species (Nottage & Robertson 2005; Doody 2008; Spencer *et al.* 2008). Managed realignment schemes have had varying success. For example, on the Crouch Estuary, Essex, saltmarsh did not form at Wallasea Island, while at Clementsgreen Creek saltmarsh formed and then eroded (Hughes 1999).

Natural rates of sediment accretion and erosion differ between locations (Best *et al.* 2007). Accretion can be accelerated by planting lower marsh species, and rapid cover of vegetation is required to prevent erosion and to attenuate wave action, but the correct type of sediment and sufficient elevation is needed for transplanted plants to establish (Broome *et al.* 1988; Garbutt *et al.* 2006; Maynard *et al.* 2011). Garbutt *et al.* (2006) observed that seeds and plants sown straight into agricultural soil after managed retreat were unsuccessful as the soil became waterlogged. Former agricultural soil also has many pits and depressions, allowing standing water to accumulate around the plantings, and the mud has low organic matter content. Dredged material may be introduced to a site, but the earth moving equipment causes compaction and the mud generally has low organic matter content (Garbutt *et al.* 2006).

A combination of methods may be required for saltmarsh restoration and managed realignment to work, including faunal exclusion, the use of new sediment and seagrass transplants (Hughes & Paramor 2004). New techniques are needed to increase sedimentation from the water column, and raise the elevation of low-lying sites, allowing marsh communities to develop.

## The use of artificial seagrass beds

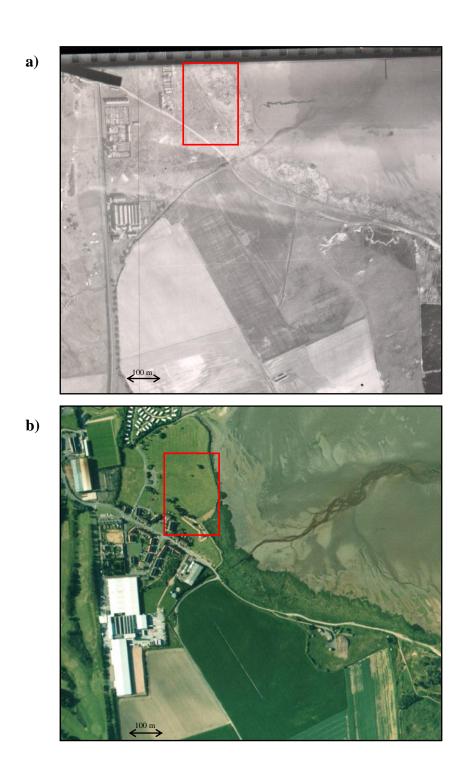
An alternative method may be needed to mimic the effects of seagrasses on water and sediment motion, and on the faunal community. Subtidal trials of artificial seagrasses have shown that mimics can reduce the mean flow velocity and increase sediment deposition (Almasi *et al.* 1987; Campbell & Paling 2003). Almasi *et al.* (1987) found that while *Thallasia* sp. seagrass in Florida Bay increased deposition by 63% in comparison to bare sediment, artificial beds increased deposition by 79%. In Western Australia it was observed that artificial beds stabilised sediment grain size, increasing the survival of subsequent *Posidonia australis* seagrass transplants to up to 50% survival, compared to no survival in bare sediments (Campbell & Paling 2003). Seagrass mimics can also have positive effects on biodiversity, providing feeding areas and protection from predators for benthic invertebrates and juvenile fish (Sogard & Able 1994; Talbot & Wilkinson 2001; Cardoso *et al.* 2007).

Compared to transplantation, artificial seagrass beds would be non-destructive and cost effective, and potentially it would be possible to deploy them easily over large spatial scales. They could also be used year-round, while intertidal seagrasses lose most of their leaves in winter. Where areas are naturally unvegetated, the use of seagrass transplants would mean substituting one habitat type for another. The absence of seagrass from a site usually means that it cannot support seagrasses, and that transplants would be unlikely to survive (Fonseca *et al.* 1998). This may be due to low lights levels caused by sediment resuspension and associated high water turbidity (Moore *et al.* 1997; Peralta *et al.* 2002), suffocation by algal mats in high-nutrient environments (den Hartog 1994; Cardoso *et al.* 2004), or exposure to strong currents and waves (Fonseca & Kenworthy 1987; Davison & Hughes 1998). Artificial beds could be utilised where seagrass would not normally grow. In turbid waters the beds will encourage sediment accretion, improving the environment for natural colonisation by seedlings or for future transplants, and as a tool for saltmarsh restoration.

# **6.1.4** Relevance of the study

The Firth of Tay and Eden Estuary is referred to as a 'European marine site' or marine Special Area of Conservation (SAC) and was designated as an SAC in 2005 for the Annex I habitats "Estuaries" and "Mudflats and sandflats not covered by sea water at low tide" which encompass a number of priority habitats including saltmarsh and beds of the intertidal seagrasses *Zostera marina* var. *angustifolia* and *Z. noltii* (SNH 2006).

In the Tay Estuary, at both Tayport and Tentsmuir, areas of saltmarsh have been lost as a result of lateral erosion of the seaward margin and internal dissection of the marshes. Since the drainage and "reclamation" of Tayport Common in the 1930s this process has led to the loss of up to 100 m of saltmarsh from the upper shore in some places (Figure 6.1). The remaining saltmarsh cliff at Tayport is eroding (Figure 6.2), and a similar process may lead to the release of contaminated waste from a disused landfill site adjacent to the nearby Eden Estuary.



**Figure 6.1**: Aerial photographs of the shoreline at Tayport, on the south shore of the Tay Estuary Scotland, taken in a) 1946 and b) 1999. Saltmarsh losses at Tayport due to land reclamation are highlighted. (Photographs courtesy of SNH).

During the survey of *Zostera* spp. in the Tay estuary (Chapter 2, Section 2.3.1) *Z. noltii* was found growing up to, and sometimes encroaching into, areas of saltmarsh where it

may function as a pioneer species, stabilising and raising the sediment level. It has already been shown (Chapter 3) that sediment deposition was greater in patches of *Z. noltii* during the growth season, and erosion was less in winter than in patches of bare sediment. The presence of *Z. noltii* in front of the remaining areas of saltmarsh suggests this may be a suitable site for habitat restoration. Physical measurements taken in 2008 showed few significant differences between sediments where *Zostera* was growing at Tayport, and sediments where it was not (Chapter 2). Therefore it may be possible to increase seagrass cover in the Tay estuary by transplantation. However, *Z. noltii* loses its leaves in winter, effectively leaving bare sediment from November to April. Artificial beds may be a way to encourage year-round sediment deposition.

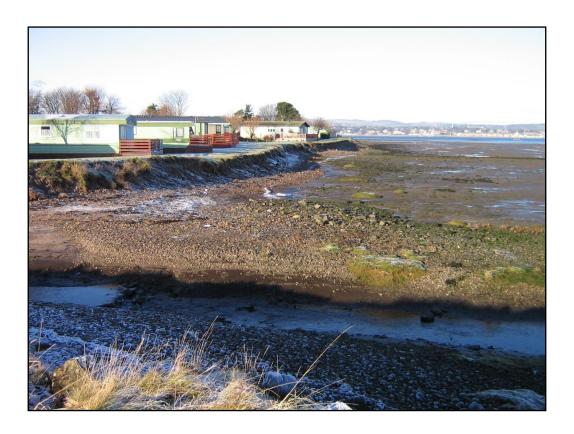


Figure 6.2: Erosion of the saltmarsh cliff at Tayport on the Tay Estuary, Scotland.

### **6.1.5** Aims

In the Tay Estuary *Z. noltii* transplants and artificial seagrass beds were compared with naturally occurring beds and bare sediment to investigate the role of *Z. noltii* as an ecosystem engineer by increasing deposition in front of saltmarsh habitat, and to compare the effectiveness of habitat restoration techniques.

The following hypotheses were tested in order to determine the efficacy of habitat restoration techniques:

- H<sub>1</sub> Sediment deposition and erosion over 1 year will be similar in patches of naturally occurring *Z. noltii* and transplanted *Z. noltii*.
- H<sub>2</sub> There will be sediment deposition in beds of artificial seagrass all year round.

The influence of restoration techniques on sediment characteristics, and on saltmarsh cliff protection were also examined.

This study offers valuable data which can be utilised for future conservation policies, habitat restoration schemes and management planning of the area.

### **6.2** Materials and methods

The experiment was carried out in front of the saltmarsh at Tentsmuir Forest (56°26'N 02°51'W; Figure 6.3). Measurements were taken between 28<sup>th</sup> July 2009 and 30<sup>th</sup> June 2010.

## **6.2.1** Experimental plots

There were five replicates each of four experimental plot types: bare sediment; naturally occurring *Z. noltii*; transplanted *Z. noltii*; and artificial seagrass. Each plot was 3 m

wide x 1 m broad, situated ~1 m in front of the saltmarsh cliff where possible. Plots comprised either bare sediment, to which transplanted or artificial grass could be added, or naturally growing *Z. noltii*. There were no significant differences between plots for sediment water content, organic content, wet bulk density, median grain size or shear strength (Table 6.1). While a fully randomised complete block design (Quinn & Keough 2002) would have been optimal, the distribution of suitable patches of *Z. noltii* in front of the saltmarsh meant this was not fully possible.



**Figure 6.3**: Restoration trial plots in front of the saltmarsh at Tenstmuir in the Tay Estuary, Scotland. Plot numbers are given followed by plot type: (V) natural *Zostera noltii*; (T) transplanted *Z. noltii*; (B) bare sediment; and (A) artificial seagrass.

**Table 6.1:** Descriptive statistics of sediment organic content, percentage water, wet bulk density (g cm<sup>-3</sup>), median grain size ( $\mu$ m), shear strength (kPa) and flow (a proxy measurement based on the reduction in mass of a plaster block (g)) in experimental plots before the commencement of the restoration trial.

Variable	n	p	Mean	StDev	SE	
Organic mater	20	0.631	5.38	1.35	0.30	
Water content	20	0.808	26.15	1.73	0.39	
Wet bulk density	20	0.052	221	0.11	0.02	
Shear strength	20	0.630	3.00	1.35	0.30	
Flow	20	0.066	1.41	0.14	0.03	
			Median	1st Quartile	3rd Quartile	
Median grain size	20		192.7	187.7	197.2	

## Transplantation of Z.noltii

On 11<sup>th</sup> June 2009, 380 vegetated sediment cores with 103 mm internal diameter and 100 mm depth (making a total donor area of 3.17 m<sup>2</sup>) were removed from *Z. noltii* beds throughout the shore at Tayport. Turfs were removed at intervals to allow recolonisation and holes were infilled using bare sediment from outside the bed to prevent scouring. The turfs were transplanted into five plots of bare sediment, in a random pattern (Figure 6.4). Patch dimensions throughout 2009 and regrowth in spring 2010 were monitored.

### Construction of artificial seagrass beds

Artificial seagrass leaves were made from polypropylene machine strapping (www.rajapack.co.uk) 12 mm x 0.6 mm split into strips approximately 2 mm x 22 mm (folded over this makes two fronds). Frond length and width were comparable to those of *Z. noltii*. Bundles of 3 strips were folded in half, and looped around PVC coated chicken wire, hexagonal mesh size 25 mm. Each loop was secured at the base with a compressed aluminium ring, internal diameter 4.8 mm, gauge 1.63 mm (www.beadsisters.co.uk). Shoot density was 600 shoots m<sup>-2</sup> (the average density of *Z.* 

*noltii* growing in front of the marsh in 2008). On 27<sup>th</sup> July 2009 the beds were secured in place (Figure 6.4), using twisted steel ground anchor stakes 0.4 m long.

a)



**Figure 6.4**: a) Transplanted *Z. noltii* in plot 17 on  $12^{th}$  June 2009, one day after planting; and b) artificial seagrass in plot 16 at Tentsmuir on  $27^{th}$  July 2009. The plots measure 3 m x 1 m.

## **6.2.2** Sediment deposition/erosion measurements

The method for measuring sediment deposition and erosion was adapted from Brown (1998). On 27<sup>th</sup> May 2009 three 15 m x 15 m x 0.15 cm steel plates were buried at ~10 cm depth along the mid-Section of each plot. Before plate burial, the absence of any other metal objects at the site was confirmed using a metal detector. The plates were left to settle for two months. The first (baseline) measurements were taken on 28<sup>th</sup> July 2009, and measurements were taken approximately every two months thereafter. The sediment height over each plate was measured at five points using a metal rod pushed through a foam disc. Any leaves were carefully moved aside using the metal rod. The rod was inserted into the sediment over the plate at right angles. The disc was pushed down to the sediment surface, and the rod and disc were carefully removed. The distance from the base of the disc to the tip of the rod was measured with a ruler to provide the sediment height over the plate.

# **6.2.3** Sediment sampling

Sediment samples were taken at the beginning and end of the experiment. Sediment sampling and analysis was carried out following the protocols outlined in Chapter 2, section 2.2.2. Water content, wet bulk density, organic matter and sediment grain size were determined.

# **6.2.4** Sediment shear strength

A 33 mm diameter shear vane was used to measure the shear strength of surface sediment (Aspden *et al.* 2005). The shear vane was held at a right angle to the sediment surface. The vane was inserted into the sediment until the base of the vane was level with the sediment surface, and the total depth of the vane head was 50 mm. Using one hand the torque head was turned clockwise at a speed of 1 rotation min<sup>-1</sup>. The torsion device was released as soon as the sediment sheared. This force was reported as the

shear strength (kPa) of the sediment on the calibrated scale of the meter. Sediment shear strength was measured at the beginning of the trial only – measurements were not taken at the end due to oversight.

## 6.2.5 Intensity of water motion comparison

A proxy measurement of the intensity of water motion in experimental plots was taken before the beginning of the trial only, following the protocol outlined in Chapter 3, section 3.2.3. One 10 g plaster block was used per plot. Blocks were left *in situ* for 24 hours (2 tides). The blocks were dried at 40 °C for 48 h and reweighed, and the differences in weight between plots were compared.

## 6.2.6 Flow reduction – comparing Zostera noltii and artificial seagrass beds

The influence of artificial seagrass on flow reduction investigated in an 8 m linear saltwater flume (Armfield Research, Flume, Figure 5.2), at three flow velocities (~0.05, ~0.15, ~0.45 ms<sup>-1</sup>). Flow was measured using the method outlined in Chapter 4 (Section 4.2.1). Results were compared to those for natural *Z. noltii* of the same density (600 shoots m<sup>-2</sup>) and bare sediment (Section 4.3.1).

#### 6.2.7 Saltmarsh cliff seaward advancement or erosion

A steel measuring tape was extended between the two marker posts at the rear corner of each plot. The distance to the base of the saltmarsh cliff, or nearest occurring saltmarsh vegetation, was measured from the beginning of the tape, and at 10 cm intervals for the 3 m length of the plot. Measurements, and photographs of each saltmarsh cliff, were taken at the beginning and end of the experiment.

## 6.2.8 Statistical analysis

The statistical software used was GenStat 12 for repeated measures MANOVA, and Minitab 16 for all other tests.

### Sediment percentage water content, organic content and wet bulk density

Sediment percentage water content, organic content and wet bulk density in each plot before the start of the trial were compared using a descriptive statistics. At the end of the experiment, the differences between values for before and after the restoration trial were calculated for each variable. The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. Data for organic content were transformed using a reciprocal transformation to fulfil the requirements of homogeneity of the variance. Statistical analysis was carried out using one-way ANOVA (Analysis of Variance). Results were considered significant at p < 0.05.

### Changes in sediment grain size

The median sediment grain sizes in each plot before the start of the trial were compared using a one-sample sign test. At the end of the experiment, the differences between values for before and after the restoration trial were calculated for each sediment grade. The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to determine whether the data were normally distributed and had equal variances. Since this was the case, statistical analysis was carried out using one-way ANOVA and a Tukey's *post hoc* test was used to determine where there were significant differences. Results were considered significant at p < 0.05.

### Sediment deposition and erosion

The mean sediment levels relative to the baseline measurement were compared, although statistical analysis covers the period up to 1<sup>st</sup> February 2010 only, due to the

displacement of the artificial beds on  $31^{st}$  March 2010. The mean sediment deposition or erosion between measurements for the duration of the experiment were also compared for each plot type; however, measurements for artificial plots for  $8^{th}$  April 2010 were omitted from the analysis. The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. Data were transformed using log(x+c) to fulfil the requirements of homogeneity of the variance. Statistical analysis was carried out using a repeated measurements multivariate analysis of variance (MANOVA). Tukey's *post hoc* test was used to determine where there were significant differences between means. Results were considered significant at p < 0.05.

## Saltmarsh cliff seaward advancement or erosion

The mean differences between distances to the saltmarsh cliff at the end of the restoration trial were calculated for each plot. Data for plot 15 (bare sediment) were removed as outliers, due to extreme erosion behind this plot only. The Anderson-Darling normality test and Bartlett's test for homogeneity of variances were carried out to test whether the data were normally distributed and had equal variances. Since this was the case, statistical analysis was carried out using one-way ANOVA and a Tukey's post hoc test was used to determine where there were significant differences between means. Results were considered significant at p < 0.05.

## 6.3 Results

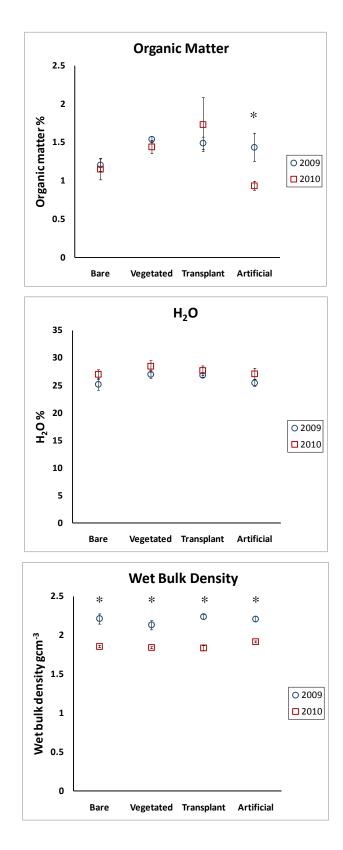
## **6.3.1** Sediment properties

Neither the transplantation of *Z. noltii* nor the presence of artificial seagrass beds affected sediment organic content, water content or bulk density (Table 6.2), although there was a greater decrease in organic content in artificial plots compared to all other treatments (Figure 6.5). In artificial plots the proportion of sediment grains under 125

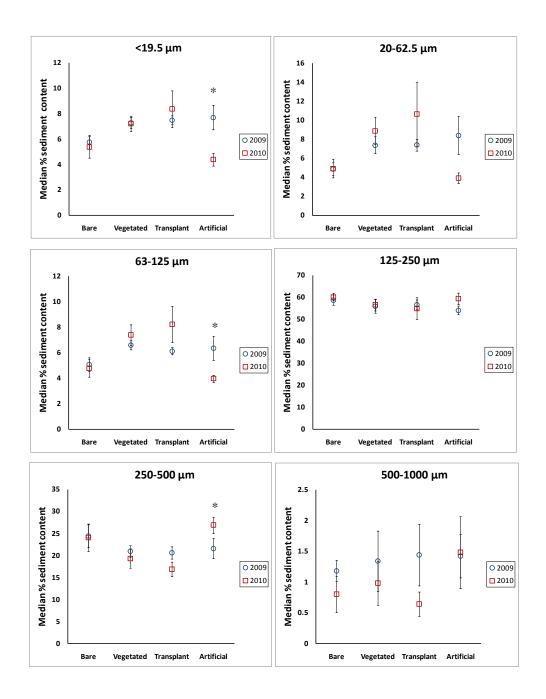
 $\mu$ m (very fine sand, silt and clay) decreased significantly (Figure 6.6), while medium sand increased. In transplanted treatments the proportion of very fine sand and clay was significantly greater, while medium sand decreased. Overall, there were significant changes in the percentage of medium sand (250-500 μm) (ANOVA,  $F_{3,19} = 5.41$ , p < 0.01, n = 20) (Table 6.3), very fine sand (62.5 to 125 μm) ( $F_{3,19} = 4.96$ , p = 0.013, n = 20), and clay (< 19.5 μm) ( $F_{3,19} = 3.27$ , p = 0.049, n = 20). There was no significant difference in silt content.

**Table 6.2:** One-way ANOVA of the changes in experimental plotsbetween the start and the end of the restoration trial, for percentage sediment organic content, percentage water, wet bulk density (g cm<sup>-3</sup>), and distance from the saltmarsh cliff.

Variable	n	F	р	Mean difference ± SE	Residual d.f.
Organic matter	20	0.50	0.688		16
Artificial seagras	S	0.0		$-0.50 \pm 0.16$	
Z. noltii (natural)				$-0.10 \pm 0.11$	
Z. noltii				$0.25 \pm 0.31$	
Bare sediment				$-0.05 \pm 0.06$	
Water content	20	0.14	0.936		16
Artificial seagras	S			$1.64 \pm 1.37$	
Z. noltii (natural)				$1.42 \pm 0.99$	
Z. noltii				$0.83 \pm 1.20$	
Bare sediment				$1.81 \pm 1.00$	
Wet bulk density	20	1.08	0.386		16
Artificial seagras	S			$0.29 \pm 0.03$	
Z. noltii (natural)				$0.29 \pm 0.06$	
Z. noltii				$0.40 \pm 0.06$	
Bare sediment				$0.35 \pm 0.05$	
Saltmarsh cliff	19	1.15	0.360		15
Artificial seagras	S			$-0.04 \pm 0.03$	
Z. noltii (natural)				$-0.11 \pm 0.04$	
Z. noltii				$0.00 \pm 0.03$	
Bare sediment				$0.00 \pm 0.04$	



**Figure 6.5**: Comparisons between sediment organic matter content (%), water content (%) and wet bulk density (gcm $^{-3}$ ) in restoration trial plots in June 2009 and 2010, with SE bars. Significant changes (p < 0.05) between 2009 and 2010 measurements are indicated (\*).



**Figure 6.6**: Comparisons between sediment grain size fractions in restoration trial plots in June 2009 and 2010, with SE bars. Significant changes (p < 0.05) between 2009 and 2010 measurements are indicated (\*). Classification of the samples was done after the Wentworth Scale (1922).

**Table 6.3:** One-way ANOVA of the differences in sediment grain sizes fractions (%) before and after the restoration trial, with Tukey's *post hoc* tests. Means that do not share a letter are significantly different (p < 0.05).

Variable	n	F	p	Mean difference ± SE	Residual d.f.	Grouping	
Clay (< 19.5µm)	20	3.27	0.049		16		
Artificial seagrass				$-3.30 \pm 1.19$			В
Z. noltii (natural)				$-0.07 \pm 0.73$		A	В
Z. noltii (transplanted)				$0.88 \pm 1.30$		A	
Bare sediment				$-0.39 \pm 1.59$		A	В
Silt (19.5 to 62.5 μm)	20	2.97	0.068		16		
Artificial seagrass				$-4.48 \pm 2.01$			В
Z. noltii (natural)				$1.51\pm1.26$		A	
Z. noltii (transplanted)				$3.24 \pm 3.16$		A	В
Bare sediment				$0.04 \pm 0.75$		A	В
Very fine sand (62.5 to 125 µm)	20	4.96	0.013		16		
Artificial seagrass				$-2.39 \pm 0.75$			В
Z. noltii (natural)				$0.80 \pm 0.64$		A	В
Z. noltii (transplanted)				$2.10\pm1.32$		A	В
Bare sediment				$-0.30 \pm 0.43$		A	
Fine sand (125 to 250 µm)	20	1.18	0.348		16		
Artificial seagrass				$5.32 \pm 3.29$		A	
Z. noltii (natural)				$0.66 \pm 1.73$		A	
Z. noltii (transplanted)				$-1.84 \pm 3.66$		A	
Bare sediment				$1.39 \pm 1.61$		A	
Medium sand (250 to 500 μm)	20	5.41	0.009		16		
Artificial seagrass				$5.30 \pm 1.25$		A	
Z. noltii (natural)				$-1.60 \pm 1.80$			В
Z. noltii (transplanted)				$-3.74 \pm 2.23$			В
Bare sediment				$-0.36 \pm 1.12$		A	В
Course sand (500 to 1000 µm)	20	1.11	0.377		16		
Artificial seagrass				$0.06 \pm 0.24$		A	
Z. noltii (natural)				$-0.36\pm0.16$		A	
Z. noltii (transplanted)				$-0.80 \pm 0.36$		A	
Bare sediment				$-0.38 \pm 0.29$		A	

## **6.3.2** Sediment deposition and erosion

For the duration of the trial, the mean differences in sediment height were highly significant both between plot types (MANOVA,  $F_{3,88} = 6.78$ , p < 0.001, n = 93) (Tables 6.4 & 6.5; Figure 6.7), and between measurement dates ( $F_{4,89} = 3.40$ , p = 0.012, n = 93). By October, bare plots were accumulating much less sediment than artificial plots, and over winter losses from bare plots were significant. Deposition in naturally vegetated and transplanted plots was initially similar. Leaf-loss occurred after October and plots were covered in ice from mid-December 2009 to the end of January 2010. There was erosion in bare (- 7 mm  $\pm$  3 SE) and natural *Z. noltii* (-1 mm  $\pm$  11 SE) plots over this period, while deposition occurred in artificial (4 mm  $\pm$  7 SE) and transplanted treatments (8 mm  $\pm$  8 SE).

Differences in mean sediment levels relative to the baseline measurement were also highly significant between plot types (MANOVA,  $F_{3,56} = 11.78$ , p < 0.01, n = 59) (Tables 6.4 & 6.5) (Figure 6.7), and between measurement dates ( $F_{2,55} = 5.66$ , p < 0.01, n = 59). The rate of sediment accretion was slowest in the first two months, but increased significantly afterwards. The greatest mean height above the baseline measurement was observed in artificial plots on  $1^{st}$  February 2010, and was 38 mm  $\pm$  5 SE, compared to 26 mm  $\pm$  7 SE in transplanted plots, and 15 mm  $\pm$  8 SE in naturally vegetated plots and on the same date.

Over winter, artificial plots consistently had significantly higher sediment levels than all other treatments. Bare plots had lower sediment levels throughout, with erosion to below the baseline during winter, to -4 mm  $\pm$  6 SE. Four of the five artificial beds were washed off in a storm on 31<sup>st</sup> March 2010 and had to be re-fastened; consequently the data for artificial plots was not continuous for the duration of the experiment. However, the mean sediment height above the baseline in the one remaining bed was 35 mm  $\pm$  8 SE after the storm, despite erosion of up to 70 mm over some measurement plates. After the beds were reinstated, deposition occurred in artificial plots, with up to 61 mm deposition over one plate over 12 weeks. All other plots underwent erosion in spring

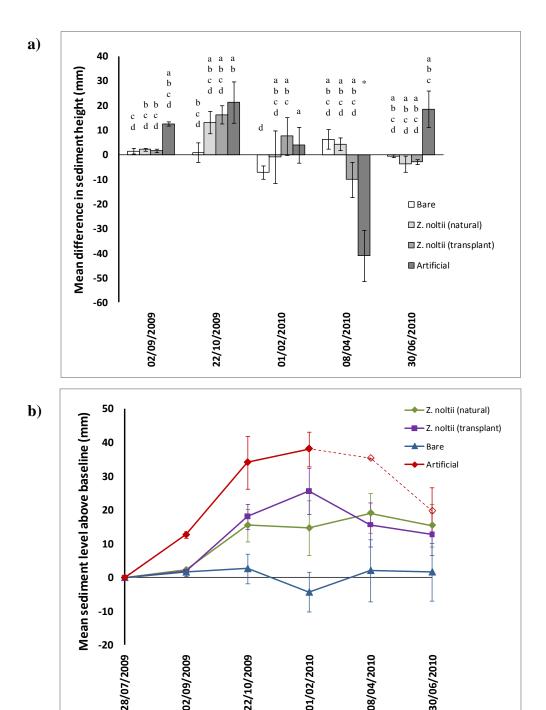
2010. Excepting the bed-loss in March, sediment deposition occurred in artificial plots throughout the experiment.

**Table 6.4:** Repeated measurements MANOVAs of the mean sediment deposition or erosion between measurements for the full duration of the experiment, for each plot type; and of the mean sediment levels relative to the baseline measurement, up to 1<sup>st</sup> February 2010.

	Variable	n	F	р	Residual d.f.
Difference l	between measurements:				
Plot type		93	6.78	< 0.001	85
	Artificial seagrass				
	Z. noltii (natural)				
	Z. noltii (transplanted)				
	Bare sed iment				
Measureme	nt date	93	3.40	0.012	85
	September 2009				
	October 2009				
	February 2010				
	April 2010				
	June 2010				
Total differ	ence from ba seline:				
Plot type		59	11.78	< 0.001	53
	Artificial seagrass				
	Z. noltii (natural)				
	Z. noltii (transplanted)				
	Bare sediment				
Measurement date		59	5.66	0.006	53
	September 2009				
	October 2009				
	February 2010				
			-		

**Table 6.5:** Tukey's *post hoc* tests and coefficient of variation (CV) (%) of the difference in sediment height between test plots and measurement dates, and of the mean sediment levels relative to the baseline measurement. Means that do not share a letter are significantly different (p < 0.05).

	Plot type	Measurement	n	Mean (mm) ± SE	CV	Grouping			
Difference between									
measurements	A	February	5	$4 \pm 7$	10.09	A			
	A	October	5	$21 \pm 8$	8.17	A	В		
	T	February	5	$8 \pm 8$	8.75	A	В	C	
	A	June	5	$19 \pm 6$	6.36	A	В	C	
	T	October	5	$16 \pm 4$	3.29	A	В	C	
	V	April	5	$4 \pm 3$	3.26	A	В	C	D
	V	October	5	$13 \pm 4$	4.63	A	В	C	D
	T	April	5	$-10 \pm 7$	1.93	A	В	C	D
	V	June	5	$-4 \pm 3$	5.02	A	В	C	D
	A	September	5	$13 \pm 1$	1.02	A	В	C	D
	V	February	5	-1 ± 11	4.93	A	В	C	D
	T	June	5	-3 ± 1	1.50	A	В	C	D
	В	April	4	$2 \pm 9$	4.65	A	В	C	D
	В	June	4	$0 \pm 1$	0.98	A	В	C	D
	V	September	5	$2 \pm 1$	0.79		В	C	D
	T	September	5	$2 \pm 2$	1.17		В	C	D
	В	October	5	$1 \pm 4$	6.06		В	C	D
	В	September	5	$2 \pm 1$	1.66			C	D
	В	February	5	-7 ± 3	5.20				D
Total difference									
from baseline	A	February	5	$38 \pm 5$	4.67	A			
	A	October	5	$34 \pm 8$	9.02	A			
	T	February	5	$26 \pm 7$	8.57	A	В		
	T	October	5	$18 \pm 4$	5.36	A	В	C	
	V	October	5	$15 \pm 5$	8.41	A	В	C	
	A	September	5	$13 \pm 1$	1.84	A	В	C	
	V	February	5	$15 \pm 8$	21.19	A	В	C	
	V	September	5	2 ± 1	1.74		В	C	
	T	September	5	2 ± 1	2.60		В	C	
	В	September	5	$2 \pm 1$	3.62		В	C	
	В	October	5	$3 \pm 4$	14.67		В	C	
	В	February	4	-4 ± 6	21.01			C	



**Figure 6.7**: Comparisons between plot types for a) the mean differences in sediment height and b) mean sediment levels relative to the baseline measurement. In figure 6.7a data for artificial plots on 8th April (\*) was not included in the statistical analysis, after four beds were removed by a storm prior to that measurement. In figure 6.7b, data points (\*) are indicative of the one remaining artificial plot measurement on 8<sup>th</sup> April, and of the sediment height above the baseline at the end of the trial on 30<sup>th</sup> June, despite sediment losses due to the temporary removal of the artificial beds.

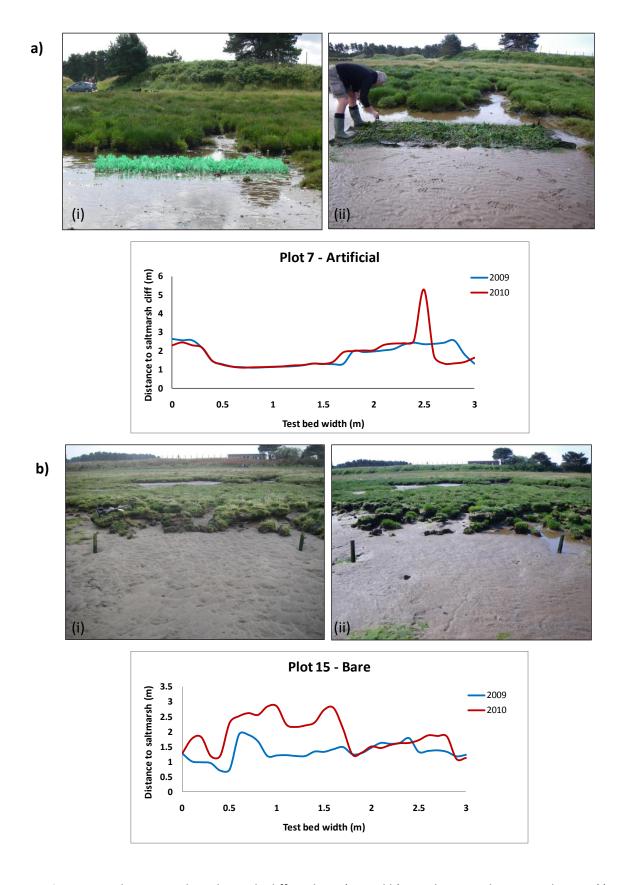
There was no significant difference between natural and transplanted *Z. noltii* treatments: at the end of the trial the mean sediment increase above the baseline measurement in transplanted *Z. noltii* patches was 13 mm  $\pm$  6 SE, in naturally vegetated *Z. noltii* patches it was 15 mm  $\pm$  6 SE, while for artificial beds it was around 20 mm  $\pm$  7 SE. Sediment heights in bare plots were close to the baseline, with an increase of 2 mm  $\pm$  9 SE.

### 6.3.3 Saltmarsh cliff seaward advancement or erosion

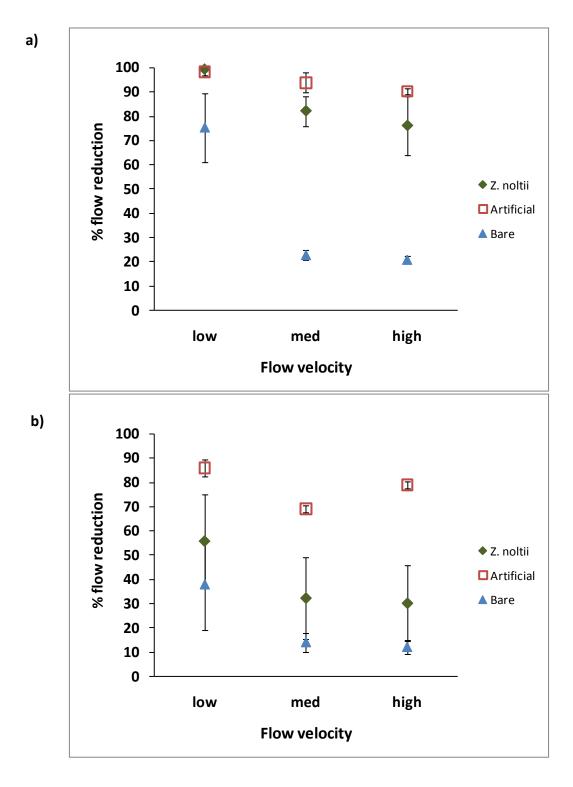
The saltmarsh cliff behind plot 15 (bare sediment) had mean erosion rates of 0.59 m  $\pm$  0.13 SE (Figure 6.8); however, this plot was not included in the statistical analysis due to the high proportion of outlying data it produced. Over the whole site there was mean erosion of 0.04 m  $\pm$  0.02 SE with erosion of the cliffs behind 15 out of the 20 plots, although there was no significant difference between plot types (Table 6.2).

## 6.3.4 Flow reduction – comparing Zostera noltii and artificial seagrass beds

At low velocity, flow reduction at 1 cm above the sediment surface in *Z. noltii* and artificial beds was similar at ~ 99% (Figure 6.9). As velocity increased, the reduction in flow decreased over *Z. noltii* and bare sediment. In artificial beds, flow at 1 cm above the bottom was reduced by > 90% at all flow speeds. It was not possible to determine full boundary layer profiles for artificial grass due to the limited depth of the flume, so flow reduction up to 5 cm (the mean boundary layer height within the *Z. noltii* beds) was compared to boundary layer flow reduction within *Z. noltii* and bare beds. In low velocity conditions flow was reduced by  $86\% \pm 3.5$  SE in artificial beds, compared to  $56\% \pm 19.2$  SE in *Z. noltii* beds and  $38\% \pm 18.7$  SE over bare sediment. At medium and high velocities, artificial seagrass reduced flow by > 69%, while *Z. noltii* reduced flow by ~ 30%.



**Figure 6.8**: Changes in the saltmarsh cliff in plots a) 7 and b) 15. Photographs were taken on (i)  $28^{th}$  July 2009 and (ii)  $30^{th}$  June 2010.



**Figure 6.9**: The mean percentage flow reduction with SE bars at a) 1 cm above the sediment surface and b) within the boundary layer above test plots of bare sediment, *Zostera noltii* and artificial seagrass at low, medium and high velocities. Flow velocities were low (0.05 ms<sup>-1</sup>), medium (0.15 ms<sup>-1</sup>) and high (0.45 ms<sup>-1</sup>).

# 6.3.5 Transplantation of Zostera noltii

Z. noltii was present in all transplanted plots until October 2009, although it grew sparsely in plots 11, 14 and 17. In plots 2 and 5, seagrass growth led to the merging of transplanted cores into a continuous bed (Figure 6.10). All transplanted plots were bare over winter 2009/10. In spring 2010 full regrowth was evident only in plots 2 and 5. Plot 14 regained around 5% coverage on one edge where there was standing water.

## 6.4 Discussion

### 6.4.1 Zostera noltii transplantation

Sediment accretion in plots of transplanted Z. noltii was similar to that in naturally occurring Z. noltti, supporting the hypothesis (H<sub>1</sub>), and both of these treatments showed greater accretion than bare sediment. The increase in the very fine sand and clay fractions in transplanted plots suggest that grain size modification was due to the presence of Z. noltii. A similar increase in the fine sediment fractions was also found during previous transplantation experiments using Z. marina (Bos et al. 2007; van Katwijk et al. 2010). However, all transplanted plots were bare over winter 2009/10, and full regrowth occurred in only two plots afterwards, suggesting that sediment grain size was linked to hydrodynamic conditions rather than the presence of seagrass. Previous Z. noltii transplantation trials have had mixed success in terms of survival and coverage (Ranwell et al. 1974; Nicholls 2003; van Katwijk et al. 2009), and during this experiment transplanted Z. noltii survived in only three out of five test plots (Figure 6.10), with only two retaining extensive vegetative cover. Plots 2 and 5 went on to have full regrowth in 2010, with plot 17 retaining ~ 5% coverage. In June 2011, a year after the end of the trial, regrowth patterns in these plots was similar to 2010, but with additional growth extending out of the plot boundaries.

The hydrodynamics of the site can influence plant development, with transplants into more sheltered sites having greater shoot density (Schanz & Asmus 2003; Kosche 2007). However, in this study no differences were found between flow conditions in

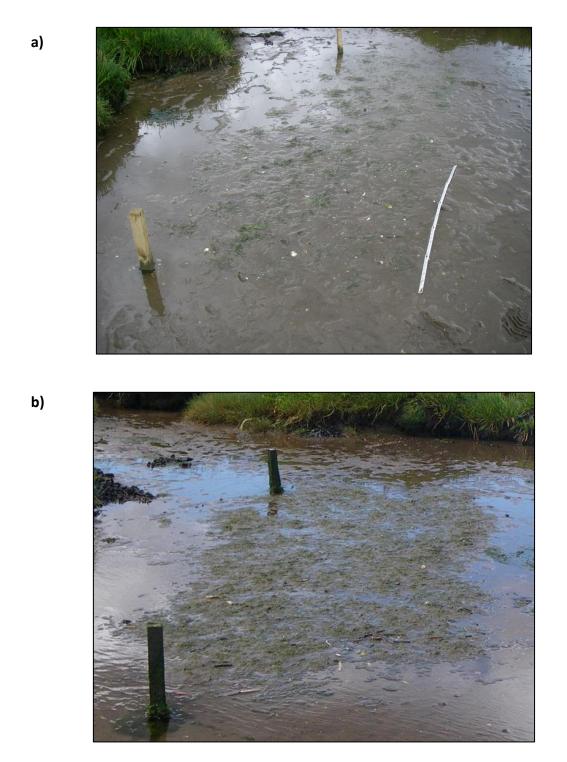


Figure 6.10: Transplanted Zostera noltii in plot 2 in a) July 2009 b) September 2010.

plots before the start of the trial and both pre- and post-transplant sediment characteristics were similar in all plots. By October dry conditions were evident in plots 11, 14 and 17, continuing for the rest of the trial. It should be noted that plot elevation was not measured, and that these plots are noticeably at a slightly higher elevation on the shore than the two successful transplantation sites. Plots at higher elevation would remain uncovered by tides for longer periods, and may not be covered at all during neap tides. Although Z. noltii is tolerant to desiccation – it may dry out completely between tides (Davison & Hughes 1998) – increased exposure to warm and/or dry conditions decreases Z. noltii photosynthetic rates and biomass (Pérez-Lloréns & Niell 1993; Cardoso et al. 2008). Over the winter of 2009/2010 there was high deposition (up to 54 mm over one plate) in plot 11, one of the plots that underwent full transplant mortality. This was followed by erosion of 64 mm over the same plate after the March storm. However, if scouring by ice or waves had removed sediments containing rhizomes and seeds, the natural Z noltii plots would also have experienced reduced shoot regrowth. It should also be considered that coastal sediment habitats are highly dynamic, thus suitable areas for transplantation may become unsuitable as a result of changes in geomorphology caused by wave energy and high tides (van Katwijk et al. 2009).

In other *Z. noltii* transplantation experiments, elevated nutrient levels (Brun *et al.* 2008), and bioturbating polychaete worms, such as *Arenicola marina* (Philippart 1994) and *Nereis diversicolor* (Hughes *et al.* 2000), have been shown to have negative effects on transplant survival. However, at no time throughout the trial was *Ulva* sp., an indicator of high nutrient levels (den Hartog 1994), present in any of the bare or vegetated plots, and there were very few polychaete worm casts.

Guidelines for seagrass transplantation suggest that the chances of transplant success can be increased by transplanting from donor locations that are environmentally similar to the reception sites, and that transplanting from genetically diverse donor material can enhance long-term survival by increasing resilience to diseases and other disturbances (Fonseca *et al.* 1998; Christensen *et al* 2004). Thus, lack of genetic diversity or use of maladapted genetic stock may reduce survival (McKay *et al.* 2005). During this trial,

the cores for transplantation were taken from over a 3 ha donor area at Tayport, 1 km west of Tentsmuir, and planted randomly. If transplant mortality was due to lack of genetic diversity, it would be expected that transplants in all plots would fail to thrive. The success in plots 2 and 5 suggest that survival or failure was related to environmental factors affecting the plots.

It is advisable that before undertaking any habitat restoration, the reasons for the initial decline should be alleviated or reversed to provide favourable conditions for growth (Hemminga & Duarte 2000; Christensen *et al.* 2004). It is also preferable that the recipient site has previously supported seagrass (Christensen *et al.* 2004; van Katwijk *et al.* 2009). Although some *Z. noltii* grew naturally in front of the saltmarsh, there were large gaps between beds. The two successful transplants were situated near naturally occurring *Z. noltii* beds.

## **6.4.2** Artifical seagrass beds

Throughout the experiment, sediment accretion in artificial seagrass beds in the Tay Estuary was significantly higher than in plots of *Z. noltii* and bare plots. The results support the hypothesis (H<sub>2</sub>) that deposition in artificial beds would occur throughout the winter, while plots of bare sediment and naturally occurring *Z. noltii* were eroded. Although the artificial leaf frond length and width, and shoot density were similar to those of *Z. noltii* growing at the site in 2008, the rate of deposition was consistently greater. This could be an effect of the greater shoot stiffness of the polypropylene mimics. Stiff shoots reduce flow more than flexible ones because within beds of stiff shoots, drag forces (Bouma *et al.* 2005), and the height of the boundary layer subject to these forces (Hughes 2001; Peralta *et al.* 2008) are greater. Bouma *et al.* (2005) observed that stiff leaves were three time more effective at attenuation of wave energy than flexible leaves of the same length. In addition, while flexible *Z. noltii* shoots bend andcan reconfigure, deflecting flow and sediment above the canopy, the stiff artificial leaves allow relatively higher volumetric flow through the upright canopy, providing a source for sediment deposition (Peralta *et al.* 2008).

Almasi *et al.* (1987) observed that mud fraction (< 63 μm) was higher in both natural and artificial *Thalassia* sp. beds than in bare sediment. However, at the end of this experiment, the percentage of small grains had decreased in artificial plots, and was lower than in bare plots. At this time (30<sup>th</sup> June 2010) artificial plots were covered by a layer of *Ulva* sp. (Figure 6.11) which would have deflected flow over the top of the bed (*sensu* Morris 1955), impeding exchange between the water column and the sediment surface (den Hartog 1994), and decreasing the amount of small sediment particles reaching the bed. The absence of *Ulva* around the plots suggests that the artificial leaves trapped floating algal filaments. If artificial beds with stiff leaves were to be used in conjunction with natural transplants as a seagrass restoration tool, the trapping of *Ulva* could cause shading and anoxic condition, "suffocating" the seagrass (den Hartog 1994; Cardoso *et al.* 2004).



**Figure 6.11**: Artificial seagrass plot covered with *Ulva* sp. on 30<sup>th</sup> June 2010.

The artificial beds were constructed from strips of polypropylene attached to chicken wire. Epiphytic leaf cover was evident by the second visit, and it has been shown in previous studies that plastic substrate is not inhibiting to epiphyte growth (Almasi *et al.* 1987; Ceccherelli & Cinelli 1999; Cardoso *et al.* 2007). Biofilms on *Z. noltii* leaves trap small sestonic particles (Chapter 5), therefore epiphytic cover on artificial fronds is likely to be important when comparing sediment accretion in artificial and natural *Z. noltii* beds during this experiment.

Although the artificial seagrass enhanced sediment deposition, the effectiveness of the artificial beds was reduced by the wire base, which was very flexible and light-weight. Although the bed was fastened down well, it was lifted by the pull of the water on the leaves; any sediment covering the plot was disturbed by the bed lifting when the tide came in. When taking sediment depth measurements it was noticeable that deposited sediment was mostly underneath the bed, and only covered the mesh near points where the bed was pegged down. Also, although the wire had a plastic coating, saltwater made contact with the metal, and the wire began to rust and become brittle, breaking easily after 8 months. Drag forces are greater on stiff shoots (Bouma *et al.* 2005), and this should be considered when selecting materials to construct the artificial bed from. The design could be improved by using biodegradable "leaf fronds" with coir mesh as a base. Coir would absorb water and be weighed down when wet, allowing deposited sediment to cover it. The beds could remain *in situ* where they would eventually decompose. The polypropylene beds needed to be removed at the end of the trial, disturbing the sediment when they were lifted.

#### 6.4.3 Saltmarsh cliff seaward advancement or erosion

Over one year there was erosion of the cliffs behind 15 out of the 20 plots, although there was no significant difference between plot types. Comparison of the cliff profiles from before and after the experiment show some changes in the morphology of the seaward face of the marsh (Figure 6.8), but overall the amount of erosion was insignificant. Changes generally occurred where creeks were present, highlighting the

importance of saltmarsh creek systems in the interchange of sediment between marshes and adjacent estuaries (Adam 1990; Garbutt & Boorman 2009). For example, plot 15 (bare sediment) had mean erosion of 0.59 m  $\pm$  0.13 SE, and has a substantial creek system behind it. When developing techniques to restore saltmarsh, it must be considered that cliff erosion is not only caused by the sea.

#### **6.4.4** Conclusions

The results of this habitat restoration trial support the hypotheses  $(H_1)$  that sediment deposition and erosion over one year would be similar in patches of naturally occurring Z. *noltii* and transplanted Z. *noltii*; and  $(H_2)$  that there would be sediment deposition in beds of artificial seagrass all year round.

 $Z.\ noltii$  transplantation cannot be considered a fast-acting technique for increasing sediment deposition in front of saltmarshes. After one year, sediment height in transplanted  $Z.\ noltii$  patches had increased by 13 mm  $\pm$  6 SE, and in naturally vegetated  $Z.\ noltii$  patches it was 15 mm  $\pm$  6 SE. Only two out of the five transplanted plots were successful, with regrowth two-years after planting, expanding out of the plot boundaries. Pre-trial sediment characteristics at the experimental site were similar to those of the donor site. However, plot elevation was not considered, and transplanted seagrass may have succumbed to desiccation. Transplantation may be considered as a tool for  $Z.\ noltii$  habitat restoration, but the elevation and hydrodynamic conditions of the planting site must be similar to those of the donor site for the seagrass to survive.

It has been demonstrated that, in principle, artificial seagrass beds could be used to increase sediment deposition in front of saltmarsh. Artificial beds accumulated 21 mm  $\pm$  8 SE during a seven week period, and this accumulation could have been greater with improvements to the design. However, *Ulva* trapped by the artificial leaves may have impeded exchange between the water column and the sediment surface, decreasing the number of sediment particles reaching the bed.

Over the relatively short time-span of one year there was no evidence that either of these restoration techniques offer protection to the saltmarsh cliff behind them.

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## Chapter 7

### **General discussion**

### 7.1 Significance of the study

In temperate European coastal waters most seagrass research is done in France, Denmark, and the Netherlands (e.g. Welsh *et al.* 1996, 1997; Bouma *et al.* 2005; Widdows *et al.* 2008; Lebreton *et al.* 2009). However, very little seagrass research has been carried out in the UK, especially in Scotland. Therefore, this project was undertaken with little prior knowledge of the distribution or ecology of intertidal seagrasses in the Tay estuary, or their role in sediment deposition and stability. The work carried to produce this thesis has increased the available knowledge of the ecology of *Zostera* and its influence on the sediment dynamics of the site considerably.

Throughout this research the concept of ecosystem engineering (Jones *et al.* 1994, 1997) in seagrass habitats was explored, building on previous research into the influence of seagrass on sediment deposition and stability (e.g. Gacia *et al.* 1999; Bouma *et al.* 2005; Bos *et al.* 2007). These ideas were taken further by including leaf and sediment biofilms in the study, as experimental results (Chapter 4) suggested that seagrasses do not work in isolation to trap and retain small sestonic particles (< 63  $\mu$ m).

During the study previous experimental techniques, such as the use of fluorescent tracer particles (Yasso 1965), were adapted to measure sediment deposition and retention in *Z. noltii* patches (Chapters 4 and 5). Novel approaches such as the use of magnetic particle induction (MagPI) to test the strength of leaf and sediment biofilms (Chapter 5), and the use of artificial seagrass as a restoration technique (Chapter 6) were also tested.

This study provides an insight into the ecology of eelgrass in the Tay estuary and its role as an ecosystem manager. It may offer valuable data which could then be utilised

for future conservation policies, habitat restoration schemes and management planning of the area.

# 7.2 Review of experiment results and recommendations for future work

#### 7.2.1 Physical effects of intertidal seagrass on sediments

The Zostera survey and the comparison of sediments in areas of Z. noltii and Z. marina (Chapter 2) highlighted their ecological plasticity, with both species tolerating a range of shore heights (tidal exposure), salinities and sediment grain sizes. The analysis of Z. noltii sediments showed higher levels of organic content and colloidal carbohydrate content, and lower redox potential than adjacent bare plots, suggesting that the plants are ecosystem engineers to some extent. At the two Z. marina sites there were no significant differences between vegetated and bare plots. However, from these results it is not possible to say that Z. marina does not modify its sediment environment. The lack of replication in Z. marina sediment sampling, due to the limited number of sites, may have led to insignificant results. To fully assess the effects of Zostera communities on sediments in the Tay estuary a larger number of samples should have been taken, and seasonal affects should also have been considered. The analysis of sediment characteristics could then be used to determine the optimal conditions for seagrass growth, providing data for future habitat restoration.

# 7.2.2 The influence of *Zostera* spp. on sediment deposition and stability in the Tay estuary

The survey of intertidal seagrass habitat (Chapter 2) revealed that there was little overall coverage of *Zostera* in the Tay estuary, with around 3 ha of *Z. noltii* and 1 ha of *Z. marina*. The total area of the sand- and mudflat at Tayport/Tentsmuir is ~300 ha, thus seagrass covers only just over 1% of the shore. From these figures it could be assumed that seagrass does not play a significant role in coastal stability in the Tay estuary.

Additionally, *Z. noltii* was found growing in discrete patches (0.25 m<sup>2</sup> to 25 m<sup>2</sup>) on the upper shore at Tayport. The results of the flume study in Chapter 4 suggest that this "patchiness" limits the effects of shoot density on deposition. The influence of patchy growth has been considered previously (e.g. Fonseca *et al.* 1982; Bouma *et al.* 2005; Fonseca & Koehl 2006) where the effects on flow and sedimentation were explored, but this approach is not the norm with most studies taking place in, or extrapolating their results to, large areas of seagrass. The results of the flume study may be indicative to conditions in the Tay, and other estuaries where *Z. noltii* does not occur in large meadows, but they are not indicative of larger meadows found elsewhere.

However, measurements of sediment accretion over one year in an area of Z. noltii (Chapter 3) demonstrated that the presence of seagrass on the upper shore decreased the loss of sediment over winter, and may actually result in net gains in sediment height in summer. In some places the elevation of the sediment surface in front of saltmarsh may lead to saltmarsh succession (Hughes et al. 2000). However, the ongoing erosion of the saltmarsh cliff suggests that this is not the case at Tayport. Elsewhere on the shore there is also evidence that sediment is not accumulating, because if deposition was continuous, seagrass patches would ultimately develop into emergent islands (Koch 1999). However, observations over one year cannot accurately predict patterns of gradual change. The IPCC have predicted sea level rise of between 18 cm to 59 cm during the 21<sup>st</sup> Century, with an increase the in the occurrence of extreme high tides (IPCC 2007). The predicted rise of sea level is within the vertical growth rate of most seagrass species, where particle trapping and retention elevates the sediment surface (Hemminga & Duarte 2000). To assess the effects of sea level rise on intertidal seagrass habitat, and the influence of the plants on sediment deposition and stability, long-term monitoring of the shore height at Tayport, and in other estuaries, is required.

#### 7.2.2.1 Further recommendations for future work

It is possible to suggest some alterations to the experimental methods which would improve future similar studies:

#### Flume study

The accuracy of the velocity profiles and near-bed hydrodynamics data would have been greater if ADV readings had been taken nearer the bed and at smaller increments e.g. every 1 mm from 1 to 10 mm and then every 5 mm thereafter. Interference by leaves may have affected readings within the bed, and removal of leaves around the ADV probe or placing the probe directly downstream of the bed may be a consideration. The flume recirculated water and this may have contributed to the particle load, although magnets were present at the flume exit to catch particles not trapped in the test bed. In addition the experiment had low statistical power to detect significant effects; therefore more replication would be required. It must be noted that measurements were carried out using small test beds 0.20 x 0.25 m; therefore the results may illustrate only meadow-edge effects. It would be of interest to investigate the mechanisms of trapping of particles of different sizes in larger beds, perhaps in situ. The high number of small particles found in low density beds could be due to sampling error; cores were taken from random areas of the bed, and some may have been taken from too near the edge where the core-well trapped particles. Fluorescent particles adsorbed onto the leaf surfaces and a count of the particles trapped by the leaves would have greatly improved this study. Although samples were taken, on this occasion it was not possible to count them due to equipment failure at the time of the experiment and subsequent degradation of the samples. However, particle capture by leaves is discussed in Chapter 5.

#### In situ study

During application some particles were scattered on the *Z. noltii* leaves, although care was taken to avoid this. Future studies may be improved by counting the particles remaining adsorbed to the leaf surface over time, although it would not be possible to separate those present from the initial application from those caught there during the experiment. A comparison of particle retention under different flow conditions could be carried out either in the flume or *in situ* - if wind and tide conditions were known in advance.

#### Particle adhesion to sediment and leaf biofilms using MagPI

MagPI (Larson *et al.* 2009) is a relatively novel experimental approach, with various potential applications. In Chapter 5 sediments samples were taken from a limited area of the Tayport shore, from *Z. noltii* patches only. Future comparisons should be made between sediments from different parts of shore, and between sites of both seagrass species, taking seasonality into consideration. In Chapter 5 the technique was used to measure sediment stability under conditions of no flow. The effects of stressors, such as waves and strong currents, on biofilm strength and cohesiveness could be assessed by placing sediment cores in a flume under different flow regimes.

# 7.2.3 Can artificial seagrass be used to restore sediment habitats? A comparison of restoration techniques

#### Zostera noltii transplantation

The transplantation trial of *Z. noltii* was partially successful, with full regrowth in only two out of five plots. Prior to habitat restoration, the causes of the initial decline should be identified and alleviated (van Katwijk *et al.* 2009), and the lack of this knowledge may have contributed to the failure. Although the use of seeds and seedlings would be preferable to transplantation, avoiding damage to existing seagrass beds, less research has been done on restoration methods using seeds. Studies suggest that the number of seed predators must be low either naturally, or through their physical exclusion (Hughes 1999; Nicholls 2003; Hughes & Paramor 2004). Seedling trials have worked best in low energy environments (Fonseca *et al.* 1998) so a future study may be appropriate for the Tay estuary intertidal area. The cultivation of seedlings, the methods for planting out and the exclusion of predators, if required, would not be possible within the timescale of this PhD project. Transplantation requires standing water, to prevent desiccation (Nicholls, 2003), and this factor along with sediment elevation should be assessed before transplantation to give the plants the best possible chance of survival.

### Artificial seagrass beds

This study has demonstrated that artificial seagrass beds increase sediment accretion and retention throughout the year, and they could be developed as a tool for enhancing sediment deposition. In conjunction with in-bed flow measurement - either *in situ* or in a flume - leaf length (Fonseca & Fisher 1986; Fonseca & Cahalan 1992), leaf width and morphology (Leonard & Luther 1995; Hendriks *et al.* 2009), and shoot density (Peterson *et al.* 2004) could be adjusted to suit the specific requirements of the site, maximising the effect on flow and on sediment deposition.



**Figure 7.1:** Biodegradable seagrass mimics; a) reed and coir mesh, b) reed on bamboo anchors, and c) test plots in the Tay estuary.

In this initial trial, budget restraints led to the use of polypropylene fronds on a wire base; materials which were too buoyant to be buried by deposited sediment. The use of plastics in coastal restoration projects should be avoided; instead biodegradable materials could be used, allowing the beds to remain *in situ* for a number of years without risk to sea birds and mammals which may be harmed through accidentally consuming plastics (NOAA 2011; United Nations Environment Programme 2011). With funding from the Spragge Conservation Scholarship, initial trials of biodegradable seagrass mimics have taken place in the Tay Estuary to test the burial potential (lack of buoyancy) and longevity of natural materials such as coir mesh, bamboo and reeds (Figure 7.1). To date, materials have been in place for over one year.

In the restoration trial, plots for all experimental conditions were 3 m x 1 m. Meadow edge effects (Fonseca *et al.* 1982) dictate that larger plots would lead to increased flow reduction and sediment deposition. However, manufacturing the artificial beds was incredibly time- and labour-intensive, and researchers producing seagrass mimics for other studies have similar experience (*pers. comms.*). Therefore, as well as trialling different materials, manufacturing methods must also be explored.

#### 7.2.4 Seagrass survey and monitoring

As biodiversity action plan priority species, and Annex I habitats (JNCC 1995) intertidal *Zostera* beds are under-recorded in the UK, and there is a general lack of monitoring. The UK Biodiversity Group Tranch 2 Action Plan for Maritime Species and Habitats (UK Biodiversity Group 2008) states that "there should be no further loss of extent of intertidal ecosystems", and in the Action Plan, SNH are required to "determine the extent and quality of the seagrass resource which falls within protected areas and notify further sites". However, the total coverage of seagrass is unknown.

In the summer of 2008 the first dedicated survey and mapping exercise was carried out for *Zostera* spp. habitat in the Tay estuary. As well as determining the extent and distribution of seagrass beds on the shores of the estuary, the results were used to plan

experimental work for this thesis. This survey data from Chapter 2 could be used as baseline for future monitoring of the habitat in the Tay estuary, and the dataset will be made available to various organisations including Scottish Natural Heritage and the Botanical Society of the British Isles, and will be uploaded to the National Biodiversity Network (www.nbn.org.uk). Further surveys may give an insight into how dynamic Zostera populations are, and how their distribution changes in relation to sea level rise. SeagrassNet (www.seagrassnet.org) are an organisation who co-ordinate a worldwide seagrass monitoring program, with volunteers, universities and research organisations undertaking surveys in 122 sites in 33 countries. The data is used to increase scientific knowledge and public awareness of seagrass ecosystems. There are currently no UK sites involved in SeagrassNet - the nearest European sites being in Denmark and Portugal - and organisers are enthusiastic about having British involvement in the project due to the lack of available data on species distribution in the UK.

#### 7.2 Conclusions

The main recommendation for future work resulting from this thesis is that SNH, the Fife Coast and Countryside Trust and/or one of the local universities (either the University of St Andrews or the University of Dundee) continue to monitor seagrass habitat in the Tay.

The results of this study suggest that in the Tay estuary, the intertidal seagrasses *Z. noltii* and *Z. marina* do not modify their environment to render it more suitable to their requirements, and therefore are not ecosystem engineers as defined by Jones *et al.* (1994, 1997).

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