THE UNIVERSITY OF HULL

Use of MicroCT to Determine the Functional Ecology of Intertidal Infauna

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Abstract

Intertidal infauna through the process of bioturbation, play a major role in estuarine ecosystem functioning. Despite this, methods to both quantitatively and qualitatively assess the features produced from bioturbation have been limited. This study builds upon the recently developed technique by Mazik et al. (2008), by stabilizing sediment cores and using µCT scanning to obtain burrow parameters along a salinity and elevation gradient. In-situ agar stabilization reduced the collapse of large infaunal biogenic features in sediment cores on intertidal mudflats and accurately determined burrow volumes and surface area of these structures through µCT and three dimensional image processing software, demonstrating that the presence of the polychaete *Hediste diversicolor* can increase surface area of bioturbated sediments to over 50%. It is likely that agar stabilization may also be an adequate substitute for several other stabilizing techniques used on sub-tidal sediment cores.

Acknowledgements

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Table of Contents

List of Figures

List of Tables

1 Chapter 1: Background

1.1 Introduction

Benthic invertebrates play a key role in ecological functioning, for example: nutrient cycling and the distribution and decomposition of organic matter (Rhoads, 1974; Pearson and Rosenberg, 1978; Dauwe et al., 1998). Furthermore, their burrowing activity, often through bioturbation, defined by Kristensen et al. (2012) as 'all transport processes carried out by animals that directly or indirectly affect sediments matrices', modifies the physical properties of the sediment in terms of particle size distribution, porosity and permeability (Rhoads, 1974; Rhoads and Boyer, 1982; Jones et al., 1997) compaction, surface roughness and cohesion and adhesion between the particles (Rhoads and Young, 1970; Eckman et al., 1979; Boyer, 1980; Nowell et al., 1981). Burrowing and burrow irrigation causes the redistribution of particles and interstitial water in sediment and increases the surface area available for exchange of nutrients and contaminants at the sediment–water interface, this changes the absorption characteristics and alters the redox potential of the sediment (Benoit et al., 2006).The ability of bioturbating organisms to influence surrounding sediments has given rise to the ecosystem engineer concept, whereby the organisms modify habitat to suit their needs (Jones et al., 1994). The importance of bioturbation by infaunal organisms and the biogenic structures they produce, on marine sediment properties and biogeochemical cycling is well researched (Rhoads, 1974; Aller, 1982; Volkenborn et al., 2007; Montserrat et al., 2008; Reise et al., 2009). Various techniques have been developed to quantify bioturbation, such as sediment profile imaging (Solan et al., 2004), luminophore tracers and laser telemetry (Hollertz and Duchêne, 2001; Maire et al., 2007). Mazik et al. (2008) used novel research using high resolution micro computer tomography (µCT) as one of the most promising studies yet into standardising the measurement of bioturbation features; this technique involved three dimensional imaging to accurately quantify burrow parameters such as volume and surface area.

The proposed study focuses on the characterisation of bioturbated sediments at two different sites on the Humber estuary UK with differing salinity and elevation gradients, the functional ecology of organisms that inhabit these areas via various feeding modes and quantification of bioturbation structures using µCT imaging techniques.

1.1.1 Aims

This thesis aims to further develop the high resolution µCT technique first developed by Mazik et al. (2008), with a view to optimise scanning method, to relate burrow structures to a species level in a mixed assemblage and determine quantifiable parameters of bioturbation; such as burrow volume and area, comparing function at each site.

1.1.2 Objectives

1) To increase sample image quality via sample stabilisation;
2) To cross reference of scanned images against sieved core biota;
3) To establish any change in bioturbation parameters over different localities.

1.2 Introduction to Bioturbation

Benthic organisms play several key roles in aquatic ecosystems, for example organic matter processing or nutrient cycling at the water–sediment interface (Gilbert et al., 1998; Mermillod-Blondin et al., 2004). The physical, chemical and biological aspects of sediments are coupled through the process of bioturbation (Figure 1.1). Rhoads and Boyer (1982) describe this simply as 'the transport of particles as well as pumping water into and out of the bottom'. However a previous definition by Richter (1952 in François et al. 2002) is more encompassing: 'all manner of displacements within unconsolidated sediments and soils that are produced by the activity of organisms, and is recognized as one of the major processes that affect aquatic ecosystem functions'. Consequently bioturbation occurs as an organism moves through the sediment, feeds and excavates or irrigates burrows.

Benthic organisms exhibit different modes of life depending on the sediments they inhabit and the hydrodynamics of the system, typically species richness increases in finer cohesive

sediments compared to that of larger mobile sediments. As sediment particle size becomes smaller and more stable organisms can burrow. In sands and muds these can be a multitude of suspension or sub surface deposit feeding bivalve molluscs, crustaceans, oligochaetes and polychaetes (Little, 2000).

Infaunal species may construct tubes or burrows for protection against desiccation or predators, for bioirrigation to facilitate feeding and oxygenation of their microenvironment, or to simply move through the sediment (Duchêne and Rosenberg, 2001). Bioturbation resulting from this burrowing and burrow irrigation activity causes the redistribution of particles and interstitial water in sediment and increases the surface/ area available for sediment–water interface exchange of nutrients and contaminants, in turn modifying the absorption characteristics of the sediment and altering the reduction-oxidisation potential discontinuity (RPD) (Benoit et al., 2006).

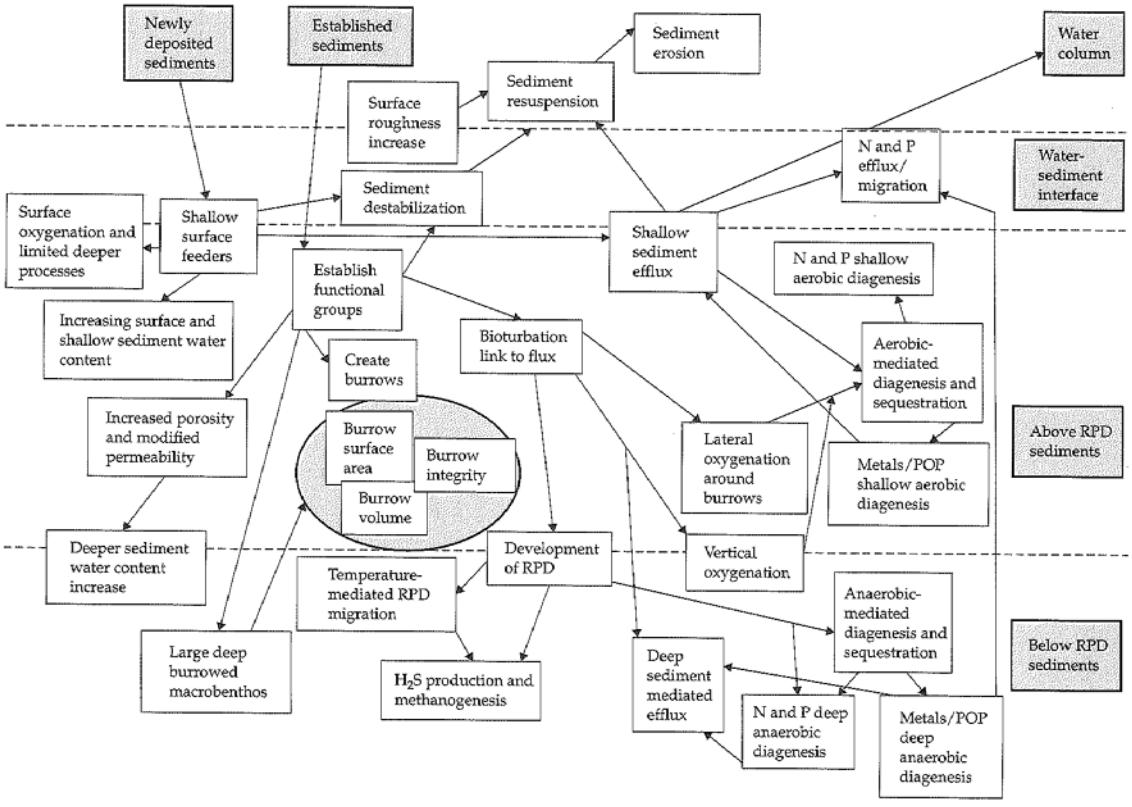


Figure 1.1 The relationships that link ecological, biogeochemical and sedimentary features of sediment modified by bioturbation activity (taken from Gray and Elliott, 2009)

In particular, bioturbation affects the fluxes of organic matter, nutrients and contaminants across the sediment– water interface and within the sediment column (François et al., 2002). The depth of bioturbation varies according to species composition, body size, relative abundance of individual/total number of species and habitats; consequently this can change the spatial distribution of biogeochemical zones, up to 20m cm of depth (Dauwe et al., 1998).

In summery bioturbation effects sediment transport processes such as resuspension, erosion and deposition cycles, nutrient/chemical fluxes in sediments and into the overlying water column, which alters benthic microbial activity. In turn this influences community structure of the system permitting or preventing different functional groups of organisms from settling and inhabiting bioturbated areas.

1.3 Functional Groups in Bioturbation

Ecosystem functions such as nutrient cycling is dependent on the functional ecology of interactions between and within individual species that inhabit an area. In soft sediment faunal communities these functional attributes are in the main related to its type of feeding mode and its position within the sediment, for example surface deposit feeder, subsurface deposit feeder, suspension feeder, predatory or grazing (Thrush et al, 2006). In regards to bioturbation these can be further categorised into four more functional groups based on particle movement (Figure 1.2): downward-conveyors, upward-conveyors, biodiffusers and regenerators (François et al., 2002; Gérino et al., 2003). These are the result of different behaviours and feeding mode exhibited by a species. An organism may display behavior of more than one feeding mode group i.e. an individual could be an predatory subsurface upwards conveyor and biodiffusor that also suspension feeds.

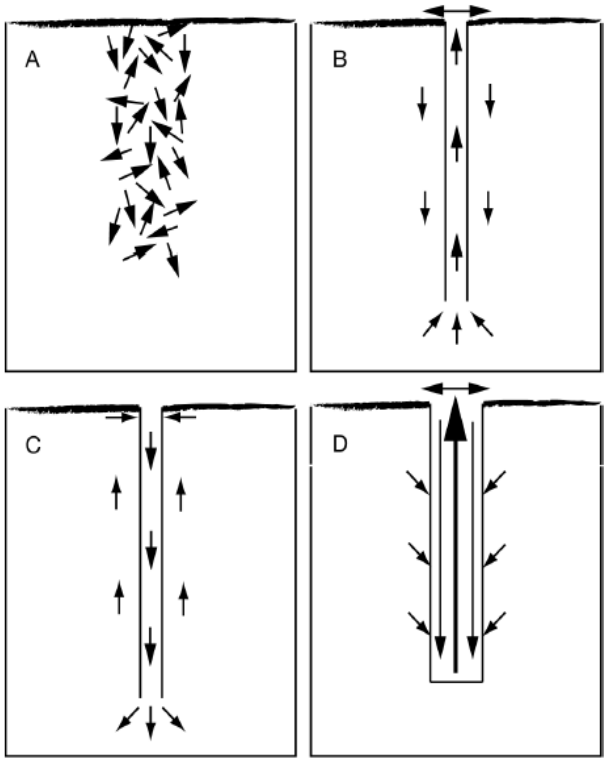


Figure 1.2 The 4 major types of particle reworking performed by bioturbators. (A) biodiffusers, (B) upward-conveyors, (C) downward-conveyors and (D) regenerators (from François et al., 1997)

1.3.1 Biodiffusers

Biodiffusers (Figure 1.2A) are organisms with life traits that produce a continuous, random local sediment bio-mixing over a short distance, this results in a net transport of particles, that is similar to molecular diffusion (Kristensen et al., 2012). Depending on the behaviour of the organisms involved, they can be separated into one of 3 distinct groups:

(1) Epifaunal biodiffusers largely inhabit areas above the sediment–water interface. They are confined to upper surface sediments and typically redistribute fine particles indiscriminately over very short distances along the surface (Kristensen et al., 2012). Typically fiddler crabs (*Uca* spp.) exhibit this method of particle transport (Penha- Lopes et al., 2009).

(2) Surficial biodiffusers are predominantly limited to the top few cm of the sediment, seldom venturing above the sediment–water interface (François et al., 1997). Examples include species from Clypeasteroida, flattened echinoderms that move throughout the upper 5 cm of sediment, relocating particles via specialized spines used for locomotion (Kristensen et al., 2012).

(3) Gallery biodiffusers are burrow inhabiting animals that perform diffusive, local bio-mixing of particles due to burrowing activity within the upper 10 to 30 cm of sediment deposits. In time they allow for transportation of particles vertically, from the near the surface of the sediment downward, at the base of the burrow (Michaud et al., 2005). Many polychaetes are gallery biodiffusers, such as the polychaete *Hediste diversicolor*, which construct complex burrow networks that connect to the surface.

1.3.2 Upward Conveyors

Upward conveyors (Figure 1.2B) are vertically-oriented species that feed head-down at depth in the sediment. They transfer particles from deep in the sediment to the surface. The particles are actively transported upwards either by passage through the gut of the organism or when subsided material is expelled from the ingestion cavity. Conversely, gravity restores these particles to depth, a local advective process; as a result subsurface feeding voids are backfilled with material from above (Rhoads, 1974; Kristensen et al., 2012). In particular *Arenicola marina* is noted for its role as an upward conveyor in shallow and intertidal sediments in NW Europe (Timmermann et al., 2002).

1.3.3 Downward Conveyors

Downward conveyors (Figure 1.2C) demonstrate the opposite feeding approach from that of upward conveyors. Vertically-oriented head-up feeders select and ingest particles at the surface and egest these non-locally as faeces in deeper within the sediment (Kristensen et al., 2012). Non-selective upward particle movement occurs via constant burrow maintenance, providing space to accumulate faecal material (Shull, 2001). Shull & Yasuda (2001) showed that the polychaete *Cirriformia grandis* gathers particles by means of its tentacles from the surface as a food source, depositing them at depth within its burrow.

1.3.4 Regenerators

Regenerators (Figure 1.2D) constantly excavate material to sustain burrows within the sediment; this action relocates sediment from depth to the surface. The excavated sediment is

exchanged with surface sediment either through current-driven infilling or during the breakdown of burrow walls. Examples of regenerators include ghost crabs of *Ocypode* spp. (Kristensen et al., 2012).

1.3.5 Functional groups and managed realignments

The voids created in the sediment from feeding can be quantified through µCT imaging. Managed realignments are a 'soft' engineered flood defence management technique, whereby an area of land adjacent to a water body usually an estuary, at which 'hard' coastal defences such as flood walls are relocated landwards of their existing position, allowing tidal inundation to occur. This permits salt marsh and intertidal mudflat habitats to develop further in land than those in inhabiting the seaward side of the previous flood defence route (French, 2006). Managed Realignments in ecological terms are the subject of several studies (French, 2006; Garbutt et al, 2006; Mazik et al, 2007, Mazik et al, 2010). As this is a newly created habitat, it can be investigated for the development of community structure and ecological functioning, such as a change in community structure from that of pioneer species to a typical intertidal community (Mazik et al, 2007;2010) or the establishment of trophic levels and species recruitment (French et al. 2006). The ecological functioning with a managed realignment site is likely to be different in comparison to that of a mature mudflat in a neighbouring location. It is for this reason that this study will attempt to use the variation between managed realignment sites and adjacent established mudflat to determine change in ecological function through feeding modes.

1.4 Physical Characteristics of Sediments in Relation to Bioturbation

The effect of benthic organisms on the physical properties of granular sediments is well documented, for example: Rhoads (1974); Volkenborn et al. (2007); Montserrat et al. (2008); Bouma et al. (2009); Reise et al. (2009) show a range of effects. These studies relate the effects of benthic species to changes in: grain size, sediment sorting, water content, compaction and benthos stability. Those autecological parameters that appear to be correlated with physical

modification of sediments include: method of feeding, feeding selectivity (active selection of certain particle sizes by suspension and deposit feeders), feeding level relative to sediment-water interface, degree of mobility, organism size, population density and burrowing depth (Rhoads and Boyer, 1982).

The ability of bioturbating organisms to influence surrounding sediments has given rise to the ecosystem engineer concept (Jones et al., 1994), comprising of two different modes: Autogenic engineering organisms change the environment via their own physical structures and are thus part of the engineered habitat whereas allogenic engineering organisms transform living or non-living materials from one physical state to another e.g. *Littorina littorea* removing sediment from hard substrates, preventing sedimentation from occurring (Jones et al., 1994). Ecosystem engineers tend to be most dominant in high physically disturbed environments (Jones et al., 1997). Benthic ecosystem engineers inhabiting coastal sediments can cause many biogenic habitat transformations such as sediment stabilisation and destabilisation (Reise, 2002; Montserrat et al., 2008). Volkenborn *et al.* (2007) found that the exclusion of certain species can stop habitat succession. In general, benthic engineers can be divided into epibenthic and endobenthic infaunal organisms depending on whether they spend most of their lifetime above or below the sediment, respectively. Many infaunal macroinvertebrate species modify the sediment through their activities and can be considered allogenic ecosystem engineers. Endobenthic species affect the flow of resources mainly through bioturbation and bioirrigation (Rhoads, 1974; Reise, 2002).

Bioturbation-mediated change in sedimentary properties can greatly influence substratum stability through producing bed roughness, increased modal grain size due to flocculation of faecal pellets/ mucopolysaccharides and changes in sediment packing, shear strength and water content. Organisms produce a variety of physical features e.g. burrowing mounds, on the seafloor related to burrowing and foraging. These activities increase bed roughness and in turn can reduce bed stability (Eckman et al., 1979; Boyer, 1980; Nowell et al. 1981). The effect of bioturbation on specific sediments is related to the rate of burrowing versus the rate of

consolidation; muds compact less rapidly than cohesionless sands, therefore the former would be expected to be affected more than the latter. Burrowing in mud produces sediment with increased water content (Rhoads and Young, 1970).

As a result an increase in several autogenic bioturbation feeding modes may influence the benthos stability. Consequently an increase in burrow voids quantified via µCT imaging may indicate a reduction in bed stability also.

1.5 Role of Disturbance in Bioturbation

Physical organism-sediment interactions coincide with ecological succession. Benthic communities are temporal and spatial mosaics, parts of which are at different levels of succession. Hence a community is the remnants of previous disturbance (Johnson, 1971). Pearson and Rosenberg (1978), Bremner et al. (2003), Pearson (2001), for example have helped to define the taxonomic and functional structure of these mosaics. Figure 1.3 illustrates the Pearson-Rosenberg model as the development of organism-sediment assemblage after an organic enrichment disturbance which, although usually occurs in near shore systems can be found in any depth of water (Jumars and Hessler, 1976).

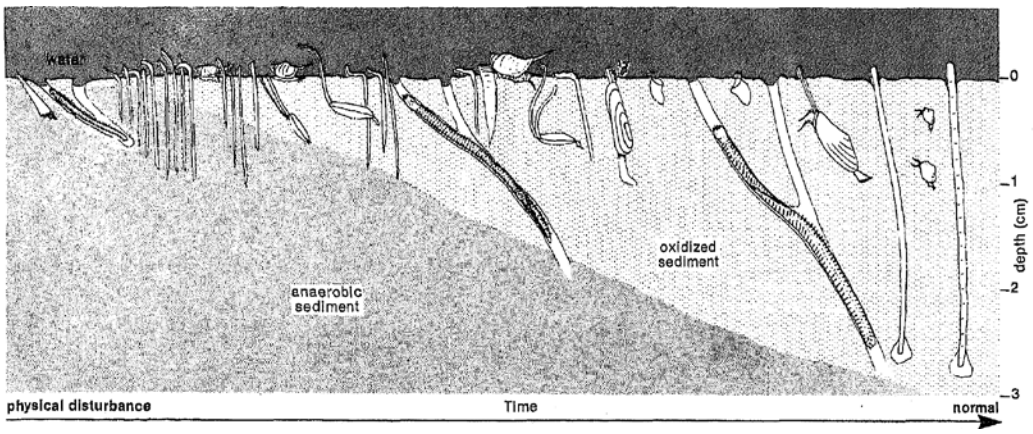


Figure 1.3 Infaunal succession over time following organic enrichment (from Rhoads et al, 1978).

Infaunal succession occurs as a two phase process of pioneering species followed by equilibrium species. Rhoads and Boyer (1982) describe several sedimentary effects of

pioneering species including: species are limited to less than 2cm to the near surface of the bottom, construct dense tube aggregations affecting micro topography and bottom roughness. Through these aggregations water is pumped into and out of the sediment so that particle bioturbation is of reduced importance and faecal pellets are produced from surface deposit and suspension feeding. Equilibrium stages are characterised by intensive particle mixing producing homogeneously mixed sediments, many particles of which at or below the surface may comprise of faecal pellets. There is also a transfer of water and particles over vertical distances through the substratum, which subsequently increases the depth of oxygenated sediment. Head-down feeding produces feeding pockets at depth within the sediment. Lastly the surface micro-topography can have reduced features as hydrographic regimes re-suspend and smoothes over surface features although, if the effects of currents are absent, feeding pits and faecal mounds maybe present. In some cases both pioneering and equilibrium species may coexist in the same sediment if the physical disturbance involves only near-surface layers. Large and deeply burrowed infaunal may not be affected by such small scale disturbances (Rhoads and Boyer, 1982). As a result it is probable that a decrease in bioturbation would coincide with a decrease in burrow volume quantified via µCT imaging.

1.6 Chemical Properties of Sediments in Regard to Bioturbation

The composition of any environment is determined by a balance between material transport processes and chemical reactions. In the case of marine sediments, transport is provided by benthic organisms moving particles and fluids during feeding, burrowing, tube construction and irrigation (Aller, 1982). This biologically derived transport has several direct and indirect effects on the composition of sediment and their overlying waters.

Many chemical reactions taking place within sediments are associated with the decomposition of organic matter and other biogenic components for example . These reactions influence pH and the depth of sediment aeration, causing the depletion or build up of chemical reactants (Goldhaber and Kaplan, 1974). Aller (1982) describes the influence of bioturbating organisms

on the distribution of chemical reactions: material is translocated continuously within the sediment during feeding, burrowing, tube construction and faecal pellet formation. These activities alter chemical reaction gradients, creating a mosaic of biogeochemical microenvironments, as opposed to a vertically stratified distribution of chemical reactions. Reactive organic substrates in the form of mucus secretions together with microbial colonisation of these secretions may be introduced into the deposit independently of sedimentation. Lastly feeding and mechanical disturbance may also influence microbial populations that mediate reactions. The extent of which these effects become apparent is dependent on the functional groups of the organisms present and their abundance (Mazik and Elliott, 2000).

1.6.1 Faecal Pellets

A large amount of potential food for detritus-feeding invertebrates is associated with sediments which contain higher fraction of muds associated with organic material; the high surface-area to volume ratio provides a considerable area for microbial colonisation and production of mucopolysaccharides, which are ingested by many benthic invertebrates (DeFlaun and Mayer, 1983). The faecal pellets produced are generally deposited at or near the surface. The distribution of pellets in the sediment column depends on their chance of burial and preservation. When pellets or mucopolysaccharides-pellet aggregates are entrained they move as bed-load. If the bed is then suspended into the water column by wave surge, faecal pellets can increase the settling velocity of sediment (McCall, 1979).These reactive particles can then be redistributed throughout the sediment via homogeneous reworking, i.e. vertically distributed in the absence of disturbance from bioturbation or selectively reworked; during feeding and burrow construction animals are capable of selecting particles on the basis of their position in the sediment, size, shape, surface texture and density (Jumars and Hessler, 1976), consequently this selection may result in lateral and vertical segregation of distinct particles, depending on: the sizes available, functional groups present and their distribution (Aller, 1982).

1.6.2 Effects of Particle Size and Surface Area on Burrow Solutes

As chemical properties of sediments correlate with grain size any spatial segregation of particle types will result in corresponding variability in chemical composition and reaction rates (Aller, 1982). In the case of aerobic reaction rates oxygen consumption by bacteria on particles is directly proportional to particle surface area (Hargrave, 1972; Hargrave and Phillips, 1977). Due to differences between the particle surface area of course grained and fine grained sediment, the latter should have an increased reactivity of surface dependant processes on a mass or volume basis than the former.

Aller (1982) showed that solutes produced in sediments post deposition are subject to transport by molecular diffusion, advection and mixing processes caused by physical or biological activity. In the absence of biological disturbance solute distributions can be quantitatively described by transport reaction equations as mathematical models to determine interactions within or between solutes for a spatial distribution of a selected outcome (Hadeler, 2000). The construction and irrigation of burrows by macro-infauna complicates this. In bioturbated zones solutes can diffuse three dimensionally along lateral concentration gradients into biogenic cavities as well as vertically towards the sediment water interface. Overlying water drawn into burrows by irrigation may be directly exchanged with surrounding pore waters, the void space between sediment particles. This is particularly the case in permeable sands, which allow solutes to pass though freely if void spaces are large and interconnected. Direct exchange also occurs during active burrowing or tube construction. Several organisms can use permeable sediments as a filter for respiratory currents to enhance food supply (Cuny et al., 2007). If animals are closely spaced and their burrows or tubes are relatively uniformly oriented, distinct horizons within the sediment column can become preferentially irrigated by such transport mechanisms (Rhoads, 1974). This implies that solutes can be advected from relatively well-mixed and distinct reservoirs within the sediment into the overlying water column (François et al., 2002; Benoit et al., 2006). If burrow walls are permeable to solute diffusion, sedimentary reactions can alter the burrow habitat. The

continual flux of solutes from neighbouring sediment into burrows is determined by concentration gradients around burrow and sediment permeability. In order to hold the composition of burrow water constant or within a restricted range of variation different from pore water, continual irrigation of the burrow must balance the flux of solutes from the surrounding sediment (Aller, 1982). During low water, the flux of solutes from surrounding sediments partly determines differences in burrow water composition, together with metabolic activity of burrow inhabitants including bacteria (Aller and Yingst, 1978).

In intertidal locations, the irrigation effort necessary to maintain burrow waters in a steady state, or the standing concentration of sediment derived solutes in the burrow at a particular irrigation rate can all be reduced by either decreasing inter-burrow distances or increasing burrow size. Of the two strategies, crowding to form areas of high burrow abundance is more effective than increased burrow size at reducing irrigation requirements (Pearson and Rosenberg, 1978; Rhoads, 1974). The construction of impermeable burrow walls would accomplish a similar result (Aller, 1982).

1.6.3 Intertidal Areas

The addition of excess nutrients or chemical pollution is likely to adversely affect a system. This is especially true of intertidal estuaries and coastal waters as they receive large volumes of waste water and contain fine, cohesive sediments. This causes contaminants to be adsorbed onto particles and incorporated within sediments. Increased levels of contamination decreases species composition, organism survival, growth, biomass and therefore bioturbation potential (Pearson and Rosenberg, 1978; Mazik and Elliott, 2000). O'Brien et al. (2009) found that *Arenicola marina* can reduce the effects of nutrient enrichment on benthic habitats by liberating nutrients into the water column for dispersal and can facilitate breakdown of organic pollutants. Cuny et al. (2007) also found a similar bioturbator facilitation using *Hediste diversicolor*, to aid bacterial communities to metabolise oil in contaminated sediment. Although Cardoso et al. (2008) determined that *H. diversicolor* bioturbation did not re-liberate other compounds such as mercury.

1.7 Quantification of Bioturbation

Characterizing and quantifying bioturbation is of important to explain the complex mechanisms that control benthic ecosystem functioning at different spatial and temporal scales. To date many laboratory and field methods have been developed to assess bioturbation. The choice of a method depends on the characteristics of the site and organisms that are studied, and on the scientific purpose. There is no 'standard' method for the assessment of sediment reworking (Maire et al., 2008).

1.7.1 Particle Tracers

Particle-tracers can be used to study the vertical component of sediment reworking. Their use is based on the measurement of their vertical distribution within the sediment column. Tracers initially deposited at the sediment–water interface or at any horizon within the sediment column, are redistributed due to the action of benthic fauna (Gilbert *et al*, 2007). The tracer is then recovered and a mathematical model is used to attain a rate of sediment reworking (Wheatcroft et al., 1990; François et al., 1997; Meysman et al., 2007).

1.7.1.1 Radionuclides

Various natural particle tracers have been used: radionuclides have widespread use as particle tracers to assess sediment reworking rates in aquatic environments (Green et al., 2002). Some radionuclides occur naturally in the water column (Aller, 1982) and most originate from atmospheric fallout. For example, is produced from decay in the atmosphere and by decay in the water column. All are quickly scavenged from the water column by suspended particles, and transported to the sea floor, where they are rapidly incorporated and mixed into the sediment. Their vertical distribution within the sediment column depends on external supply rates, *in situ* production, half-life and sediment reworking (Maire et al., 2008). If the first three factors are known an assessment of reworking can be made through vertical concentration profiles. Key radionuclides used to assess sediment reworking rates in marine environments derive from nuclear testing; long half-lives allow sediment reworking rates to be assessed over

temporal scales (Thomson *et al*, 2000). As opposed to natural radionuclides, which are continuously supplied to the sea floor, deliberately introduce radionuclides are not characterized by steady-state distributions and can be considered a pulse input (Aller, 1982).

1.7.1.2 Microtektites

Glass et al. (1973) used microtektites for evaluation of sediment reworking; these small glass spheres deposited on the sea floor following meteorite showers are similar in density to sediment particles, but rounded in shape. Their transparent glassy appearance makes them easy to distinguish and count under a microscope. Since their input to the sediment column is restricted in time, their subsequent vertical concentration profiles result from a balance between sediment reworking and sedimentation following the event. The main limitation of this technique is that their temporal resolution is low due to meteorite showers occurring over a geological time-scale, thus are more suited to evaluation of past sediment reworking (Maire et al., 2008)

1.7.1.3 Deliberately Introduced Tracers

Many frequently used tracers are deliberately introduced into sediment (Gérino et al., 1994, Mugnai et al., 2003, Ouellette et al., 2004). Their main advantage is that they allow a direct quantification of sediment reworking over short time-scales after the input of a tracer pulse at the sediment surface. However the addition of deliberate tracers unavoidably modifies sediment conditions and therefore could affect organism behaviour and sediment reworking (Maire et al., 2008).

D'Andrea et al. (2004) used heavy mineral sand as particle tracers to assess sediment reworking in coarse sediments although density affects particle handling by benthic infauna (Jumars et al., 1982). Isotopically- labelled sediment particles can also be used: natural sediment particles labelled with radioisotopic elements, typically 51Cr (Sandnes et al., 2000), with the vertical distribution of the tracer monitored over time. Similarly isotopically labelled organic matter is utilized with the same approach, with labelling algae with or (Blair et al.,

1996). Glass beads with a size representative of the average sediment grain size are used as particle tracers (Shull and Yasuda, 2001; Mazik, 2004). This technique is used in evaluating relatively recent sediment reworking. Limitation of results can occur as the exotic natures of the tracers have different surface properties from those of typical sediment particles (Maire et al., 2008). Although Berg et al. (2001) has shown this disparity can be alleviated to some degree by developing a biofilm on the surface of the beads prior to the experiments.

Wheatcroft (1991) introduced the use of microtaggants, inert and non-toxic rough plastic particles with a size range of 50 to 125 µm with a specific gravity comparable to natural sediment particles. They are coated with paint to aid recovery and similar to luminophores which are natural sediment particles covered in a thin layer of UV fluorescent paint, with diameter between 10 and 500 µm (Mahaut and Graf, 1987) similarly microspheres are UV fluorescent balls of polystyrene that have a much smaller size and lower density (Ciutat et al., 2005). Due to their specific properties, the addition of microspheres does not modify initial sedimentary conditions unlike microtaggants and luminophores. This makes them ideal for examining sediment reworking processes resulting mainly from feeding activity, as microspheres can be ingested together with surrounding sediment (Ciutat et al., 2005). Luminophore particles and microspheres can be visually counted under a microscope (Mahaut and Graf, 1987; Gérino, 1990; François et al., 1999; Ciutat et al., 2005). However Solan et al. (2004) developed image analysis techniques to facilitate this process.

1.7.2 Direct Collection

Cadée (1976) used the direct collection of sediment brought by organisms to the sediment–water interface to estimate *in situ* sediment reworking by the lugworm *Arenicola marina*. Castings were collected at low tide; their amounts were standardized relative to immersion duration and sediment area. This form of collection is inaccurate as it assumes that all the sediment expelled at the sediment–water interface can be collected separately from the surrounding sediment, difficultly arises as much of reworked sediment may be unconsolidated and spread laterally (Cadée, 1976). The use of entrapment which consists of placing a trap

around the site of sediment expulsion, typically the opening of a burrow or tube, can help reduce this affect and enable a more complete description of sediment reworking, although a portion of material can be lost through re-suspension and the method is restricted to sessile or discretely motile organisms (Maire et al., 2008). As these methods do not allow for high-frequency measurements or determine sediment origin, microtopography mapping and image analysis have been developed to overcome these issues.

1.7.3 Microtopography Mapping

Microtopography mapping estimates the amount of sediment that is reworked via temporal changes in microtopography of the sediment surface. This can be performed by two different techniques: Roy et al. (2002, 2005) projected a laser line onto the sediment surface, comparing its position between successive images. This allows the mapping of sediment microtopography up to 50 µm vertically with high temporal resolution. Maire et al. (2007) used a laser telemeter mounted on motorised tables. This provides both high horizontal (200 µm) and vertical (15 µm) resolutions. The main limitation of the first approach is that it cannot provide measurements behind sediment mounds or within sharp pits (Roy et al., 2002; 2005). The main disadvantage of laser telemetry is its reduced temporal resolution due to time-consuming scans (Maire et al., 2007). Furthermore when used during *in situ* experiments it produces inaccurate sediment reworking, as reworked sediment may be transported out of or into the monitored area (Maire et al., 2008).

1.7.4 Resin Casting

Several studies have used resin casting to establish the shape and length of bioturbation structures (Gerino and Stora 1991; Lee and Koh 1994; Barros 2001). This involves injecting polyester resin mixed with hardener, into biogenic features of a sediment sample. This can be done in the lab or field. The technique has been utilized on organisms that produce larger structures for example Thalassinidean shrimp (Nickell and Atkinson, 1995) or Solemyoid bivalves (Seike et al., 2012). However the lack of resolution prevents an accurate

determination of the space occupied by small macro-fauna due to difficulty in filling smaller voids with resin (Dufour et al., 2005).The fragility of set resin can also be problematic when extracting from the surrounding sediment.

1.7.5 Sediment Profile Imaging

Lohrer et al., (2005) and Hollertz and Duchêne (2001) used surface image analysis for large organisms living immediately beneath the surface of the sediment. This involves the recording of the movements of benthic fauna at the sediment surface using a video sensor. In each image the position of the organism is automatically detected and its coordinates within the image are recorded. At the end of the experiment all coordinates within successive images are used to assess displacements. Its main advantage is that it allows for the assessment of short-term temporal changes in sediment reworking (Hollertz and Duchêne, 2001).

Rhoads and Germano (1982) achieved success with sediment profile cameras in situ experiments (Figure 1.4). This involves a support frame to which a prism imaging module is attached. This penetrates the sediment water interface, when lowered by cable from a vessel. At the back of the prism a mirror is mounted at a 45° angle that reflects the sediment profile up to the camera (Rhoads and Cande, 1971). Images can be captured real-time in video or time-lapse stills, allowing a variety of physical, chemical and biological parameters to be ascertained (see Rhoads and Germano, 1982). Solan et al. (2004) combined this method to great effect with luminophores, whilst Birchenough et al., (2006) used Hamon grabs and side scan sonar. The only constraint of SPI is the production of two dimensional images, where bioturbation features can be counted, but other 3D sedimentary characteristics such as burrow volume cannot be determined (Solan et al., 2003; Mazik et al., 2008).

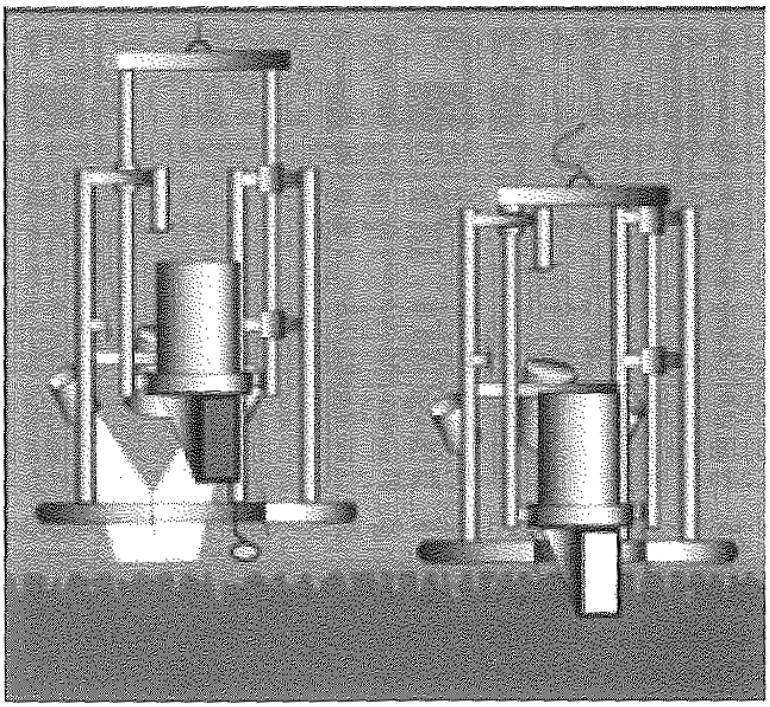


Figure 1.4 Sediment profile imaging camera rig (from Gray and Elliott, 2009)

1.7.6 Computer Tomography

A relatively new area of research has arisen to resolve the lack of three dimensional quantification of bioturbation, computer tomography (CT) has 3D scanning capabilities and was used by Perez et al. (1999) to determine the health of sediment via categorizing burrow structures along pollution gradients. Similar techniques were also used to establish volumes of bioturbator-inhabited structures within sediment cores (De Montety et al., 2003; Mermillod-Blondin et al., 2003; Dufour et al., 2005; Rosenberg et al., 2007; Weissberger et al., 2009). These studies primarily used commercial medical CT scanners, which employs the use of fan beam X-ray technology that creates a series of axial image slices, with a typical minimum axial slice thickness of 15mm (Perez et al., 1999).

Recent research by Mazik et al. (2008) has developed this process further by the use of micro-CT scanner (µCT) on sediment cores. µCT is normally used to investigate internal (trabecular) bone found in larger bones; it allows a greater resolution 3D image than a conventional CT scan. It thus allows the three-dimensional geometry of the burrows were ascertained and used to accurately quantify the properties of the burrows, such as burrow wall surface area, burrow density, volume and diameter (Mazik et al., 2008).

Current CT techniques have been used foremost on sub-sea sediments; only Mazik et al. (2008) studied intertidal areas using µCT. A key limitation of using CT is the fragility of the burrow structures created by infaunal organisms, which are prone to collapse when excavated from the surrounding sediments. This collapse is exacerbated when using CT scanners which perform scans horizontally. Several attempts to stabilize sediment features in the field have been performed with varying results for sub-sea CT scans (Perez et al. 1999; Michaud et al. 2003; Dufour et al. 2005; Rosenberg et al. 2007; Mazik et al. 2008; Weissberger et al. 2009). No such stabilization has occurred for µCT.

1.7.7 Summary

The use of methods such as direct collection and micro-topography mapping to quantify bioturbation lack the ability to measure the vertical component of the benthos therefore can only be used at the sediment-water interface and are subject to bias from resuspension and deposition of expelled burrow material. Particle tracers, for example radionuclides and luminophores do measure the vertical component and can have high temporal resolution, but are exotic and consequently are may not be manipulated by burrowing organisms in the same manner as other sediment particles. Sediment profile imaging has high spatial and temporal resolution that can be performed in-situ. However this technique only produces two dimensional images, whereby biogenic features: burrows, voids and tubes can be enumerated but properties such as burrow volume cannot. CT scanning techniques allow such properties to be determined, with µCT increasing resolution further. For this reason µCT is an improvement on previous methods to accurately determine changes in burrow volume and therefore bioturbation.

2 Chapter 2: Technique Development - Sample Stabilization and Optimisation

2.1 Introduction

As discussed previously bioturbation affects nutrient cycling and microbial activity by increasing the surface area available for exchange (Rhoads, 1974; Aller, 1982; Volkenborn et al., 2007; Montserrat et al., 2008; Reise et al., 2009), through bioturbation an increase of these can processes occur (Benoit et al., 2006), therefore to assess bioturbation there is a need to measure changes in surface area and volume. As section 1.7.7 demonstrates the techniques to achieve this are the subject of many studies (Rhoads and Germano, 1982; Barros 2001; Hollertz and Duchêne, 2001; Roy et al., 2002, 2005; Solan et al., 2004), though these methods are two dimensional in nature, thus all rely on estimation of burrow volumes (Rosenberg and Ringdahl, 2005). µCT scanning is the only technique that can accurately quantify burrow volume without the use of estimation (Mazik et al., 2008).

Due to the variable physical nature of intertidal sediments, for example differences in shear strength and water content (Hall, 1994); there is the need to stabilize the sediment core samples which were to be scanned using µCT (Dr K. Mazik, IECS pers. comm.). This is to ensure that biologically derived features within the core did not collapse when removed from situ and transported to the scanning facility.

The use of stabilization techniques in relation to CT scanning has been well researched (Perez et al. 1999; Michaud et al. 2003; Dufour et al. 2005; Rosenberg et al. 2007; Mazik et al. 2008; Weissberger et al. 2009), these studies relate to horizontal and vertical stabilization. However due to the reduced size of a µCT scanner samples are scanned vertically only. The advantage being that there is a reduced chance of burrow collapse as biogenic features are kept in their naturally excavated position, unlike that of CT scanners that generally scan in the horizontal position, thus increases the chance of feature collapse. In both µCT and CT the size of a

scanned core is limited by the dimensions of the scanning machine itself. µCT scanners are smaller therefore need core that are smaller in length.

All methods of stabilization have involved capping the core at both ends and infilling the topmost section with a semi solid material (Table 2.1). In several cases this has preserved the surface features of the sediment, but does not take into account loss structure further down the length of the core (Rosenberg et al. 2007). No stabilization of samples occurred when using the µCT technique (Mazik et al., 2008) or on CT scans performed by Perez et al. (1999) and Dufour et al. (2005). The previously developed methods of Michaud et al. (2003) and Rosenberg et al. (2007) were carried out using large cores on sub-tidal sediments which contain a greater size of benthic invertebrates.

Table 2-1 Stabilization Techniques of Sediment Cores for CT scanning

Reference	Stabilization Technique	Scanning Position	Scanner Type
Michaud et al. 2003; Rosenberg et al. 2007	1:1 ratio of Paraffin and Vaseline®	Horizontal	CT
Rosenberg et al. 2007; Weissberger et al. 2009	Cream of Rice®(ground rice) and Seawater	Horizontal	CT
Perez et al. 1999; Dufour et al. 2005	None	Vertical	CT
Mazik et al. 2008	None	Vertical	µCT

2.1.1 Aim

Improve the scanning method and determine a method of stabilization for vertically scanned cores for intertidal sediments when using high resolution µCT.

2.1.2 Objectives

1) Establish criteria for an optimum stabilization material.
2) Trial several stabilization materials to obtain the most favourable one.

2.2 Stabilization Material

2.2.1 Criteria for Stabilization Material

Using previous studies (Perez et al. 1999; Michaud et al. 2003; Dufour et al. 2005; Rosenberg et al. 2007; Mazik et al. 2008; Weissberger et al. 2009), it was recognised that in order to ascertain a material that could stabilize intertidal sediment cores, a number of criteria must be established in order to assess their suitability. These comprised of scientific and practical considerations:

Scientific

1) A material that would be fluid enough to flow into biologically derived features such as burrows and dense enough to displace water from the features, but also become solid/semi-solid within a fixed time to maintain burrow structure and avoid collapse.
2) Allow for the removal of intact organisms from the core for subsequent macrofaunal analysis with conventional sieving techniques.
3) Appear distinct from the surrounding sediment of a core for further scan analysis

Practical

4) Have a minimal environmental impact with regard to working near aquatic ecosystems and possible use in protected sites, for example, Special Area of Conservation (SAC), Site of special scientific interest (SSSI).
5) Have low Health and Safety concerns e.g. no requirement for specific transport, or specialized additional training.
6) Be a cost effective yet practical solution to working in intertidal locations.

2.2.2 Potential Trial Materials

Using the above criteria several different potential materials were established summarised in Table 2.2. Four of the potential materials were gathered from previous studies of either: sediment CT scanning (Michaud et al. 2003; Dufour et al. 2005; Rosenberg et al. 2007) or

investigation into biogenic sediment features (Wiltshire et al. 1997; Seike et al. 2012). Two of the materials, Gelatine and Agar were deemed a possibility as they met the stabilization conditions.

Table 2-2 Potential Stabilization Materials for Vertical µCT Scanning

Potential Material	Reference
Agar	-
Cream of Rice®	Rosenberg et al. 2007; Weissberger et al. 2009
Gelatine	-
Liquid Nitrogen	Wiltshire et al.1997
Paraffin/Vaseline®	Michaud et al. 2003; Rosenberg et al. 2007
Polyester resin	Seike et al. 2012

Using literature (Table 2.2) and expert judgement, each of the possible materials was graded on a scale of 1-3 according to their ability to meet the stabilization criteria, weighted to 40% for setting point/density, 20% practicality, with the remainder 10%. Table 2.3 shows these scores with a scale range of 100 to 300: the higher the total the more suitable a material potentially could be (see appendix Table 6.1 for scoring scale).

Liquid Nitrogen scored low mainly due to high health and safety aspects when in use, together with its associated costs and difficulty in transporting over intertidal sediments, specifically mudflats. It was also noted that as muds freeze fast they dry rapidly, this changes their physical structure producing brittle sediment layers. The Paraffin/Vaseline® technique also scored low due health and safety, environmental and organism retrieval concerns as it could degrade structure in the uppermost part of a core (Rosenberg et al. 2007).

Polyester resin scored high for setting time but low in organism retrieval as it would possibly encase organisms making identification difficult. Cream of Rice®, gelatine and agar were all highly graded; being derivatives of natural products and so environmental, health and safety would not be an issue. However Cream of Rice® and gelatine did score low in setting time.

Table 2-3 Weighted Scoring of Potential Stabilization Materials for Vertical µCT Scanning

Stabilization Method	Set Point	HSE Concern	Environment Concern	Organism Retrieval	Cost	Practic-ality	Total
Agar	120	30	30	30	30	40	280
Gelatine	80	30	30	20	30	60	250
Cream of Rice®	40	30	30	30	30	60	220
Liquid Nitrogen	120	10	30	10	10	20	200
Polyester resin	120	10	20	10	10	20	190
Paraffin/Vaseline®	80	20	10	20	20	40	190

Using the scores above a decision was made to preliminary trial the use of Cream of Rice®, gelatine and agar in stabilizing sediment cores for µCT analysis. Cream of Rice® is not available in the UK therefore a substitute of ground rice was used, this is the main constituent of the product.

2.3 Preliminary trial

2.3.1 Sample Site

The preliminary trial site was located near Paull, on a small intertidal mudflat approximately 10km South East of Hull (Figure 2.1), along the middle section of the Humber estuary, UK (Allen et al. 2003). The area is typically inhabited by several different macrofaunal polychaetes, oligochaetes, nematodes and bivalves, similar to other areas in the middle Humber. These primarily consist of the polychaete *Hediste diversicolor* and the bivalve *Macoma balthica* with smaller numbers of the bivalve *Abra tenuis*, polychaetes *Manayunkia aestuarina*, *Streblospio shrubsolii* and oligochaetes *Heterochaeta costata* , *Tubificoides benedii* (Fujii, 2007). Trials were performed on the upper shore which consists of consolidated very fine, silty mud or mud (Allen and Mazik, 2005).

Figure 2.1 Preliminary trial location

2.3.2 Method

2.3.2.1 Sample Collection

3 plastic cores measuring 200mm in length, internal diameter of 56mm were taken, situated 10m apart and approximately 20m onto the mudflat from a shingle flood defence embankment. The core sizes were developed to the constraints of the scanner dimensions and the density of the scanning material. Cores were placed in the sediment in an area with an elevated number of burrow entrances as the exercise was to determine the efficiency of the stabilization materials rather than to characterise faunal distribution. To minimize disturbance cores were placed in intact areas of the mudflat away from footprints. The cores were left in situ while any surface water was drawn off and the stabilization material was cautiously injected into any obvious burrow structures using a syringe (ratios of material to water Table 2.4). A header of approx. 20mm was left in the top of the core to pack the remaining space and to act as a reservoir in case burrows were not completely filled. The materials then were left to set for up to 2 hours. The cores were lifted, capped and placed in cardboard and polystyrene packaging for transport back the scanning facility at the University of Hull. Collection of samples was in January 2011 and transfer was completed in a temperature close the external air, in less than 2 hours. The cores were placed in a cold store (8°C +/-1°C) until scanning took place, a maximum of 48 hours later.

Table 2-4 Ratios Used of Preliminary Materials

Preliminary Material	Ratio *(g/ 500ml)
Agar	14
Ground Rice	60
Gelatine	23

*Ratios determined from product instructions

2.3.2.2 Sample Scanning Procedure

The scan protocol followed the basic method from Mazik et al. (2008) using an X-Tek HMX160 µCT system (X-Tek, Tring, UK). The µCT system uses an X-ray generator to fire a cone of X-rays

through a specimen, that is then collected by a photo-detector on the opposite side. To scan a core it was positioned upright in the µCT system and X-rays are accelerated through it, some of these X-rays are absorbed by the material while others pass through. The absorption rate is dependent on the density of the material and the configuration of volts and amps used to generate the X-rays. This produced a 2D digitised greyscale X-ray image. Black and white light calibrations were obtained prior to scanning that provide the upper and lower extremes of greyscale limits, consequently the digitised X-ray images produced during the scan consisted of varying shades of grey between black and white. Each sample was rotated by 0.36°. The X-rays acceleration and capture process was repeated to attain a further 2D image. This is replicated until the core has been rotated through 360°, 2D X-ray images are collected at every step to a total of 1000. Background noise can be produced on the images as the X-rays pass through the air, this was minimised by taking 128 images at each scanning step and averaging the results. Taking approximately 3 hours to complete, the scanning procedure used a voltage of 70 kV, a current of 18 µA, and an aperture setting of 75%. For the duration of the scan a 0.1mm copper filter was applied to eliminate low voltage X-rays. Images were reconstructed via NGI CT Control software (X-Tek, Tring, UK). This adjusts the raw digital X-ray images and converting them into a stack of µCT slice images; with a resolution of 1000×1000 pixels in 2D along the X and Y axis. Using the gap between each slice along the Z axis, a 3D image can be created. The stacks of images are stored as 16-bit tiff files (Tagged Image File Format).

2.3.3 Results

To determine which of the stabilization materials were more successful in fulfilling stabilization criteria, a simple comparison of the quality of the image, in regard to clarity of burrows was conducted. An image was taken from each of the cores, at the same point immediately below the stabilization material/sediment interface (Figure 2.2).

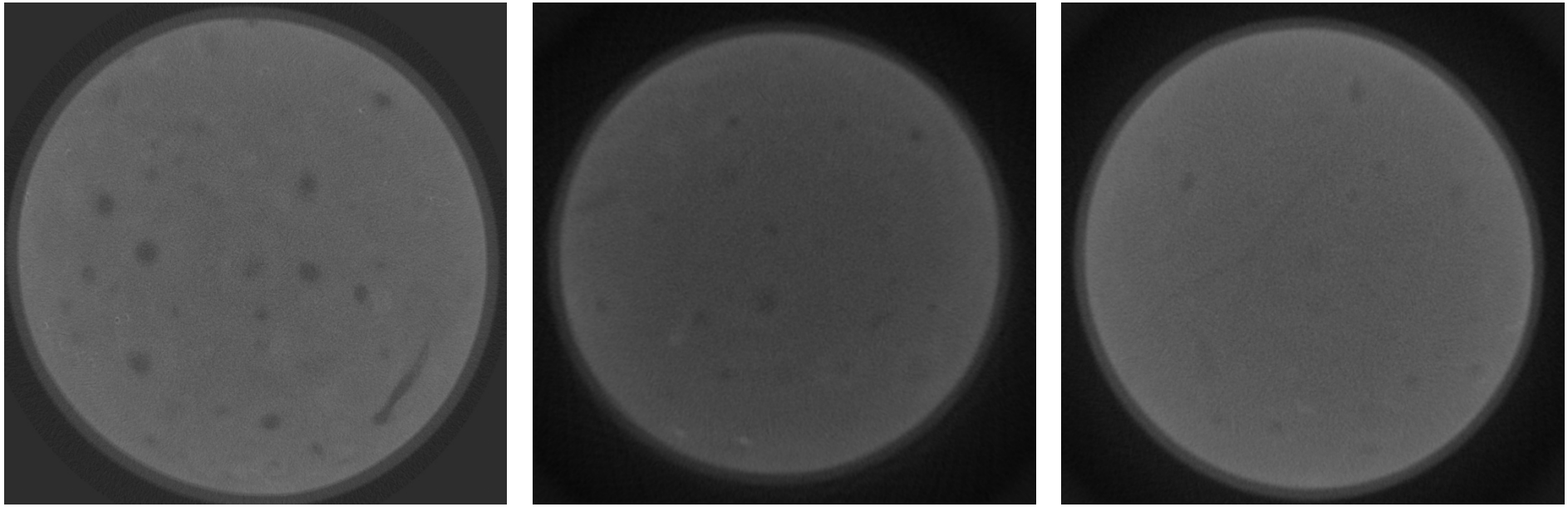


Figure 2.2 Images of µCT scanning with stabilization method: A- Agar, B-Ground Rice, C- Gelatine.

Table 2-5 Setting Points of Preliminary Stabilisation Materials

Preliminary Material	Setting Point
Agar	20 min.
Ground Rice	>48 hours
Gelatine	>24 hours

This point was chosen as the amount of bioturbation from an organism increases towards the surface, consequently burrows further down the core would be situated in slightly more consolidated sediment, therefore upper burrows would stand a greater chance of collapse.

2.3.3.1 Stabilization Method: Agar

The use of agar as a stabilization material (Figure 2.2A) produced clear voids in the sediment with definable edges and in a definite contrast to the surrounding sediment. Agar also set within twenty minutes (Table 2.5) allowing for a prompt removal of the core from the mudflat and return to the scanning facility.

2.3.3.2 Stabilization Method: Ground Rice

Although it was possible to observe voids in the sediment core scan stabilized with ground rice, it is difficult to accurately determine their extent (Figure 2.2B). The voids closely resemble the density of the adjacent sediment. Ground rice took the longest of all the materials to set: solidification occurred after 48 hours (Table 2.5).

2.3.3.3 Stabilization Method Gelatine

Gelatine produced images of similar quality than that of ground rice, void structures could be viewed; however they were of an indeterminate area with almost the same density as the adjoining sediment (Figure 2.2C). The product hardened after 24 hours (Table 2.5).

2.4 Conclusion

It is assumed that the reduction in the detection of voids in scans B and C (Figure 2.2) is due to either infilling from feature collapse via extraction and transportation from the mudflat, or that the stabilization material is of a similar density to the sediment in the cores. Ground rice and gelatine also performed poorly in regard to their setting points, as it took a minimum of 24 hours for them to set. This is not practical on an intertidal mudflat as the sample would be covered by the tide at least twice. Agar achieved good results: setting sufficiently fast to

extract the core before the oncoming tide and with adequate time to perform the stabilization technique. It also had superior void clarity with easily definable edges.

For the above reasons, agar is used to stabilise sediment cores for the next phase of the project.

3 Chapter 3: Technique Development- Identification of Bioturbation Structures

3.1 Introduction

Several studies have shown the use of CT scanning for sediment cores to ascertain biogenic structures within (Perez et al. 1999; Michaud et al. 2003; Dufour et al. 2005; Rosenberg et al. 2007; Weissberger et al. 2009). Only Mazik et al (2008) has made extensive use of three dimension visualisation software to create images of these biological features; by using a 3D image analysis package called Avizo®, they demonstrated it is possible to gather specific quantifiable data of these structures in upper shore areas of mudflat. However the 3D images have no classification regarding what constitutes a specific structure, the organism which made it and its life traits. The ability to ascertain bioturbation features such as burrows, bivalves and voids, is necessary to establish the amount of bioturbation that is taking place within the sediment (Pearson and Rosenberg, 1978). An increase in the quantity, volume and surface area of these features would correlate to an increase in bioturbation and therefore differences between locations could be related to different levels of benthic activity, with possible implications for the physical and chemical properties of the sedimentary environment.

3.1.1 Aim

1. Establish the extent of biogenic features within sediment cores from µCT scanning via 3D visualisation from different locations along the shore, linking them to the functional ecology of bioturbating organisms.
2. Determine if the use of Agar is a viable stabilization material for use on an intertidal mudflat.

3.1.2 Objectives

1. Use Agar to stabilize sediment cores.

2. Using Avizo®, identify biologically derived features in sediment cores.
3. Eliminate the non-biogenic material from core scans.
4. Generate a system of categories on to determine which species could create a specific bioturbation structure.
5. Produce qualitative and quantitative data on bioturbation structures in 3D.

3.2 Methods

3.2.1 Sampling Sites

Paull Holme Strays and Welwick managed realignment sites were chosen as sample collection areas. Situated on the Humber estuary, UK (Figure 3.1 and Figure 3.2) they consist of two parts: the realignment section itself, surrounded by an embankment that is breached at various points along the estuary side, and a fronting mudflat that leads down the shore into the main river channel. Paull Holme Strays managed realignment is located on the middle estuary with Welwick managed realignment on the outer estuary (Allen et al. 2003) Benthic fauna that inhabit these sites are typical of estuarine intertidal communities (Mazik and Elliott, 2000). Within both Paull Holme Strays and Welwick realignment these primarily consist of polychaetes, oligochaetes, bivalves, nematodes and gastropods. Along the fronting mudflats species composition is similar to that of the inside but with a more uniformly distributed abundance than that of the inside (Mazik et al, 2010).

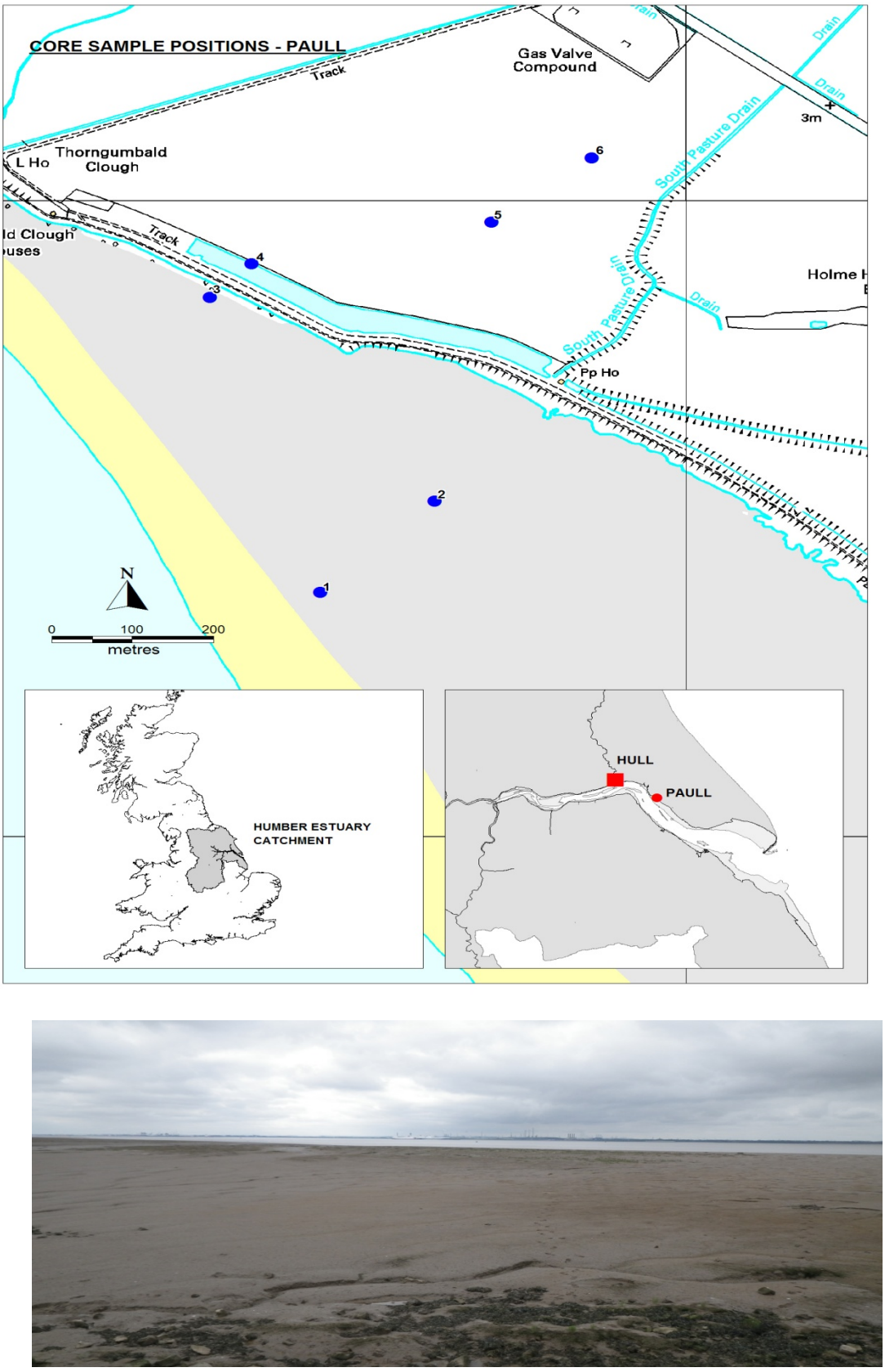


Figure 3.1 Core sample locations Paull

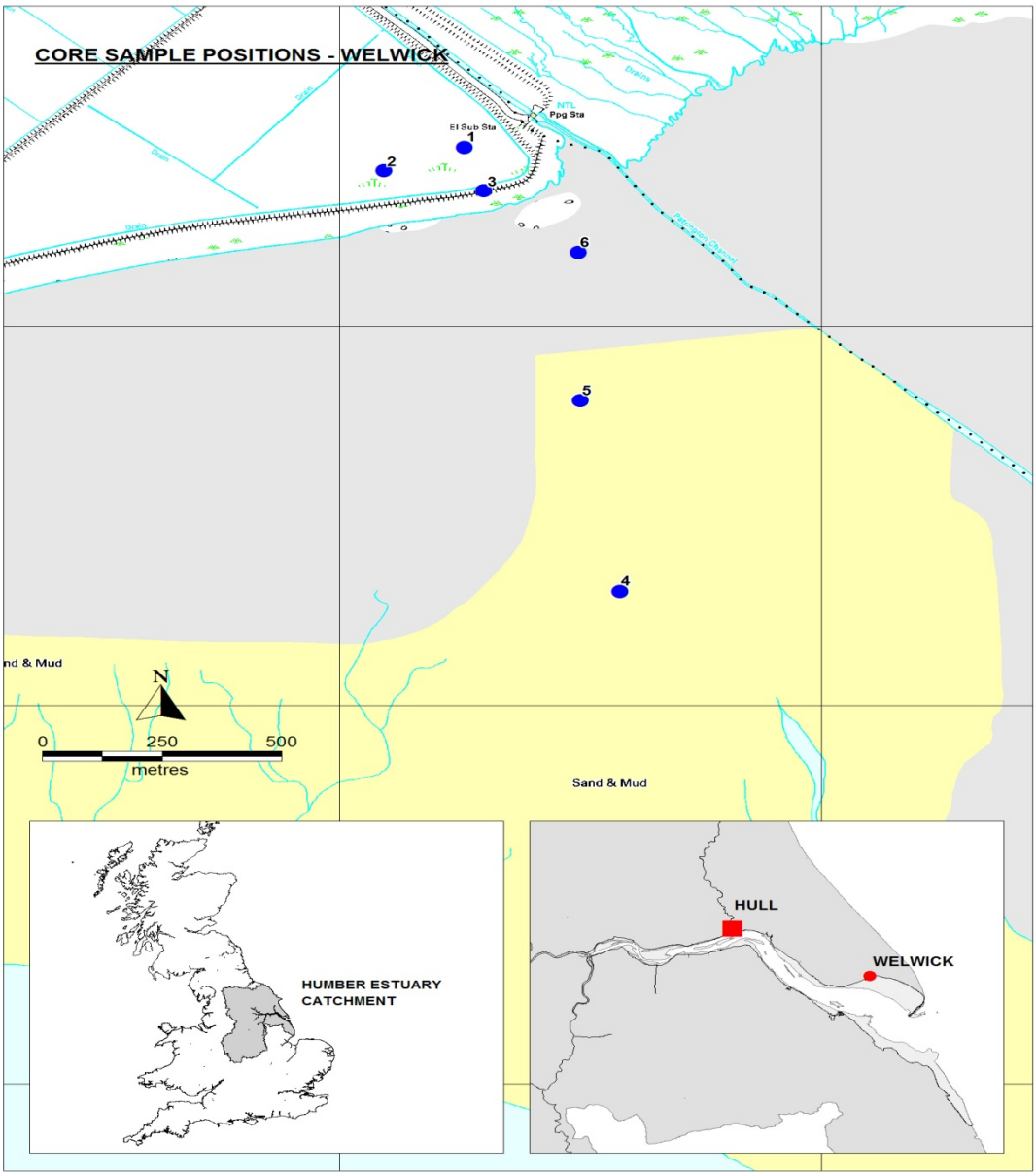


Figure 3.2 Core sample locations Welwick

These sites were chosen for two main reasons. Firstly the locations of the sites are close to the scanning facility at the University of Hull and have public access. Secondly the segregation of the fronting mudflat and the realignment area allow for different infaunal communities, with potentially different ecological functions, to occur and be sampled using µCT.

3.2.2 Sample Collection

Plastic cores measuring 200mm in length, internal diameter- 56mm were used to take 6 cores at each site; 3 in the realignment site and 3 on the fronting mudflat (Figure 3.1 and Figure 3.2). The cores outside the realignment were approximately 500m apart, inside the distances were less due to the presence of salt marsh. The core sizes were developed to the constraints of the scanner dimensions. Cores were placed in a random manner at a similar tidal level. A heated agar solution of ratio of 7g of agar to 250ml of water was prepared on site. The cores were pushed vertically into the sediment taking care to minimize disturbance, left in situ while any surface water was drawn off and the agar solution was cautiously injected into any obvious burrow structures using a syringe. A header of approximately 20mm was left in the top of the core to pack the remaining space and to act as a reservoir in case burrows were not completely filled. The agar solution was then left to set for up to 30 minutes. The cores were extracted by removing material from around the outside of the core and slicing it away from the sediment underneath. This was to prevent the suction created when lifting a core, which could possibly damage burrow structures. Then cores were then capped and placed in cardboard and polystyrene packaging for transport the scanning facility. Collection of samples was in August 2011 and transfer was completed in a temperature close the external air, in less than 2 hours. The cores were placed in a cold store (8°C +/-1°C) until scanning took place, a maximum of 12 hours later. In addition a core of the same length and diameter was taken for particle size analysis (PSA) at each of the agar core locations.

3.2.3 Sample Scanning Procedure

The scan protocol followed the same procedure as in the preliminary trial (2.3.2.2). Approximately 3 hours per sample was needed to obtain a full scan. The X-Tek HMX160 µCT system (X-Tek, Tring, UK) performs a pre-scan to assess the density of the subject material; this sets the voltage and current for the main scan allowing for an optimum scanning capability. Individual core sample scan voltage and current data are given in Appendix 6.1.2.

3.2.4 Particle Size Analysis

Using a Malvern Mastersizer 2000 (Malvern industries) particle size analysis (PSA) was conducted on all 12 cores to characterise the sediment types of the area and for cross-reference against infauna found in the cores. Samples were taken from upper 5cm of amalgamated sediment from the PSA core, a similar depth to the scanned areas of the cores. Analysis involves a laser diffraction technique to measure the size of particles via calculating the intensity of light scattered as a laser beam passes through a dispersed particulate sample. This data are then used to determine the size of the particles that created the scattering pattern.

3.2.5 Sediment Carbon Analysis

Loss-on-ignition analysis was performed on the PSA core; this entails heating the sediment sample at 84°c for 48 hours to remove water, subsequently subjecting the same sample to a temperature of over 475°c for 4 hours to eliminate carbon. The sample is weighed at different stages to calculate the quantity of carbon by loss-on-ignition. When cross-referenced with PSA it facilitates the characterisation of the sediments at the sample sites.

3.2.6 Infaunal Analysis

After scanning, cores were immediately subjected to traditional infaunal analysis: the entire core sample was sieved through a 500 micron mesh and the residue fixed in a 10% seawater buffered formalin solution containing Rose Bengal stain. They were then sorted for

invertebrate fauna which after counting were identified to species level were possible. Specimens are stored in 70% solution of industrial methylated spirit (IMS). Any other items of note from the sieve residue were also recorded i.e. stones, shell. This work was performed in parallel with another intertidal benthic fauna project, simultaneous coring occurred to give replicate samples for a better indication of fauna present, density and species richness.

3.2.7 3D Reconstruction

To reconstruct the scan data into a 3D format several different phases are required, the processes undertaken was the same for each of the cores. After the completion of the scanning process and image reconstruction, the 2D µCT slice images were exported as a stack of 16-bit tiff images into ImageJ (NIH): an open access image processing software. This was to reduce the 16-bit .tif files to 8-bit .tif files. The file size is modified to reduce the processing power needed to analyse the large files created from the X-Tek HMX160 µCT system. The average voxel resolution per scan was 93.4µm x 93.4µm x 93.4µm (±11 µm) in X, Y and Z direction respectively (Appendix 6.1.2).

The 8-bit slices are then imported into Avizo®. Only the slices below the sediment-agar interface and 5cm down the length of the core were used (Figure 3.3A), as these are the horizons at which the samples biogenic features start and where the majority of bioturbation activity takes place. Also voids created from extracting the core can become apparent passed this depth. Approximately 700 image slices per core were used.

Following this a thresholding technique was then applied to segment out the sediment material from other density structures. The aim was to quantify the voids or burrows in the core sample, thus thresholding limits were set to highlight burrows (Figure 3.3B). The minimum and maximum greyscale limits varied between specific cores but were approx set to 50 and 90 respectively.

After thresholding, smoothing options were applied to the highlighted data in Avizo®. This was to remove any small particles and individual lone highlighted voxels in the image that were not considered to be biogenic features i.e. background interference differentiated as alternating density areas of light and dark extending vertically through the centre of all the cores (Figure 3.3C).

The core sample tube was un-highlighted as it was of a similar density to that of biogenic features and was previously highlighted as part of the thresholding process. Each slice was examined for any other isolated background particles with these removed also (Figure 3.3D).

3D surface models of the burrows were then created from the segmented slice images, which were used to attain burrow structure data e.g. burrow volume.

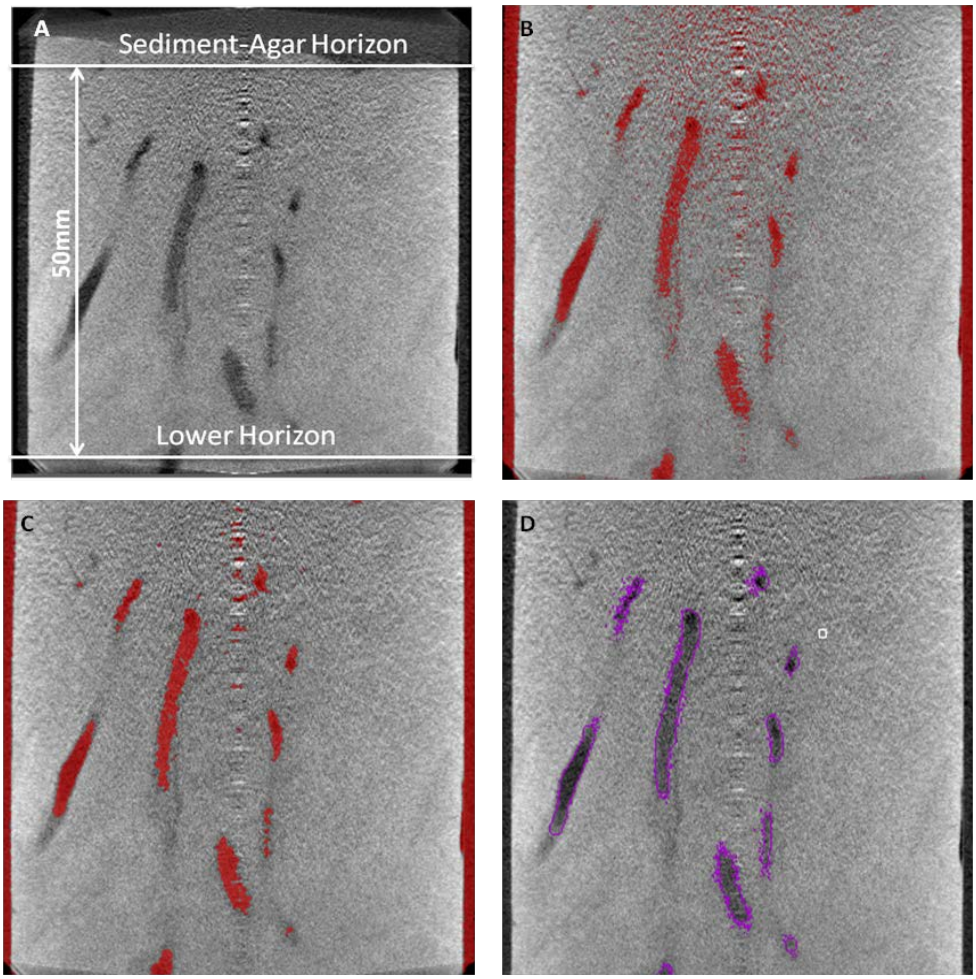


Figure 3.3 Aviso® Selection techniques: A- Slide selection area, B- Core sample slide with thresholding, C- Core sample slide with smoothing, D- Core sample slide with plastic tube and background interference removed.

3.2.8 Identification of Biologically Derived Features

To limit the highlighted material in the 3D reconstructions to that from bioturbation activity, reducing other structures being used in further analysis. This could become problematic when trying to evaluate if burrow structures were relic and/or active, if a burrow had collapsed or if it was two separate structures. For the purpose of this study a burrow is defined as: 'any structure which has clear links to the surface'. This allows for burrow parameters to be established per core for burrows and 'other burrow like structures' separately. 'Other burrow structures' may also include burrows which have entrances outside the core but a portion of the burrow is within the core.

Calculations per core sample to be established are:

1. Total burrow volume:

   = Number of burrow voxels × voxel volume ()

2. Relative burrow density (i.e. volume fraction):

   = Total burrow volume/Total section volume

3. Burrow surface area:

   = Number of external burrow voxel faces × voxel face area ()

This was performed on both burrow and the other burrow (biogenic) structures. This will allow to for a comparison between individual cores, between and within the sample sites.

3.3 Results

Unfortunately µCT scans of samples Welwick 1 and 6 were lost to a data corruption issue, therefore they are excluded from the analysis.

3.3.1 3D Core Sample Reconstruction

Figure 3.4 and Figure 3.5 shows the 3D reconstruction of the sediment cores from the sample locations at Welwick and Paull. Red structures are connected to the surface, whereas purple areas show other biologically derived (biogenic) features. These may include: burrows which are not connected to the surface and/or collapsed therefore considered relict, gas vacuoles and bivalve voids. A small number of burrows in Figure 3.4 appear in red without linking to the surface. This is due to an 'unconstrained smoothing factor' that is performed when turning 2D images into 3D images in Avizo®. This is to reduce the amount of pixels in the 3D image, therefore decreasing the computer processing power needed. The burrows do link to the surface in the software. Measurements are taken in 2D and consequently are not affected by 'unconstraint smoothing'.

3.3.1.1 Welwick Sample Reconstruction

The 3D reconstruction results from the Welwick scans were (Figure 3.4): site 2 and 5 both show 1 large well defined burrow spanning the length of the scan, with a substantial amount of biogenic features. Site 3 contains several smaller burrows with a sizeable amount of biogenic material. Site 4 possessed the least amount of both burrow and biogenic material between the four Welwick sample sites with greatly reduced features in both size and frequency. Site 4 also had the lowest features of all the core samples.

3.3.1.2 Paull Sample Reconstruction

The 3D reconstruction results from the Paull scans (Figure 3.5) were, with the exception of site 2, broadly similar: each site had multiple large well defined burrows spanning the length of the core, typical of larger polychaetes e.g. *Hediste diversicolor*. The presence of biogenic features ranged from low to moderate. Site 2 had only two small burrows with a comparatively high

quantity of biogenic material, site 2 had the least amount of biogenic features of the Paull samples.

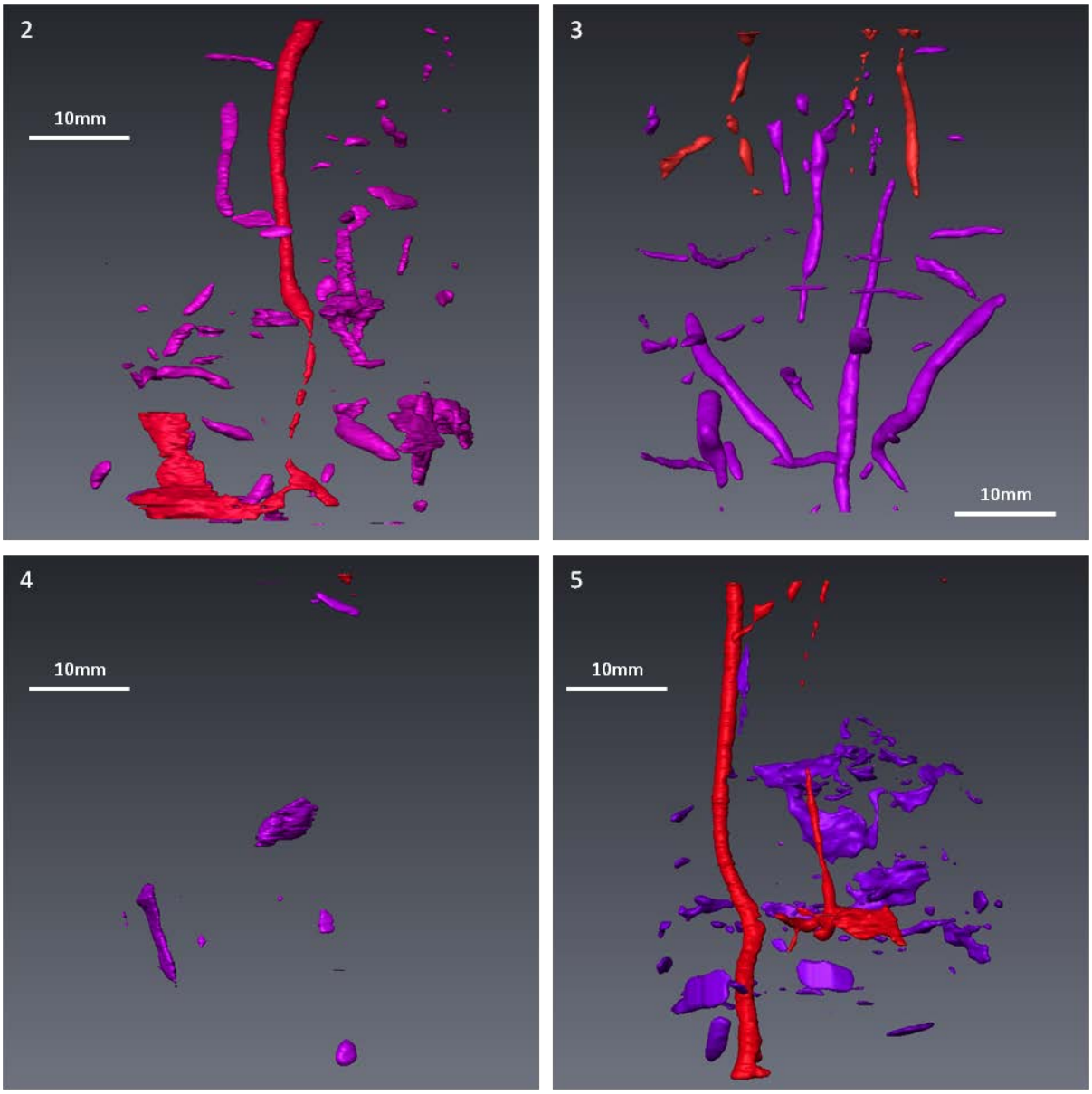


Figure 3.4 3D reconstruction of burrows (red) and biogenic features (purple) of Welwick sampling sites 2-5

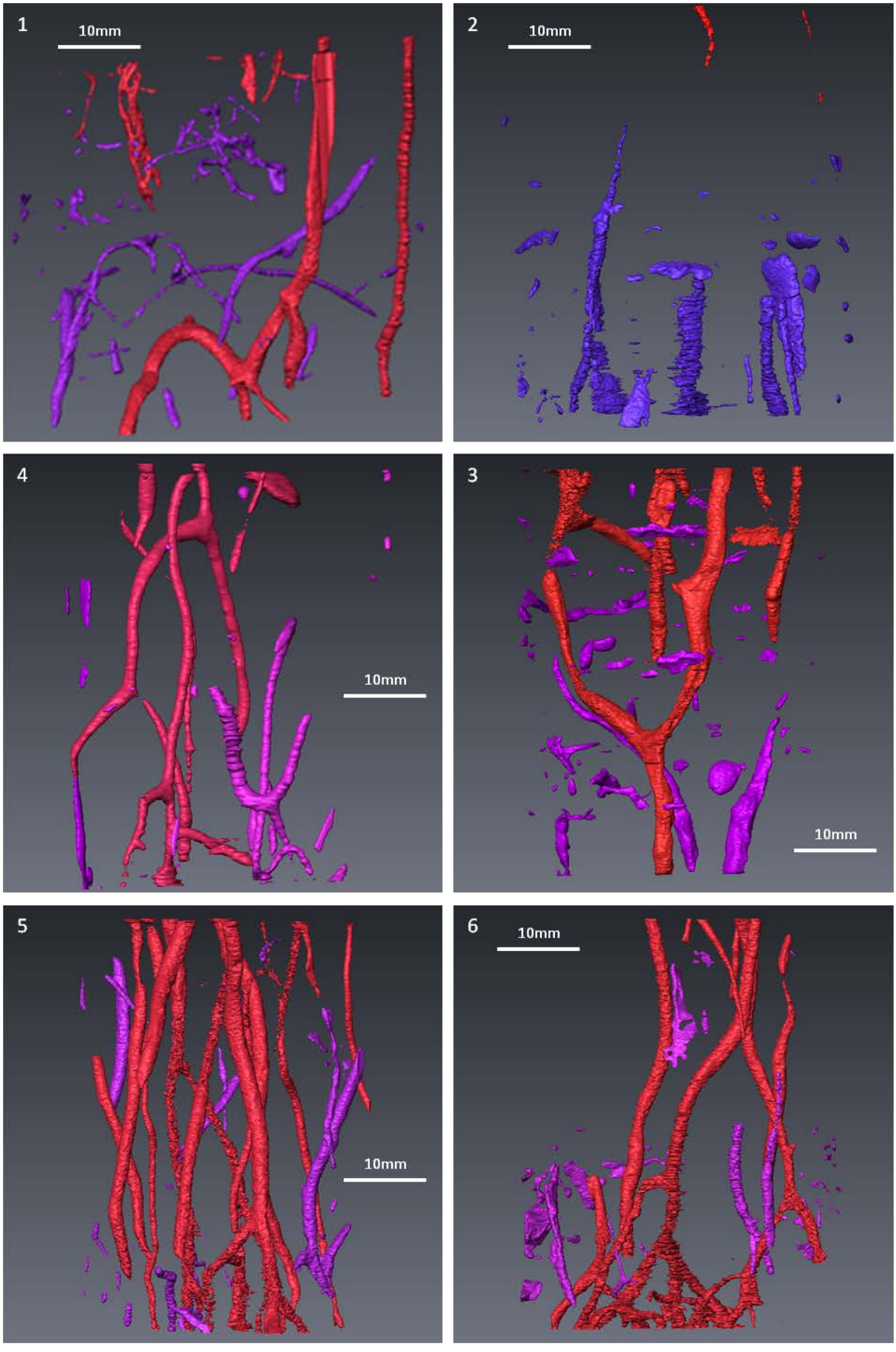


Figure 3.5 3D reconstruction of burrows (red) and biogenic features (purple) of Paull sampling sites 1-6

3.3.2 Bioturbation Structure Analysis

Volume and surface area measurements were taken from the core samples for both burrow (objects linking to the surface) and other biogenic material (collapsed burrows, bivalve voids, etc), this is calculated by multiplying the total number of voxel/voxel faces by their respective volume/area (Figure 3.6 and Figure 3.8; Appendix 6.1.3-Table 6.3). Relative burrow density by volume was also determined (Table 3.1) via multiplying total burrow volume by total volume in the scan area (Appendix 6.1.5). T-tests used to compare means between and within each site for volume, surface area and relative burrow density demonstrated no significant results, $p<0.05$ (Appendix 6.1.4).

3.3.2.1 Surface Area

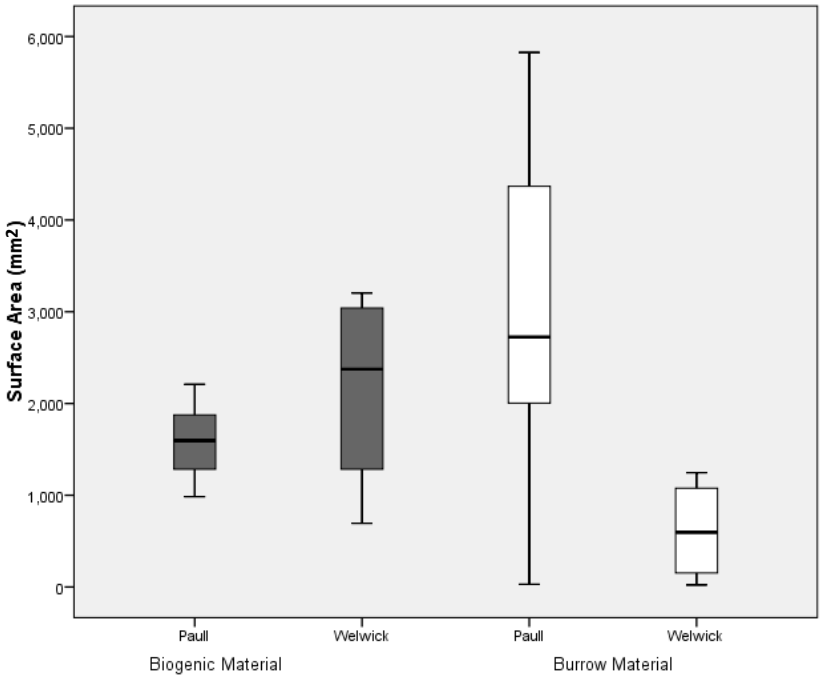


Figure 3.6 Surface area ( ) of burrow and other biogenic material in core samples

The sample sites at Paull exhibited an increased surface area of burrow material than that of biogenic material, while the opposite can be observed for sites at Welwick, where biogenic material has a higher amount of surface area than that of burrow material (Figure 3.6). At Paull the combined burrow surface area increased within the realignment compared to that of the fronting mudflat, similarly this is also true at Welwick, although not to the same extent: Paull

having a difference of between the realignment and fronting mudflat, with at Welwick (Figure 3.7).

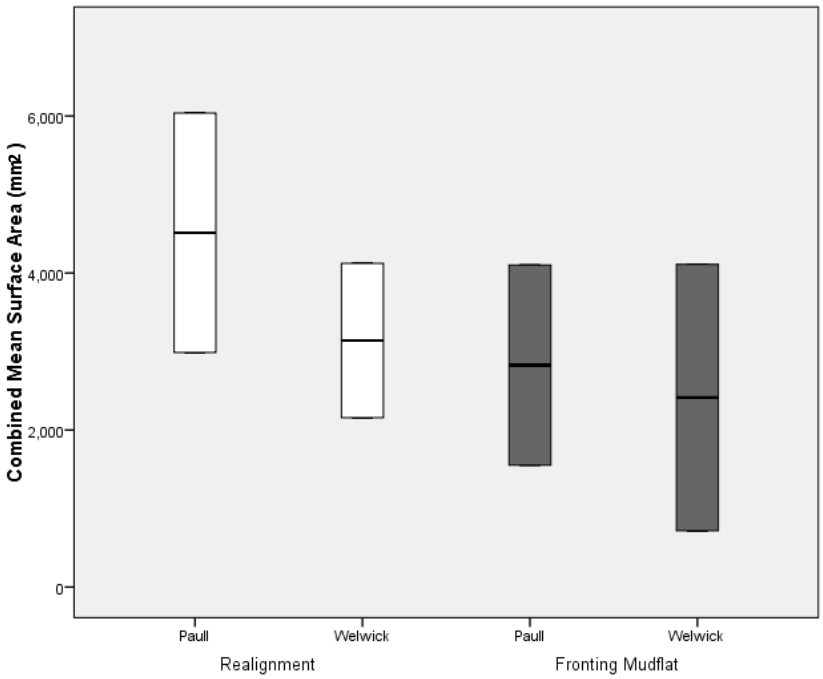


Figure 3.7 Mean surface area ( ) of realignment and fronting mudflat cores at Paull and Welwick

3.3.2.2 Volume

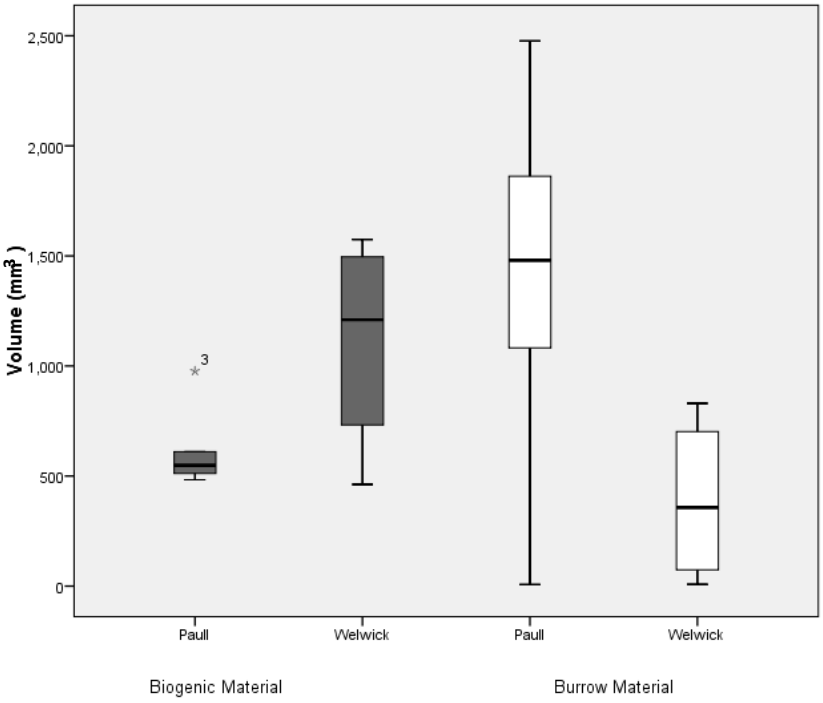


Figure 3.8 Volume ( ) of burrow and other biogenic material in core samples

Figure 3.8 shows that the sample sites Paull exhibited an increased volume of burrow material than that of biogenic material. The reverse can be observed for Welwick, where biogenic material has a higher volume than that of burrow material. The mean volume of biogenic, burrow and combined materials are: 814, 994 and respectively.

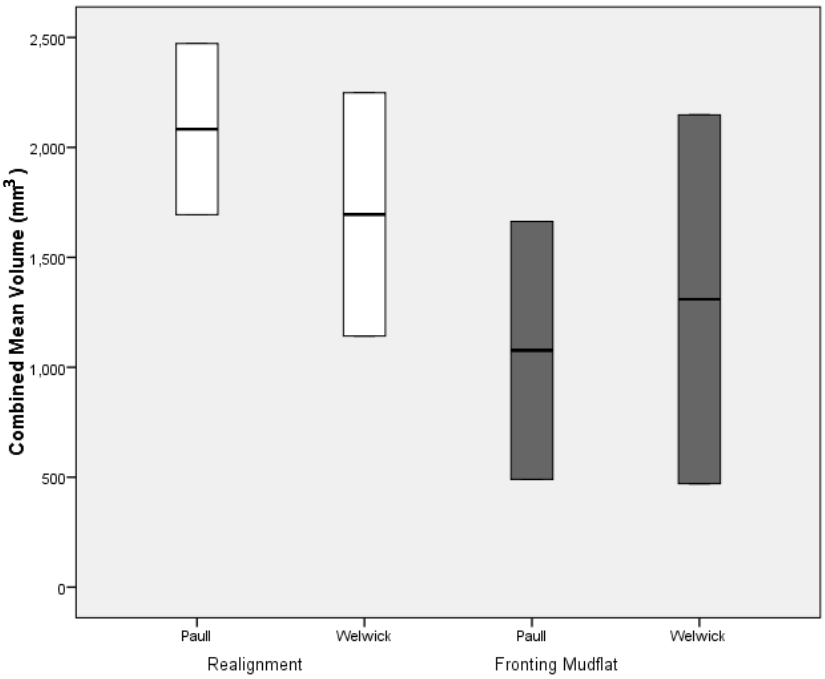


Figure 3.9 Mean Volume ( ) of realignment and fronting mudflat cores at Paull and Welwick

At Paull the combined volume increased within the realignment compared to that of the fronting mudflat, similarly this is also true at Welwick, although a lesser extent: Paull having a difference of between the realignment and fronting mudflat, with at Welwick (Figure 3.9).

3.3.2.3 Relative Burrow Density

Relative burrow density was highest in samples Paull 5 and 6, with the lowest being samples Paull 2 and Welwick 4 (Table 3.1). This was to be expected as these samples consisted of the upper and lower burrow volumes. The mean relative density was 0.$^{-1}$, which is broadly similar with relative densities found in other studies (Mazik et al, 2008), with the exception off Paull 2 and Welwick 4, which have particularly low burrow volumes and therefore relative densities.

Table 3-1 Volumes of Burrow Total Scan Volume and Relative Burrow Density Found in Core Scans

Core Sample	Volume of Burrows only ()	Total Scan Volume ()	Relative Density (Burrow Vol. / Total Scan Vol.)
Paull 1	1082.1	123150.4	0.0087865
Paull 2	8.0	123150.4	0.0000651
Paull 3	1778.8	123150.4	0.0144440
Paull 4	1181.6	123150.4	0.0095945
Paull 5	1861.5	123150.4	0.0151156
Paull 6	2476.8	123150.4	0.0201122
Welwick 2	829.9	123150.4	0.0067388
Welwick 3	139.9	123150.4	0.0011357
Welwick 4	8.8	123150.4	0.0000717
Welwick 5	574.4	123150.4	0.0046638

3.3.2.4 Use of Surface Area

Percentage use of the surface area by infaunal organisms was calculated for each core (Table 3.2). These percentages represent an increase in surface area of the sediment that without infaunal organisms would be greatly reduced. Paull 6 had the highest percentage surface area usage at 52% with Welwick 4 the lowest at 5.2%. Paull 6 had the highest burrow surface area with Paull 2 and Welwick 4 the lowest. Welwick 5 had the highest biogenic surface area and Welwick 4 the lowest.

Table 3-2 Percentage Surface Area Utilised by Infaunal organisms at Core Sample Locations

Core Sample	Surface Area Utilized (%)		
	Biogenic Material	Burrow Material	Combined
Paull 1	13.7	16.2	29.9
Paull 2	11.1	0.2	11.3
Paull 3	16.1	23.5	39.6
Paull 4	7.2	14.6	21.8
Paull 5	12.2	31.8	44.0
Paull 6	9.4	42.5	51.8
Welwick 2	21.0	9.1	30.0
Welwick 3	13.7	2.1	15.7
Welwick 4	5.1	0.2	5.2
Welwick 5	23.4	6.6	30.0

3.3.3 Infaunal Analysis

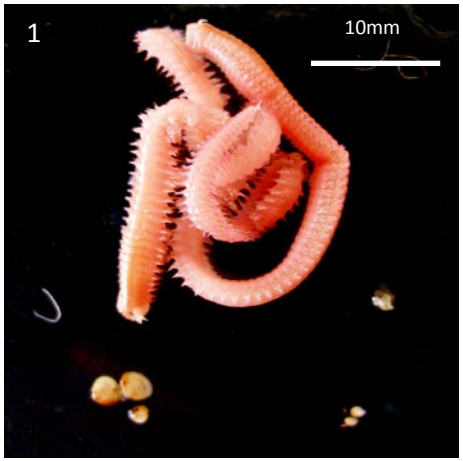
A total of 16 different species across both sites were recovered after the cores were analysed (Table 3.3). These were typical of organisms mostly found on intertidal mudflats around the UK. The main species observed were oligochaetes, polychaetes and bivalves.

3.3.3.1 Paull

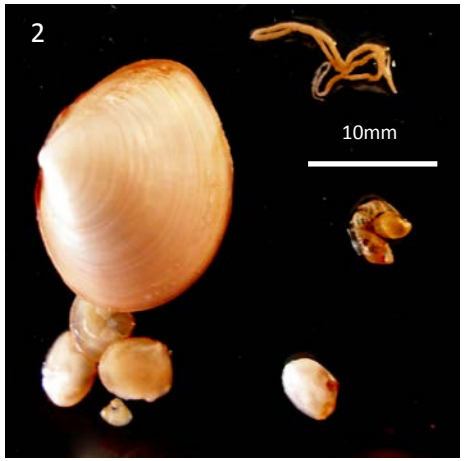
Three species: Nematoda, *Manayunkia aestuarina* and Enchytraeidae accounted for 73% of the overall number of individuals found within the Paull samples, with Nematoda the highest at 46%. Another 9 species made up the remaining 27%. Site Paull 3 had the highest total with 81 individuals, Paull 2 held the least with only 10 individuals. In regard to larger bioturbating organisms which create subsurface burrows i.e. species that have the optimum likelihood of selection by µCT at the implemented scan resolution, sample sites Paull 1 and 4-6 contained between 1 and 5 *Hediste diversicolor* and 2-7 bivalves: *Macoma balthica* or *Abra tenuis* (Figure 3.10; 1, 4,5 and 6). Sample Paull 2 (Figure 3.10; 2) consisted primarily of the bivalve *M. balthica*, with the largest specimen size of 20mm. In contrast Paull 3 (Figure 3.10; 3) comprised in the main of small polychaetes, the majority *M. aestuarina.*

Table 3-3 Species Core Counts of Infaunal Organisms from Sample Sites (Total Numbers of Individual Species)

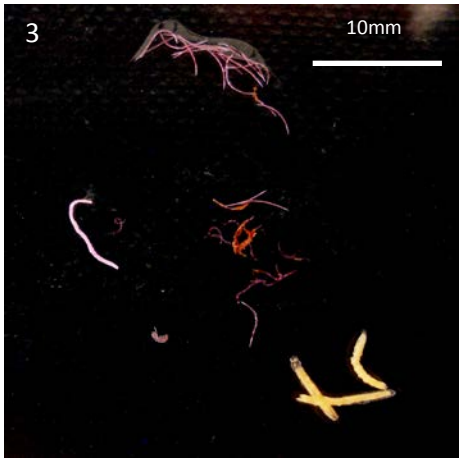
Species (Juv.- Juvenile)	Core Sample									
	Paull						Welwick			
	P1	P 2	P 3	P 4	P 5	P6	W 2	W3	W 4	W 5
Nematoda	16	0	45	6	11	1	7	130	87	99
Hediste diversicolor	2	0	0	2	5	1	1	0	0	1
Manayunkia aestuarina	0	0	29	0	0	1	1	259	0	0
Eteone flava/longa	0	0	0	2	1	0	0	0	1	0
Tubificoides benedii	0	1	0	0	0	0	0	0	1	57
Enchytraeidae	1	0	3	1	0	0	1	642	0	0
Hydrobia ulvae	1	4	0	0	3	10	5	0	0	3
Tellinoidea (Juv.)	2	0	0	0	3	0	0	0	6	20
Macoma balthica	3	5	0	0	0	0	0	0	3	7
Abra tenuis	0	1	0	7	2	0	0	0	0	1
Cardiidae (Juv.)	0	0	0	0	0	0	0	0	1	0
Diptera	0	0	3	0	0	0	3	0	0	0
Collembola	0	0	1	0	0	0	36	7	0	0
Ostracoda	0	0	0	0	0	0	0	0	1	8
Copepoda	0	0	0	0	0	0	0	0	12	14
Totals	25	10	81	18	25	13	54	1038	112	210



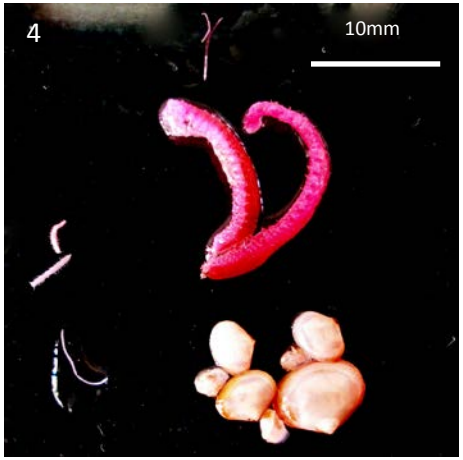
Clockwise from the top right: Nematode spp., *Hydrobia ulvae*, Tellinacea spp. (Juv.), *Macoma balthica*, Enchytraeidae spp., *Hediste diversicolor*



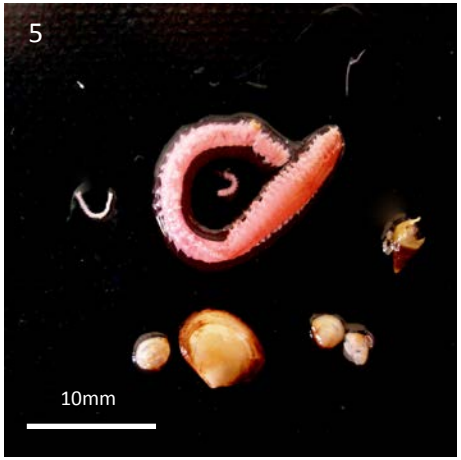
Clockwise from the top right: *Tubificoides bendii*, *Hydrobia ulvae, Abra tenuis, Macoma balthica*



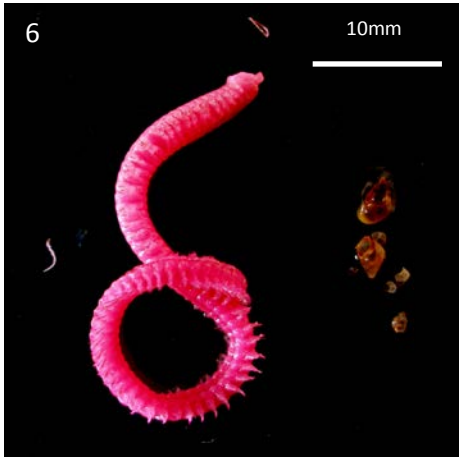
Clockwise from the top right: Nematode spp., *Manayunkia aestuarina*, Diptera spp., Collembola spp., Enchytraeidae spp.



Clockwise from the top right: Nematode spp., *Hediste diversicolor, Abra tenuis*, Enchytraeidae spp., *Eteone flava/longa*

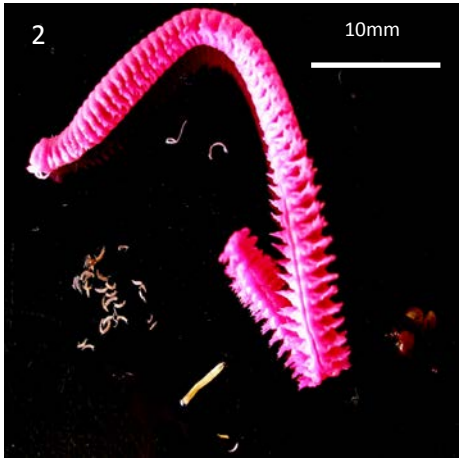


Clockwise from the top right: Nematode spp., *Hediste diversicolor, Hydrobia ulvae*, Tellinacea spp. (Juv.), *Abra tenuis, Eteone flava/longa*

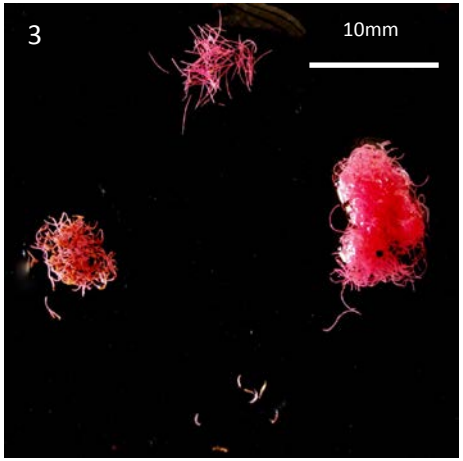


Clockwise from the top right: Nematode spp., *Hydrobia ulvae, Hediste diversicolor, Manayunkia aestuarina*

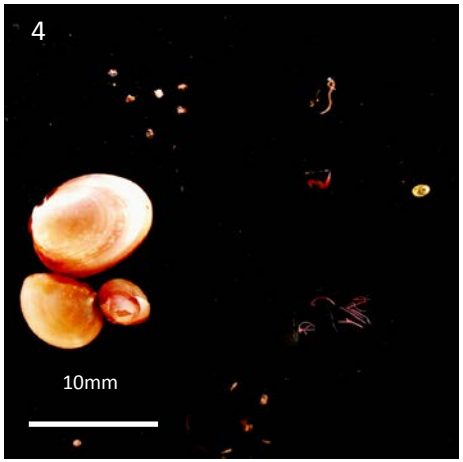
Figure 3.10 Benthic fauna found at Paull sample sites (P1-6)



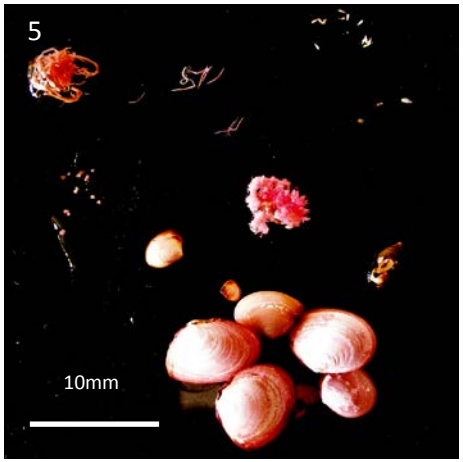
Clockwise from the top: *Hediste diversicolor*, Nematode spp., *Hydrobia ulvae*, Diptera spp., Collembola spp., Enchytraeidae spp., *Manayunkia aestuarina*



Clockwise from the top: Nematode spp., Enchytraeidae spp., Collembola spp., *Manayunkia aestuarina*



Clockwise from the top right: *Tubificoides bendii*, *Eteone flava/longa*, Nematode spp., Copepoda spp., Caridiidae spp. (Juv.), *Macoma balthica*, Tellinacea spp. (Juv.)



Clockwise from the top right: Copepoda spp., *Hydrobia ulvae*, *Macoma balthica, Abra tenuis*, Tellinacea spp. (Juv.), *Tubificoides bendii*, Nematode spp. Centre: *Hediste diversicolor*

Figure 3.11 benthic fauna found at Welwick sample sites (W2-5)

Overall Nematoda spp, M*. aestuarina* and *M. balthica* were more prevalent at sites inside the realignment, while *H. diversicolor* and *A. tenuis* were more widespread on the fronting mudflat.

3.3.3.2 Welwick

At Welwick, Enchytraeidae accounted for the most of overall numbers of individuals found within the samples, achieving 45%, with *Manayunkia aestuarina* and Nematoda accounting for 17 and 10% respectively. Another 4 species made up the remaining 8%. Site Welwick 3 had the highest total with 1038 individuals, Welwick 2 held the least with 54 individuals.

Sites Welwick 2 and 5 were the only samples from Welwick that contained *H. diversicolor (*Figure 3.11*;* 2 and 5*);* the latter also had the highest number of bivalves from all the sample sites, with a total 28 split between 3 species: juvenile Tellinoidea spp., *M. balthica* and *A. tenuis*. Welwick 4 was similar in having no large worms but small bivalves primarily of *M. balthica* (Figure 3.11; 4). In contrast Welwick 3 (Figure 3.11; 3) had the largest counts of *M. aestuarina* and Enchytraeidae spp*.* with 259 and 642 respectively with no bivalves.

In Summery Nematoda, *M. aestuarina* and Enchytraeidae were recorded at higher densities within the realignment with the bivalves *M. balthica*, *A. tenuis* and juvenile Tellinoidea along the fronting mudflat.

3.3.4 Relative Abundance of Infaunal Organisms

The relative abundance for infaunal organisms found in the sediment cores were calculated, for each site and between the fronting mudflat and realignment areas (Table 3.4).

As a whole the Paull sampling site held a higher abundance of Nematode species, the polychaetes *E. flava/longa* and *H. diversicolor,* the gastropod *H. ulvae* and the bivalves *M. balthica* and *A. tenuis* than the *W*elwick site, which had higher abundances of the oligochaete *T. benedii* and Enchytraeidae than Paull.

3.3.4.1 Paull

Inside the realignment Nematode species, the polychaete, *M. aestuarina* and the small bivalve, *M. balthica* were the most abundant organisms. On the fronting mudflat nematodes, the gastropod *H. ulvae* and the polychaete *H. diversicolor* had the highest abundances. Between both sections the realignment had higher abundances of small worms and the bivalve *M. balthica*, while the fronting mudflat had higher abundances of larger worms and the bivalve *A. tenuis*.

3.3.4.2 Welwick

In the realignment the oligochaete Enchytraeidae spp. the polychaete *M. aestuarina* and Nematode spp. were the most abundant organisms, with Nematode spp. the oligochaete *T. benedii* and juvenile bivalves Tellinoidea spp. abundant on the fronting mudflat. Between each section the realignment contained a higher abundance of Enchytraeidae spp. while the mudflat comprised of a similar abundance of Nematode spp. Only the foreshore had bivalves.

Table 3-4 Relative Abundance of Infaunal Organisms

Species	Paull			Welwick		
	Realignment	Fronting Mudflat	Both	Realignment	Fronting Mudflat	Both
Nematoda	0.526	0.321	0.459	0.125	0.578	0.228
H. diversicolor	0.017	0.143	0.058	0.001	0.003	0.001
M. aestuarina	0.250	0.018	0.174	0.238	0.000	0.184
Eteone flava/longa	0.000	0.054	0.017	0.000	0.003	0.001
T. benedii	0.009	0.000	0.006	0.000	0.180	0.041
Enchytraeidae spp.	0.034	0.018	0.029	0.589	0.000	0.455
H. ulvae	0.043	0.232	0.105	0.005	0.009	0.006
Tellinoidea spp. Juv.	0.017	0.054	0.029	0.000	0.081	0.018
M. balthica	0.069	0.000	0.047	0.000	0.031	0.007
A. tenuis	0.000	0.161	0.052	0.000	0.003	0.001
Cardiidae Juv.	0.000	0.000	0.000	0.000	0.003	0.001
Diptera larvae	0.026	0.000	0.017	0.003	0.000	0.002
Collembola	0.009	0.000	0.006	0.039	0.000	0.030
Ostracoda	0.000	0.000	0.000	0.000	0.028	0.006
Copepoda	0.000	0.000	0.000	0.000	0.081	0.018

3.3.5 Comparison of 3D Visualizations and Infaunal Organisms

3D visualizations scans of the infaunal organisms found within the core were compared to the actual species/families in the core. This became problematic as only the larger polychaete worm *H. diversicolor* produced extensive burrow structures which could be easily identified. This in itself presented a difficulty as the number of burrows varied greatly with the actual numbers of *H .diversicolor* (Table 3.5) in the cores. Samples Paull 2 and 3, Welwick 3 and 4 had large burrows with no *H .diversicolor* present. This was particularly evident in core Paull 3 which contained a typical 'Y' shape burrow. It is likely that the majority of the burrow material in Figure 3.4 and Figure 3.5 was produced by *H .diversicolor* as these burrows are of a large size needed by the species. Therefore the number of burrows does not reflect the actual number within the core. It was not possible to identify structures made by other organisms found in the cores due to their smaller size.

Table 3-5 Comparison of Burrows and *H. diversicolor* in Core Samples

Core Sample		No. of Burrows in 3D core	Actual No. of *Hediste diversicolor*
Paull	1	7	2
	2	2	0
	3	7	0
	4	5	2
	5	7	5
	6	11	1
Welwick	2	2	1
	3	2	0
	4	1	0
	5	1	1

3.3.6 Particle Size Analysis (PSA) and Sediment Carbon Analysis

3.3.6.1 PSA

The statistics package Gradistat (Blott and Pye, 2001) was used to analyse the data of grain distribution from the PSA core samples which were produced from the laser granulometer. All Phi (Φ) values were calculated using the Folk and Ward (1957) method.

Particle size analysis showed that the sediment collected from close to the scan cores was poorly sorted (1.8 sorting co-efficient) across all the sampling sites. The sediment at Paull ranged from very course silt at Paull 1 to medium silt at Paull 6. Similarly Welwick samples contained course silt (Welwick 5) to medium silt at Welwick 2.

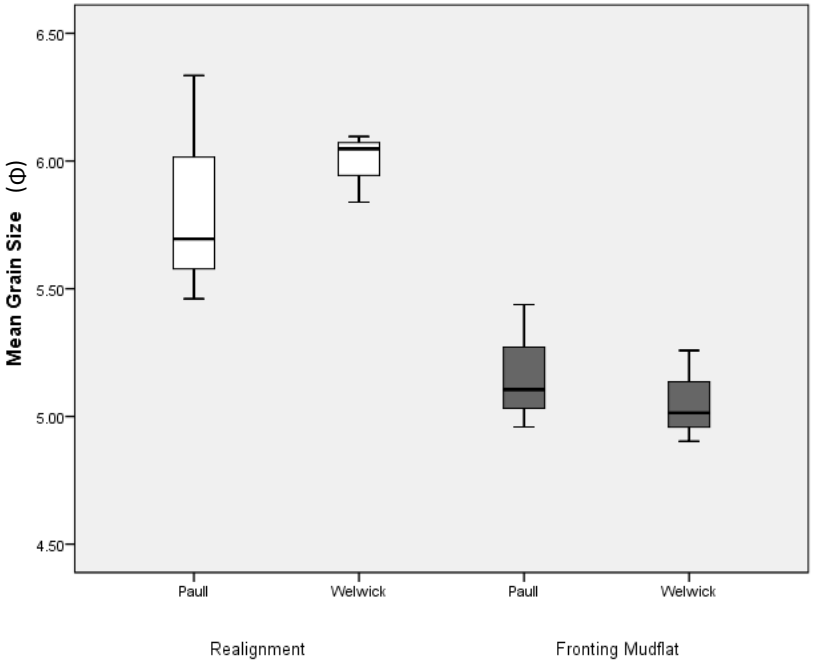


Figure 3.12 Mean grain size of sediment (Φ) collected from sample sites

At both Paull and Welwick, the sites within the realignment areas had a higher phi value than those along the fronting mudflat i.e. finer sediments; this reflects lower tidal velocities inside the site enabling finer particles to settle out of suspension (Figure 3.12). Most of the samples had a value of between 5-6Φ which classifies them as course silt. Samples Welwick 2, 3 and Paull 6 were similar in containing approx. 90% muds to 10% sands. Paull 2, 4 and 5 had approx 80% muds with 20% sand. Welwick 4, 5 and Paull 1, 3 all held < 70% muds. The core samples collected for PSA analysis all contained large bioturbation structures and small shell fragments (Figure 3.13 and Figure 3.14). The majority also show clear graduations in oxygenated (light coloured) and anaerobic/less well oxygenated sediment (dark coloured).

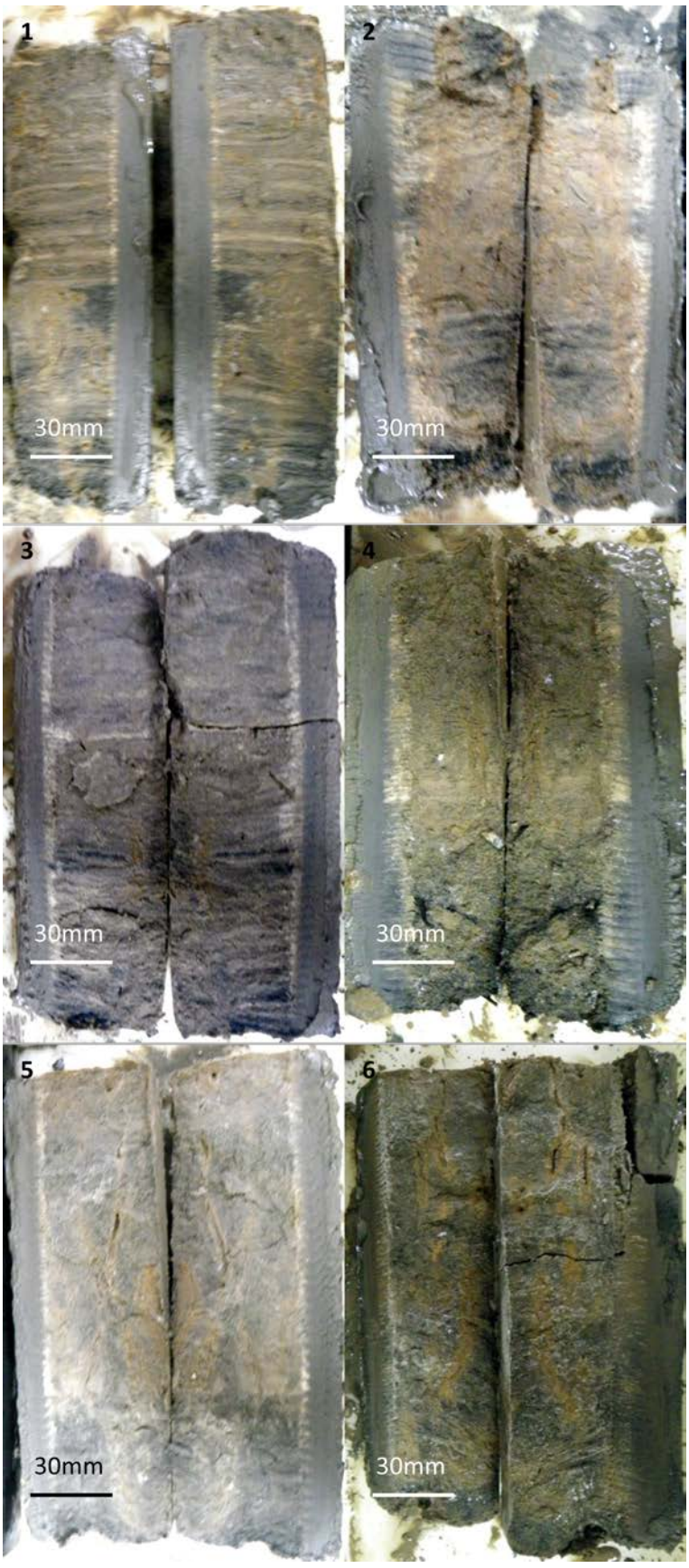


Figure 3.13 PSA cores from Paull sampling site 1-6

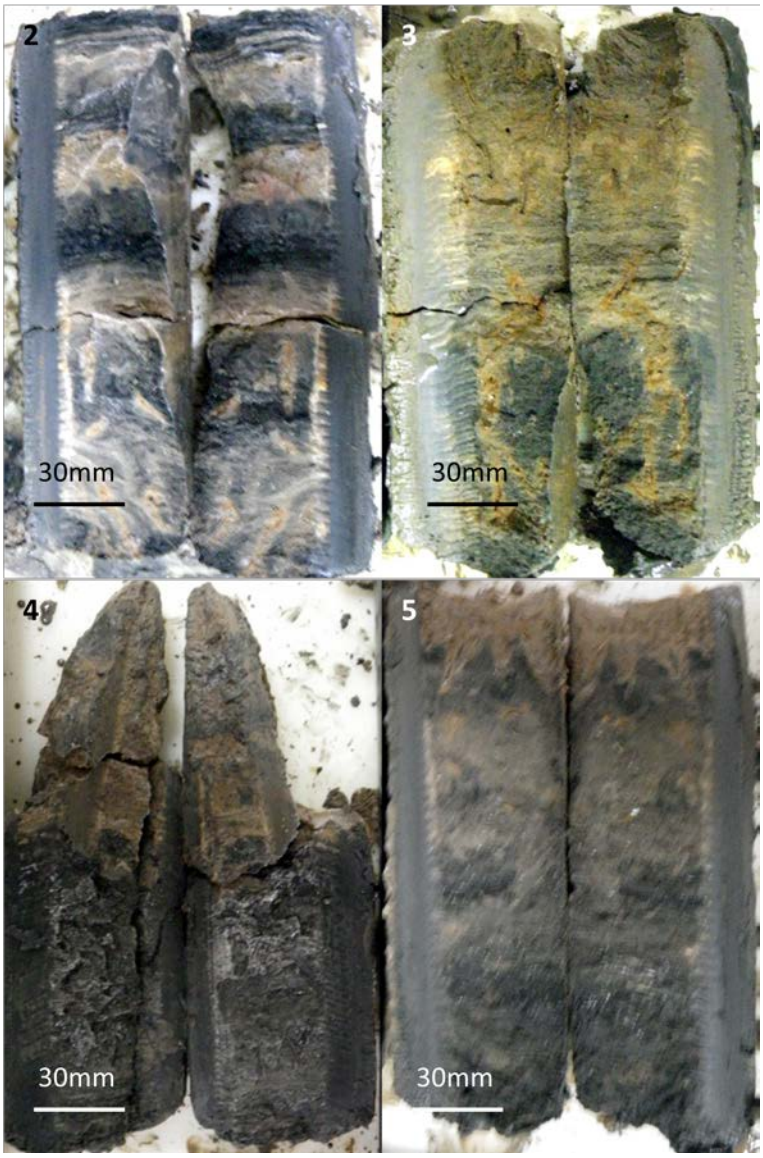


Figure 3.14 PSA cores from Welwick sampling site 2-5

In general oxygenated sediment is present throughout the cores, Welwick 2 is the notable exception (Figure 3.14.2) which has distinct horizons within the core producing alternate banding of oxygenated/anaerobic sediment.

3.3.6.2 Sediment Carbon Analysis

Sediment carbon analysis was performed by means of percentage loss-on-ignition method (LOI) on the homogenised PSA core samples. Site Paull 6, had the highest amount of organic carbon at 7.55%, and Paull 3 the least with 3.11% (Table 3.6). The mean value across all samples was 4.66%. In general the samples taken from within the Welwick realignment site had >5% organic carbon, with the samples on the fronting mudflat <5%. At Paull all samples

had <5% carbon with the exception of Paull 6 which is located at the north east corner of the site.

Table 3-6 Total Organic Carbon at Sample Sites (% Loss on ignition)

Sample	Sample Dry Weight (g)	Sample Post Ignition Weight (g)	Organic Carbon (%)
Paull 1	18.3672	17.6025	4.16
Paull 2	19.9659	19.1239	4.22
Paull 3	22.5704	21.8689	3.11
Paull 4	18.2930	17.5015	4.33
Paull 5	19.4007	18.5098	4.59
Paull 6	15.8090	14.6157	7.55
Welwick 2	18.9848	17.9952	5.21
Welwick 3	16.8751	15.9647	5.39
Welwick 4	17.8032	17.1210	3.83
Welwick 5	18.8305	18.0427	4.18

The percentage of organic carbon was plotted against mean grain size in Φ (Figure 3.15) this shows that an increase in organic carbon corresponds to an increase in Φ i.e. the smaller the grain size the more organic carbon is contained within the sediment sample.

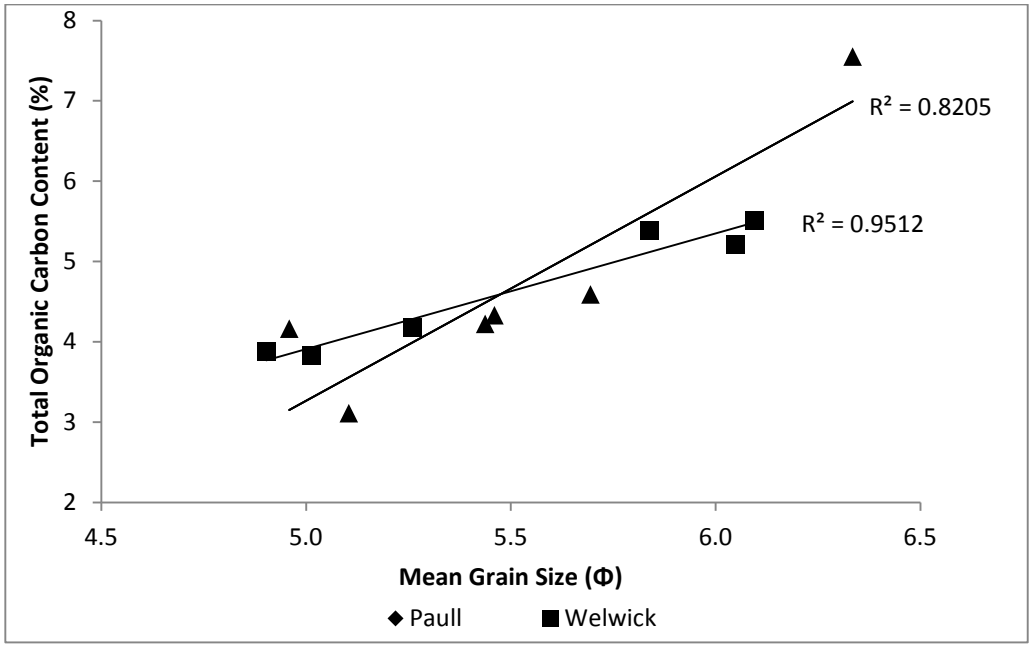


Figure 3.15 Total organic carbon (%) against mean grain size (Φ)

3.4 Discussion

The purpose of this chapter was to establish the extent of biogenic features within sediment cores from µCT scanning via 3D visualisation from different locations along the shore and link them to the functional ecology of bioturbating organisms.

The extent of the biogenic features within the core was determined via the use of µCT. By using Avizo®, 3D reconstructions of burrow structures within sediment cores were established from different locations. This data has then been used to calculate burrow volumes, surface area and relative burrow density, then cross referenced with infaunal, PSA and LOI data.

The majority of samples from Paull have extensive burrow structures with small amounts of 'other' biogenic material, therefore possessed a higher relative density compared to that of the Welwick cores, which contained elevated amounts of 'other' biogenic material and a lower number of burrows. Welwick had higher species richness than Paull, having 9 species/groups compared to 6. Although relative species abundance was lower for large worms and bivalves at Welwick.

The scans clearly show the presence of *H. diversicolor*, predominantly at the Paull sample sites. The large size of the burrows from this organism has enabled these structures to be easily identified; the values obtained from this analysis are consistent with Mazik et al. (2008) for the Paull sampling sites which examined a location near the present study. This the first time the site at Welwick has been sampled in this way. The reduced burrow statistics from these samples when compared to Paull is due to the reduction of *H. diversicolor* burrows found in the cores. Mean numbers of *H. diversicolor* per sample for Welwick and Paull are 0.5 and 1.6 respectively. Higher numbers of *H. diversicolor* were found within the realignment at Paull than on the fronting mudflat. Numbers at Welwick were equal in both sections but at a much lower abundance.

Particle size and loss-on-ignition analysis demonstrates that the sediments were homogeneously mixed, finer sediments were located inside both of the realignment sites, with coarser sediments on the fronting mudflats and that with a decrease in sediment particle size there was an increase in organic carbon content.

4 General Discussion

The purpose of this study was both methodological; to develop the high resolution µCT technique first developed by Mazik et al. (2008), optimising the scanning method with sample stabilisation and ecological; to ascertain burrow structures to a species level and determine quantifiable parameters of bioturbation. Therefore these two aspects have been discussed separately below.

4.1 Ecological aspects

4.1.1 3D Structure and Infaunal Analysis

The 3D reconstructions of burrow structures created by using Avizo® show that most samples from Paull had extensive burrow structures with small amounts of 'other' biogenic material, therefore possess a higher relative density compared to that of the Welwick cores, which contained elevated amounts of 'other' biogenic material and a lower number of burrows.

The images of the scans clearly show the presence of *H. diversicolor,* one of the most dominant macrofaunal organisms found on the mid to upper intertidal mudflats along the mid to outer Humber estuary (McLusky and Elliott, 2004). The large size of the burrows from this organism has enabled these structures to be easily identified. Although the number of burrows does not reflect the actual number of individuals within the core, it is likely that most if not all the burrow structures retrieved from the scans were from this species, as *H. diversicolor* can create burrows larger than the core diameter and as are known to maintain several smaller side burrows of one larger main burrow (Davey, 1994).

The values obtained from burrow volume and surface area analysis are similar to Mazik et al. (2008) findings, for the Paull sampling sites which examined a location near the present study. Sample Paull 6 had the highest burrow volume () and surface area () representing 52% increase in surface area; Mazik et al. (2008) found an approximate burrow volume and surface area of and respectively, for a 45mm core depth.

The reduction in burrow volume and surface area of the Welwick samples when compared to Paull (excluding P2) is due to the lower numbers of *H. diversicolor* burrows found in the cores, mean *H. diversicolor* numbers per sample were 0.5 for Welwick and 1.6 for Paull, this equates to 203-609 individuals per m^{-2}, which is within the range of density of 35-3700 $ind.m^{-2}$ estimated by Scaps (2002) for this species' populations in estuarine areas. Many infaunal organisms are known to have a patchy abundance in intertidal areas (Bulling et al., 2008); more core sampling would be needed to ascertain the extent of *H. diversicolor* burrows at this site as only one core per site was taken.

Relating the 3D visualizations of the scans to the infaunal organisms found within the core using functional ecology of the species involved was difficult to establish as only *H. diversicolor* could be clearly identified. Samples Paull 2 and 3, Welwick 3 and 4 contained large burrows with no *H .diversicolor* present. This was particularly evident in core Paull 3 which contained a typical 'Y' shape burrow. It is likely that these structures were still produced by *H .diversicolor* as these burrows are of a large size needed by the species and that animal which made the burrows was either: in another section of burrow which was connected to, but not sampled by the core or had vacated the burrow prior to the coring taking place, as *H. diversicolor* burrows are only semi permanent (Gunnarsson et al. 1999) and are known to vacate burrows to areas of organic enrichment (Bulling et al. 2008).

Infaunal activity has been shown to increase the surface area in bioturbated sediments (Biles et al. 2002), with some studies demonstrating an increase of over 300% (Davey, 1994). The current study ascertained an increase of up to 43% for burrow structures and 52% when combined with other biogenic features. Though this figure does not take into account small infaunal worms and bivalve organisms which would not have been picked up on the scan, but were included by Davey (1994), it still represents a substantial increase in surface area due to bioturbation activity. As the majority of the burrow features were *H. diversicolor,* therefore it is likely that this species solely reasonable for the 43% increase in surface area.

The infaunal analysis of core samples demonstrates that many other organisms such as oligochaete and polychaete worms, gastropods and bivalves were retrieved from the cores, which proved difficult to distinguish at the current scan resolution due to their small size and possible confusion with detritus. Characterisation of smaller burrows was only achieved by Mazik (2008) scanning a very small 10mm diameter core. This would limit the value of the technique in that a 10mm core is not sufficient to characterise bioturbation or community structure. Therefore we can scan for larger macrofauna and meiofauna separately but not for size classes in between.

Particle size and loss-on-ignition analysis demonstrates that finer sediments were located inside both of the realignment sites, with coarser sediments on the fronting mudflats and that with a decrease in sediment particle size there was an increase in organic carbon content. This was expected as lower tidal velocities allow finer sediment to settle out of suspension. It is also notable that Paull 6 had both the highest LOI carbon concentration and burrow structure statistics of all the sites; this supports Bulling et al. (2008) findings in which *H .diversicolor* spatially reposition to areas of nutrient enrichment. PSA also showed that the sediments from the core locations were homogeneously mixed i.e. they contained no large stones or larger debris and that recent bioturbation had taken place with recently active burrows extending the length of the core, therefore it is likely that the agar core were of a similar composition.

4.2 Methodological Aspects

4.2.1 Sample Stabilization

The importance of sample stabilization in the main is due to the variable physical nature in sheer strength and compaction of intertidal sediments (Hall, 1994), that can allow for the collapse of bioturbation features. Following the establishment of criteria and a scoring scale for a suitable stabilization material, three substances were trialled to determine the optimal one. This proved to be agar, which set sufficiently fast to extract the core before the oncoming tide and with adequate time to perform the stabilization technique. It also had superior void

clarity with easily definable edges than the other materials trialled. As the other materials were extracted at the same time from an area of elevated burrows, using the same techniques, it is assumed that the reduction in the detection of voids from the other materials tested is due to either infilling from feature collapse via extraction and transportation from the mudflat, or that the stabilization material is of a similar density to the sediment in the cores.

Agar as a stabilization method performed well when compared to other studies: Michaud et al. (2003) and Rosenberg et al. (2007) found that the use of a hot paraffin/Vaseline® damaged surface features and killed infaunal organisms. The technique of Seike et al. (2012) using resin to determine dimensions of burrow structures encased the organisms which died and made identification difficult. When using agar all organisms recovered from the cores were alive with surface features intact and easily identifiable. In producing live intact organisms Agar performed similarly to Cream of Rice® (Rosenberg et al. 2007 and Weissberger et al. 2009) did on larger sub-tidal cores, but has the ability to set faster for transport to be used in an intertidal environment.

4.2.2 3D Analysis Experimental Protocol

Currently there is no standard protocol for µCT in regards to software analysis, precise protocols are crucial since software such as Avizo® are user sensitive and results can be biased if standardized methods are not applied (Kallai et al, 2011), making it difficult to compare with results of other studies (Bouchet et al, 2009).

To perform the collection of agar stabilized intertidal cores and µCT scanning techniques methods 3.2.2 and 3.2.3 can be used. To establish 3D burrow measurements for macrofaunal organisms, the following protocol has been developed for using Avizo® imaging software:

- Reduce scanner 16 bit .tif image output files to 8-bit .tif with Image J (NIH).
- In Avizo® load the 8-bit .tif files that are below the sediment-agar horizon and above any scans which show damage from extraction.

- Assign a thresholding value to the cores bioturbation features, highlight burrows and other biogenic objects that appear darker than the surrounding sediment.
- Smooth all slides and remove coring tube if highlighted.
- Using expert judgement and Figure 4.1 as a guide:
    - Identify and remove highlighted material which is under 63µm or extends less than 3 slides.
    - Identify and remove cracks, large stones and shell fragments.
    - Identify and categorise obvious burrow structures and bivalves.
    - Label any other biogenic material.
- Establish surface area and volume of each category via three dimension surface generation.

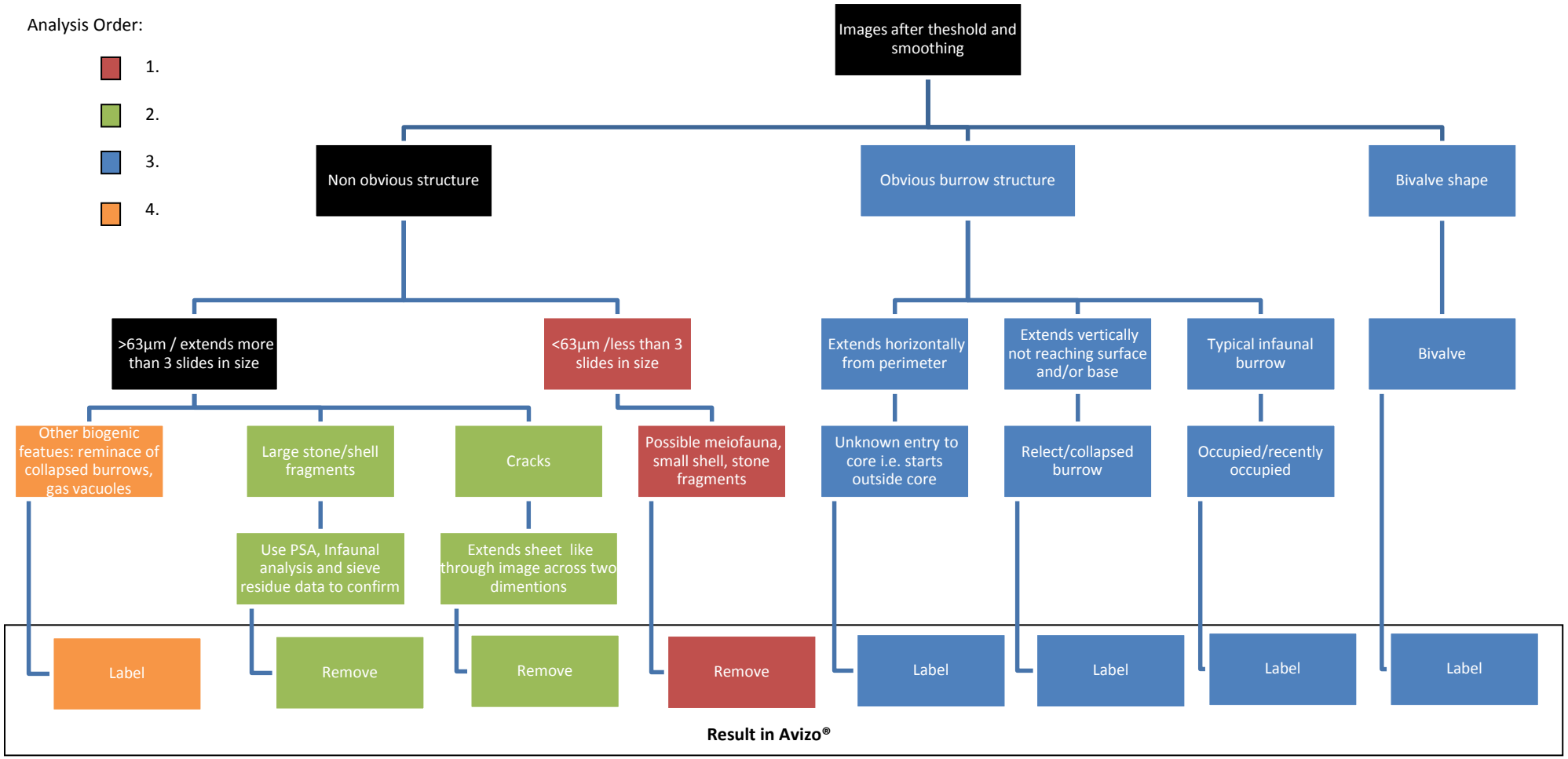


Figure 4.1 Identification and analysis of µCT agar cores using 3D reconstruction software for benthic macrofauna after thresholding

4.2.3 Limitations

The main limitation of the technique is that in its current format, large extensive burrows are easily found, whereas smaller worm and bivalve burrows are difficult to establish. This is due to the reduced size of the burrows, the movement of bivalves though sediments; infilling burrows as they travel (Tallqvist, 2001), together with possible confusion with detritus and other inorganic material e.g. stones. Particle size analysis was performed to determine if sediment near to the coring location contained other inorganic material and although it indicated that it did not, it is probable that scanning a core specifically for bivalve shell density and at a higher resolution would solve this issue and allow bivalves and smaller worm burrows to be recovered.

A time limitation occurs when using agar to stabilise cores. Agar is heated in solution to activate its setting properties (90°C), which allows solidification on cooling at approximately 35°C The rate of reduction in temperature is the timeframe in which injection into a core must occur. This rate is affected by several environmental factors such as air temperature and wind speed. Thus on a cold day with elevated wind levels the solution congeals faster than a warm day with no wind. In addition as sample sites become an increased distance from the point of heating, there is a greater opportunity for heat loss. To a certain degree this can be counteracted by heating agar on site and using an insulated heated water bath when transporting agar across the mudflat.

4.2.4 Conclusions

Using agar it is possible to stabilize in-situ bioturbation structures of large infaunal organisms in sediment cores on intertidal mudflats and accurately determine the parameters of these structures through µCT both qualitatively and quantitatively.

Agar may also be an adequate substitute for Cream of Rice® on sub-tidal sediment cores as it performed superior over the latter on intertidal cores.

4.2.5 Further Research

It would have been advantageous to the results if each of the agar cores were scanned multiple times, specifically for bivalve shell density in addition to burrow voids. This would allow for easier recognition and clearer interpretation of these features, cross referencing between each scan would also reduce confusion between biogenic and non biogenic features such as mollusc shell and small stones. This would involve refining voltage and amps of the scanning parameters. Refining the scanning technique would also be beneficial in reducing background 'noise' that result from the reflection/refraction of x-rays passing though the core to the detector. This creates a distorted image on the resulting scan and can make feature identification difficult. To ascertain a more precise infaunal distribution between the realignments and fronting mudflats at Paull and Welwick, sampling would need to include further coring stations with an increased number of replicates.

Additional research would be needed to determine if a significant amount of bivalves, smaller organisms and their associated bioturbation structures are to be revealed using µCT scans. There is also a need to establish a way of directly linking burrow structures found in scans to community composition and function to maximise the use of this technique in providing an indication of ecological functioning, for example being able to identify key suspension and deposit feeding organisms from individual burrow structures for their roles as bioindicators for monitoring purposes.

Further studies would also be needed to establish if agar can be used to stabilize burrows of other intertidal sediments of differing sizes i.e. fine/course sands, as the present project only applied agar to muds. This would allow for the study of various other organisms and an accurate comparison between similar roles of infaunal organisms in functioning on different intertidal sediments.

The use and analysis of µCT cores is widely applicable to other fields of benthic ecology and as such could be used to investigate: the link between amounts of burrow solutes produced by a

species and its relationship to burrow size, bacterial colonisation of burrow structures and the effect of heavy metals or excess nutrients on burrow development.

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6 Appendixes

6.1.1 Stabilization Scoring

Table 6-1 Scale for Determining Scoring of Potential Stabilization Materials for Vertical µCT Scanning

Number	Setting Time (mins)	HSE Concerns	Environmental Concerns	Organism retrieval	Cost per sample (£)	Practicality on a mudflat
3	0-30	Negligible	Negligible	High	2	Easily
2	30-60	Low	Low	Medium	2-4	Moderately
1	60+	Medium	Medium	None	4+	Difficult

6.1.2 Scan Parameters

Table 6-2 Core Sample Scan Parameters

Sample Number	Voltage (kV)	Current (µA)	Voxel size (µm)		
			X	Y	Z
Paull 1	75	15	82.4	82.4	82.4
Paull 2	75	15	82.4	82.4	82.4
Paull 3	70	19	93.4	93.4	93.4
Paull 4	70	19	93.4	93.4	93.4
Paull 5	70	17	94.6	94.6	94.6
Paull 6	70	17	92.1	92.1	92.1
Welwick 2	80	22	96.2	96.2	96.2
Welwick 3	80	21	104	104	104
Welwick 4	80	21	97.9	97.9	97.9
Welwick 5	80	21	97.9	97.9	97.9

6.1.3 Bioturbation Analysis Values

Table 6-3 Volume and Surface Area of Burrow and Biogenic Features

Core Sample	Volume of Biogenic Features ()	Volume of Burrows only ()	Combined Volume ()	Surface Area of Biogenic Features ()	Surface Area of Burrows only ()	Combined Surface Area ()
Paull 1	581.0	1082.1	1663.1	1876.2	2226.1	4102.4
Paull 2	482.6	8.0	490.6	1521.8	29.3	1551.0
Paull 3	977.5	1778.8	2756.3	2208.6	3222.7	5431.2
Paull 4	512.4	1181.6	1693.9	983.7	2004.6	2988.3
Paull 5	610.8	1861.5	2472.3	1669.1	4368.0	6037.1
Paull 6	515.9	2476.8	2992.7	1283.6	5825.7	7109.3
Welwick 2	1418.5	829.9	2248.4	2875.7	1246.6	4122.2
Welwick 3	1002.2	139.9	1142.0	1873.8	284.1	2157.9
Welwick 4	461.5	8.8	470.4	694.7	22.2	716.9
Welwick 5	1573.6	574.4	2147.9	3204.2	906.7	4110.9

6.1.4 Statistical comparison of bioturbation features

Table 6-4 T-test results of comparison between site features

Variable	Site				Values		Sig.
	Paull Front.	Welwick Front.	Paull Realign.	Welwick Realign.	Burrow	Combined	
Relative den.	X	X	X	X		X	.059
Volume	X	X	X	X		X	.059
Volume	X	X	X	X	X		.405
Volume	X		X			X	.237
Volume		X		X		X	.707
Volume	X	X				X	.061
Volume			X	X		X	.556
Surface Area	X	X	X	X		X	.193
Surface Area	X	X	X	X	X		.058

6.1.5 Calculating the Total Volume of the Scan Area

To determine the volume of the scan area, the volume of a cylinder had to be ascertained, using the formula:

$V = \pi r^2 h$ were by r =28mm and h =50mm

$V = \pi 2822 \quad 50$

$V = \pi 784 \times 50$

$V = 2463.00864 \times 50$

$V = \underline{123150.}$